Uniwersytet Szczeciński
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# ROZPRAWA DOKTORSKA

Ekspresja wybranych cząsteczek mikroRNA i ich genów docelowych w zakażeniu *Lagovirus europaeus* – genotypami GI.1 i GI.2

Rozprawa doktorska wykonana
w Instytucie Biologii US
pod kierunkiem
dr hab. Beaty Hukowskiej-Szematowicz, prof. US

**Szczecin 2024**

*Składam serdeczne podziękowania dla Promotora mojej pracy*
*Pani dr hab. Beacie Hukowskiej-Szematowicz, prof. US*
*za zaangażowanie, poświęcony czas i wsparcie merytoryczne*
*podczas realizacji rozprawy doktorskiej.*

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*za cierpliwość i wsparcie podczas realizacji niniejszej pracy.*

## OŚWIADCZENIE DOKTORANTA

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Wyrażam zgody na udostępnienie mojej pracy doktorskiej pt.: Ekspresja wybranych cząsteczek mikroRNA i ich genów docelowych w zakażeniu *Lagovirus europaeus* – genotypami GI.1 i GI.2

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**[P-1]** **Ostrycharz E.**, Hukowska-Szematowicz B. (2022). Micro-players of great significance-host microRNA signature in viral infections in humans and animals. *International Journal of Molecular Science* 23 (18), 10536. DOI: 10.3390/ijms231810536.

**IF- 5,6; pkt. MNiSW – 140**

Mój wkład w powstanie tej publikacji polegał na współudziale w opracowaniu koncepcji i metodologii pracy, przeprowadzeniu przeglądu literatury z zakresu omawianego tematu, przygotowaniu strony graficznej pracy, współudziale w napisaniu oraz przygotowaniu manuskryptu do redakcji, współudziale w przygotowaniu manuskryptu po recenzjach.

Mój udział procentowy szacuję na 65%

**[P-2]** **Ostrycharz E.**, Fitzner A., Kęsy A., Siennicka A., Hukowska-Szematowicz B. (2024). MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *Frontiers in Microbiology* 15:1349535. DOI: 10.3389/fmicb.2024.1349535.

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Mój udział procentowy szacuję na 65%.

**[P-3]** **Ostrycharz-Jasek E.**, Fitzner A., Siennicka A., Budkowska M., Hukowska-Szematowicz B. (2024). MicroRNAs regulate the expression of genes related to the innate immune and inflammatory response in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *International Journal of Molecular Science* 25 (17), 9531. DOI: 10.3390/ijms25179531.

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Wszystkie publikacje naukowe wchodzące w skład niniejszej rozprawy doktorskiej zostały opublikowane w otwartym dostępie (Open Access).

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## Spis treści

**Wykaz skrótów**

AC9 – cyklaza adenylowa 9

Akt – kinaza białkowa B

ALF – ostra niewydolności wątroby

ALP – fosfataza alkaliczna

ALT – aminotransferaza alaninowa

ALV – wirus białaczki ptaków

APC – regulator APC szlaku sygnałowego Wnt

AST – aminotransferaza asparaginianowa

ATF2 – czynnik transkrypcyjny 2

Bach1 – domena BTB i CNC homolog 1

Bax – białko X związane z BCL2

Bcl-2 – białko regulatora apoptozy BCL2

Bcl-6 – chłoniak komórkowy 6

BoDV-1 – wirus Borna

C/EBPα – czynnik transkrypcyjny białka wiążącego wzmacniacze CAATα

C1QTNF3 – białko 3 związane z czynnikiem martwicy nowotworu i C1q

CCL7 – ligand chemokiny C-C motyw 7

CCND1 – cyklina D1

CDK4 – cyklina 4

CFH – czynnik H dopełniacza

cFLIP – kaspazopodobne białko regulujące apoptozę

CHB – przewlekłe zapalenie wątroby typu B

COVID-19 – choroba koronawirusowa 2019

CVB – wirus Coxsackie B

DAMP – wzorce molekularne związane z uszkodzeniami

DENV – wirus Dengi

DIC – zespół rozsianego wykrzepiania wewnątrznaczyniowego

DR – receptor śmierci

DUSP6 – fosfataza 6 o podwójnej specyficzności

EBHSV – wirus krwotocznej choroby zajęcy

EBV – wirus Epsteina-Barr

ErbB2 – receptor Erb-B2 kinazy tyrozynowej

Fas – powierzchniowy receptor śmierci komórki Fas

FasL – ligand dla receptora Fas

FHF – piorunująca niewydolność wątroby

FMDV – wirus pryszczycy

GM-CSF – czynnik stymulujący kolonie granulocytów i makrofagów

GSSG/GSH – stosunek glutationu utlenionego do zredukowanego

HA – hemaglutynina

HaCV – kaliciwirus zająca

HBeAg – antygen HBV

HBV – wirus zapalenia wątroby typu B

HBx – białko X wirusa HBV

HCC – rak wątrobowokomórkowy

HCV – wirus zapalenia wątroby typu C

HGF – czynnik wzrostu hepatocytów

HIF-1α – czynnik indukowany niedotlenieniem 1α

HO-I – oksydaza hemowa I

HSC – komórki gwiaździste wątroby

HTLV-1 – wirus T-limfotropowy typu 1

IAV – wirus grypy typu A

IFN – interferon

IKK-α – inhibitor podjednostki alfa kinazy czynnika jądrowego kappa-β

IL – interleukina

JNK – kinaza c-Jun N-końcowa

iNOS – syntaza tlenku azotu

IRAK1 – kinaza związana z receptorem interleukiny 1

IRF3 – czynnik regulacyjny interferon 3

ISG – geny stymulowane interferonem

JEV – wirus japońskiego zapalenia mózgu

LDH – dehydrogenaza mleczanowa

MAFB – czynnik transkrypcyjny MafB

MAP2K7 – kinaza białkowa aktywowana mitogenem kinazy 7

MAPK – kinaza białkowa aktywowana mitogenem

MDV – wirus choroby Mareka

miR – mikroRNA

MOF – niewydolność wielonarządowa

MyD88 – pierwotne białko odpowiedzi na różnicowanie mieloidalne MyD88

NF-κβ – czynnik jądrowego κ-łańcucha lekkiego wzmacniacza aktywowanych komórek β

NLRP3 – inflamasom domeny NACHT, LRR i piryny zawierającej białko 3

NO – tlenek azotu

NPC – nieparenchymalne komórki wątroby

Nrf-2 – czynnik jądrowy związany z erytroidem 2

p53 – białko p53

p65 – czynnik transkrypcyjny p65

PAMP – wzorce molekularne związane z patogenami

PARP – białko polimerazy poli(ADP-rybozy)

PBMC – komórki jednojądrzaste krwi obwodowej

PDCD4 – programowana śmierć komórki 4

PI3K – fosfatydyloinozytol 3-kinazy

PTEN – homolog fosfatazy i tensyny

RABV – wirus wścieklizny

RCV – kaliciwirus królika

RHD – krwotoczna choroba królików

RHDV – wirus krwotocznej choroby królików

RhoB – homolog Ras członka rodziny B

ROS – reaktywne formy tlenu

RSV – syncytialny wirus oddechowy

S1PR1 – receptor 1 sfingozyno-1-fosforanu

SHIP-1 – inozytol polifosforanu-5-fosfazaty 1

SIRT1 – sirtuina 1

SOCS1 – supresor sygnalizacji cytokin 1

SOD – dysmutaza ponadtlenkowa

STAT3 – przewodnik sygnału i aktywator transkrypcji 3

TAB2 – białko wiążące kinazę aktywowaną przez TGF-β 1

T-bet – T-box w komórkach T

TGF-β – transformujący czynnik wzrostu β

THP-1 – linia komórek monocytowych człowieka

TIM-3 – domena immunoglobuliny limfocytów T i mucyny 3

TLR – receptor Toll-podobny

TNFR – receptor czynnika martwicy nowotworu

TNF-α – czynnik martwicy nowotworu α

TRAF6 – czynnik 6 związany z receptorem TNF

TRAIL – ligand TNF – członek rodziny 10

VSV – wirus pęcherzykowego zapalenia jamy ustnej

Wnt – szlak sygnałowy związany z białkiem Wingless/Int-1

WNV – wirus Zachodniego Nilu

α-SMA – α-aktyna mięśni gładkich

γ-GT – γ-glutamylotransferaza

Uniwersytet Szczeciński

Instytut Biologii

**Ewa Ostrycharz-Jasek/magister**

# Ekspresja wybranych cząsteczek mikroRNA i ich genów docelowych w zakażeniu *Lagovirus europaeus* – genotypami GI.1 i GI.2

promotor: dr hab. Beata Hukowska-Szematowicz, prof. US

## Streszczenie rozprawy doktorskiej

*Lagovirus europaeu*s (*L. europaeus*) to wirus wywołujący ciężką chorobę u królików zwaną krwotoczną chorobą królików (RHD) i należy do rodzaju *Lagovirus*, rodziny *Caliciviridae*. W obrębie *L. europaeus* związanego z RHD wyróżniono dwa genotypy – GI.1 (RHDV) i GI.2 (RHDV2). Zakażenie *L. europaeus* prowadzi do zmian patologicznych w wątrobie, płucach, nerkach i śledzionie, przypominających ostrą niewydolność wątroby i niewydolność wielonarządową u ludzi.

MikroRNA (miR) to małe, niekodujące RNA o długości 17–25 nukleotydów, które regulują ekspresję genów na poziomie potranskrypcyjnym. MiR mogą regulować ekspresję genów związanych z procesami biologicznymi zachodzącymi podczas chorób, a tym samym wpływać na aktywność szlaków sygnałowych. Dysregulacja ekspresji lub funkcji miR może prowadzić do zaburzeń ekspresji genów, co przyczynia się do patologii chorób. Wcześniejsze badania wykazały, że kluczową rolę w patogenezie RHD (wywołaną zakażeniem *L. europaeus*) odgrywa apoptoza, stres oksydacyjny oraz elementy wrodzonej i nabytej odpowiedzi immunologicznej i zapalnej. Ekspresja miR gospodarza i ich wpływ na regulację krytycznych genów docelowych zaangażowanych w procesy biologiczne może odgrywać znaczącą rolę w patogenezie chorób o etiologii wirusowej. Jak dotychczas, w literaturze naukowej brakuje danych dotyczących molekularnych sygnatur regulacyjnych interakcji między miR, a apoptozą, stresem oksydacyjnym oraz wrodzoną odpowiedzią immunologiczną i zapalną w zakażeniu *L. europaeus*, patogenezie RHD. Stąd ten problem naukowy stał się celem badań podjętych w rozprawie doktorskiej. Materiał do badań stanowiły tkanki wątroby, płuc, nerek i śledziony

pobrane po eutanazji od królików zdrowych oraz pośmiertnie lub po eutanazji od królików eksperymentalnie zakażonych *L. europaeus* genotypem GI.1 i *L. europaeus* genotypem GI.2.

W celu wyboru miR oraz selekcji i przewidywania docelowych genów dla miR zaangażowanych w procesy biologiczne zachodzące w zakażeniach wirusowych posłużono się dogłębną analizą literatury oraz metodami in silico z wykorzystaniem baz danych dedykowanych do pracy z miR. W celu zbadania wybranych kluczowych miR i ich genów docelowych zaangażowanych w apoptozę, stres oksydacyjny oraz wrodzoną odpowiedź immunologiczną i zapalną w zakażeniu *L. europaeus* dwoma genotypami – GI.1 i GI.2 w czterech tkankach (wątrobie, płucach, nerkach i śledzionie) wykorzystano metody z zakresu biologii molekularnej w postaci pomiaru ekspresji miR i genów docelowych. Takie samo podejście metodyczne wykorzystano do zbadania biomarkerów apoptozy, stresu oksydacyjnego oraz stanu zapalnego. Dla apoptozy wybrano: miR-21 (*PTEN*, *PDCD4*), miR-16b (*Bcl-2*, *CXCL10*) oraz miR-34a (*p53*, *SIRT1*); dla stresu oksydacyjnego: miR-132 (*Nrf-2*) i miR-122 (*Bach1*); dla wrodzonej odpowiedzi immunologicznej i zapalnej: miR-155 (*MyD88*, *TAB2*, *p65*, *NLRP3*), miR-146a (*IRAK1*, *TRAF6*), miR-223 (*TLR4*, *IKKα*, *NLRP3*) oraz miR-125b (*NLRP3*). Dodatkowo zbadano na poziomie mRNA biomarkery apoptozy (*Bax*, stosunek *Bax/Bcl-2*, *PARP*, *kaspazę-3*), stresu oksydacyjnego (*HO-I*) oraz stanu zapalnego (*IL-1β, IL-6, TNF-α, IL-18*) w wątrobie, płucach, nerkach i śledzionie po zakażeniu *L. europaeus* genotypami – GI.1 i GI.2.

W wyniku przeprowadzonych badań po raz pierwszy, wykazano, że podczas zakażenia *L.europaeus*/GI.1 i GI.2 dochodzi do zmian ekspresji miR-21, miR-16b i miR-34a zaangażowanych w proces apoptozy. MiR-16b i miR-34a mogą wpływać na nasilenie apoptozy w badanych narządach, a z drugiej strony hamować, co obserwowano w przypadku miR-21. Na podstawie zbadanych biomarkerów apoptozy *Bax* i stosunku *Bax*/*Bcl-2* można wysnuć wniosek, że proces apoptozy był silniej wyrażony po zakażeniu królików *L. europaeus*/GI.2. MiR-122 i miR-132 regulują dwa szlaki stresu oksydacyjnego w patogenezie RHD, wywołanej zakażeniem *L.europaeus*/GI.1 i GI.2 związane z uszkodzeniem tkanek. Biomarker *HO-I* w RHD wskazuje na oksydacyjne uszkodzenie tkanek. Wykazano, że głównymi regulatorami wrodzonej odpowiedzi immunologicznej i zapalnej w zakażeniu *L. europaeus* /GI.1 i GI.2 są miR-155, miR-223 i miR-146a. Ekspresja miR-125b była ograniczona i dotyczyła wątroby i śledziony. Wykazano, że podczas zakażenia *L. europaeus*/GI.1 i GI.2 miR-155 ma działanie pro- i przeciwzapalne w wątrobie, jak i działanie przeciwzapalne w nerkach i śledzionie; miR-146a ma działanie przeciwzapalne w wątrobie, płucach i nerkach; miR-223 ma działanie przeciwzapalne we wszystkich tkankach; miR-125b ma działanie przeciwzapalne tylko

w wątrobie. W każdym przypadku taki efekt może być wyznacznikiem patogenezy RHD. MiR mogą regulować wrodzone szlaki odpowiedzi immunologicznej i zapalnej w zakażeniu *L. europaeus*, a na wynik tej regulacji może mieć wpływ mikrośrodowisko tkankowe. W badaniach po raz pierwszy przedstawiono profil ekspresji biomarkerów stanu zapalnego (*IL-1β, IL-6, TNF-α, IL-18*) na poziomie mRNA w czterech tkankach po zakażeniu *L. europaeus*/GI.1 i GI.2. W efekcie czego zaproponowano trzy profile zapalenia (płucny, nerkowy oraz wątrobowo-śledzionowy) w odpowiedzi na zakażenie *L.europaeus*/GI.1 i GI.2.

Wyniki uzyskane w trakcie realizacji niniejszej rozprawy doktorskiej istotnie pogłębiły wiedzę dotyczącą patogenezy RHD (wywołanej zakażeniem *L. europaeus* dwoma genotypami – GI.1 i GI.2) z uwzględnieniem mikrośrodowiska tkankowego wątroby, płuc, nerek i śledziony. Przyczyniły się do częściowego poznania roli cząsteczek miR w zakażeniu *L. europaeus* i dostarczyły nowych danych pozwalających lepiej zrozumieć patogenezę RHD w zakresie molekularnej regulacji, apoptozy, stresu oksydacyjnego oraz wrodzonej odpowiedzi immunologicznej i zapalnej przez miR.

**Słowa kluczowe:** *Lagovirus europaeus*/GI.1, *Lagovirus europaeus*/GI.2, wirus krwotocznej choroby królików (RHDV), krwotoczna choroba królików (RHD), mikroRNA, ekspresja genów, apoptoza, stres oksydacyjny, wrodzona odpowiedź immunologiczna, zapalenie, biomarker, królik.

University of Szczecin

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**Ewa Ostrycharz-Jasek/master**

# Expression of selected microRNA molecules and their target genes in *Lagovirus europaeus* infection –GI.1 and GI.2 genotypes

supervisor: dr hab. Beata Hukowska-Szematowicz, prof. US

## Summary of doctoral dissertation

*Lagovirus europaeus* (*L. europaeus*) is a virus that causes severe disease in rabbits called rabbit hemorrhagic disease (RHD) and belongs to the genus *Lagovirus*, the family *Caliciviridae*. Two genotypes of *L. europaeus* associated with RHD have been distinguished: GI.1 (RHDV) and GI.2 (RHDV2). Infection with *L. europaeus* leads to pathological changes in the liver, lungs, kidneys, and spleen, resembling acute liver failure and multi-organ failure in humans.

MicroRNAs (miRs) are small, non-coding RNAs of 17–25 nucleotides in length, that regulate gene expression at the post-transcriptional level. MiRs can regulate the expression of genes related to biological processes occurring during diseases and thus affect the activity of signaling pathways. Dysregulation of miR expression or function can lead to gene expression disorders, contributing to disease pathology. Previous studies have shown that apoptosis, oxidative stress and elements of innate and adaptive immune and inflammatory responses play a key role in the pathogenesis of RHD (induced by *L. europaeus* infection). The expression of host miRs and their influence on the regulation of critical target genes involved in biological processes may play a significant role in the pathogenesis of viral diseases. So far, there is a lack of data in the scientific literature on the molecular regulatory signatures of interactions between miRs and apoptosis, oxidative stress, and innate immune and inflammatory response in *L. europaeus* infection, RHD pathogenesis. Hence, this scientific problem became the goal of the research undertaken in the doctoral dissertation. The material for the study consisted of

liver, lung, kidney, and spleen tissues collected after euthanasia from healthy rabbits and postmortem or after euthanasia from rabbits experimentally infected with *L. europaeus* genotype GI.1 and *L. europaeus* genotype GI.2.

In order to select miRs and to select and predict target genes for miRs involved in biological processes occurring in viral infections, an in-depth literature analysis and silico methods using databases dedicated to working with miRs were used. In order to examine selected key miRs and their target genes involved in apoptosis, oxidative stress, and innate immune and inflammatory response in *L. europaeus* infection with two genotypes – GI.1 and GI.2 in four tissues (liver, lung, kidney, spleen), molecular biology methods were used in the form of measurement of miR expression and target genes. The same methodological approach was used to examine apoptosis, oxidative stress, and inflammation biomarkers. For apoptosis, the following were selected: miR-21 (*PTEN*, *PDCD4*), miR-16b (*Bcl-2, CXCL10*) and miR-34a (*p53*, *SIRT1*); for oxidative stress: miR-132 (*Nrf-2*) and miR-122 (*Bach1*); for innate immune and inflammatory response: miR-155 (*MyD88*, *TAB2*, *p65*, *NLRP3*), miR-146a (*IRAK1*, *TRAF6*), miR-223 (*TLR4*, *IKKα*, *NLRP3*), miR-125b (*NLRP3*). Additionally, biomarkers of apoptosis (*Bax*, *Bax*/*Bcl-2* ratio, *PARP*, *caspase-3*), oxidative stress (*HO-I*), and inflammation (*IL-1β*, *IL-6*, *TNF-α*, *IL-18*) were examined at the mRNA level in the liver, lungs, kidneys, and spleen after infection with *L. europaeus* genotypes – GI.1 and GI.2.

Our research has unveiled novel insights into the role of miRs in the pathogenesis of RHD during *L. europaeus*/GI.1 and GI.2 infection. We observed significant changes in the expression of miR-21, miR-16b, and miR-34a, all of which are involved in the apoptosis process. MiR-16b and miR-34a can influence the intensification of apoptosis in the examined organs and, on the other hand, inhibit it, as observed in the case of miR-21. Based on the examined *Bax* biomarkers and the *Bax/Bcl-2* ratio, it can be concluded that the apoptosis process was more strongly expressed after infection of rabbits with *L. europaeus*/GI.2. MiR-122 and miR-132 were shown to regulate two oxidative stress pathways in the pathogenesis of RHD, caused by *L. europaeus*/GI.1 and GI.2 infection associated with tissue damage. The biomarker *HO-I* in RHD indicates oxidative tissue damage. The main regulators of the innate immune and inflammatory response in *L. europaeus*/GI.1 and GI.2 infection were shown to be miR-155, miR-223, and miR-146a. The expression of miR-125 was very limited and restricted to the liver and spleen. During *L. europaeus*/GI.1 and GI.2 infection, miR-155 was shown to have pro- and anti-inflammatory effects in the liver, as well as anti-inflammatory effects in the kidney and spleen; miR-146a has anti-inflammatory effects in the liver, lung, and kidney; miR-223 has anti-inflammatory effects in all tissues; and miR-125b has anti-inflammatory effects

only in the liver. In each case, such an effect may be a determinant of the pathogenesis of RHD. MiRs may regulate innate immune and inflammatory pathways in *L. europaeus* infection, and the outcome of this regulation may be influenced by the tissue microenvironment. MiRs may regulate innate immune response pathways and inflammation in *L. europaeus* infection, and the outcome of this regulation may be influenced by the tissue microenvironment. The study presented for the first time the expression profile of inflammatory biomarkers (*IL-1β, IL-6*, *TNF-α*, *IL-18*) at the mRNA level in four tissues after *L. europaeus*/GI.1 and GI.2 infection. As a result, three inflammation profiles (pulmonary, renal, and hepato-splenic) were proposed in response to *L. europaeus*/GI.1 and GI.2 infection.

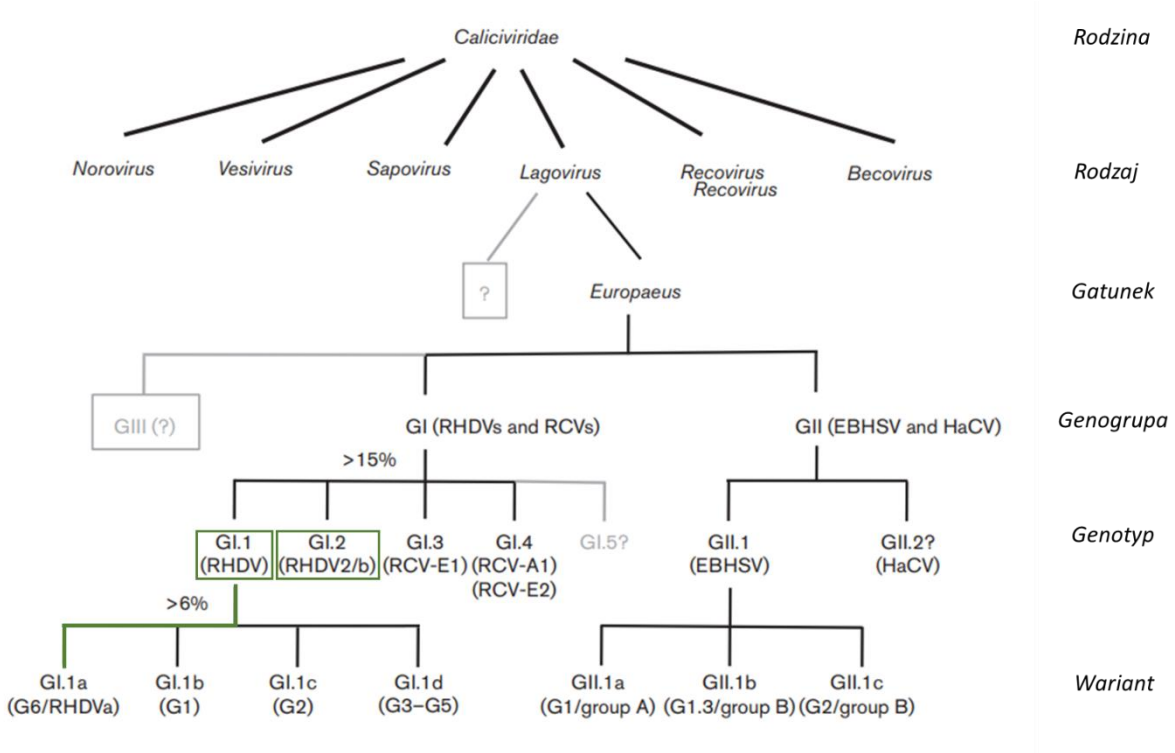
The results obtained during the implementation of this doctoral dissertation significantly deepened the knowledge of the pathogenesis of RHD (caused by *L. europaeus* infection with two genotypes – GI.1 and GI.2), taking into account the tissue microenvironment of the liver, lungs, kidneys, and spleen. They contributed to the partial cognition of the role of miR molecules in L. europaeus infection and provided new data allowing for a better understanding of the pathogenesis of RHD in terms of molecular regulation, apoptosis, oxidative stress, and innate immune and inflammatory response by miR.

**Keywords:** *Lagovirus europaeus*/GI.1, *Lagovirus europaeus*/GI.2, rabbit hemorrhagic disease virus (RHDV), rabbit hemorrhagic disease (RHD), microRNA, gene expression, apoptosis, oxidative stress, innate immune response, inflammation, biomarker, rabbit.

# 1. Wstęp

## 1.1. *Lagovirus europaeus* i krwotoczna choroba królików – RHD (ang. Rabbit hemorrhagic disease)

*Lagovirus europaeu*s (*L. europaeus*) należy do rodziny *Caliciviridae*, rodzaju *Lagovirus* [1] (Rycina 1). Zgodnie z aktualną propozycją ujednoliconego systemu klasyfikacji i nomenklatury rodzaju *Lagovirus* w obrębie *L. europaeus* wyróżnia się dwie genogrupy: genogrupę GI obejmującą wirusy krwotocznej choroby królików – RHDV (ang. rabbit hemorrhagic disease viruses) i kaliciwirusy królika – RCV (ang. rabbit caliciviruses) oraz genogrupę GII obejmującą wirus krwotocznej choroby zajęcy – EBHSV (ang. European brown hare syndrome virus) i kaliciwirus zająca – HaCV (ang. hare calicivirus), w obrębie których wyróżniono genotypy. W genogrupie GI (RHDV i RCV) opisano cztery genotypy (GI.1-GI.4), z których *L. europaeus* genotyp GI.1 (RHDV) i *L. europaeus* genotyp GI.2 (RHDV2/b) (użyte do zakażenia królików w eksperymencie i oznaczone na zielono na Rycinie 1) są patogennymi wirusami i przyczyną krwotocznej choroby królików – RHD. Podczas gdy genotypy GI.3 (RCV-E1) i GI.4 (RCV-A1, RCV-E2) są niepatogennymi kaliciwirusami królika – RCV. Ponadto wśród patogennego genotypu GI.1 (RHDV), który po raz pierwszy został wykryty u królików domowych w Chinach w 1984 roku i od tego czasu występuje na całym świecie [2] wyróżniono cztery warianty – GI.1a (G6/RHDVa) (użyty do zakażenia królików w eksperymencie i oznaczony na zielono na Rycinie 1), GI.1b (G1), GI.1c (G2), GI.1d (G3-G5). Wariant GI.1a (G6/RHDVa) tzw. wariant antygenowy pojawił się po raz pierwszy we Włoszech w 1997 roku [3]. Najpóźniej bo w 2010 roku po raz pierwszy opisano *L. europaeus* genotyp GI.2, początkowo nazwany RHDVb, następnie RHDV2, aktualnie GI.2 (RHDV2/b) [4]. Z kolei w genogrupie GII (EBHSV i HaCV) wyróżnia się dwa genotypy – GII.1 (prezentowany przez EBHSV i zawierający trzy warianty (GII.1a, GII.1b i GII.1c)) i GII.2 (prezentowany przez HaCV) [1].




**Rycina 1.** Propozycja ujednoliconego systemu klasyfikacji i nomenklatury rodzaju *Lagovirus* w obrębie rodziny *Caliciviridae* [1]. Zielonym kolorem oznaczono użyte do zakażenia królików w eksperymencie *Lagovirus europaeus* – genotyp GI.1 (RHDV), wariant GI.1a (G6/RHDVa) i *Lagovirus europaeus* – genotyp GI.2 (RHDV2/b) wyróżnione w obrębie genogrupy GI (RHDV i RCV). Propozycja nowej nomenklatury oparta jest na powiazaniach filogenetycznych, gdzie *Lagovirus* został rozpoznany i nazwany *Lagovirus europaeus*. Gatunek został podzielony na dwie genogrupy, które odpowiadają wirusom powiązanym z RHDV i EBHSV. Genogrupy zostały podzielone na genotypy (zdefiniowane na podstawie odległości genetycznej miedzy dwiema grupami filogenetycznymi wynoszącej co najmniej 15%,), które z kolei podzielono na warianty dobrze poparte filogenetycznie (odległość genetyczna co najmniej 6%).

Pod względem budowy molekularnej *L. europaeus* związany z RHD jest bezotoczkowym, wirusem o średnicy kapsydu 35-40 nm. Materiał genetyczny w postaci pojedynczej nici RNA o dodatniej polarności i wielkości ok. 7,5 kb [5].

*L. europaeus* – genotyp GI.1 (RHDV), w tym wariant GI.1a – (G6/RHDVa), powodują wysoce zakaźną i śmiertelną krwotoczną chorobę królików – RHD u królików domowych i dzikich, podczas gdy *L. europaeus* – genotyp GI.2 (RHDV2/b) wywołuje ją nie tylko u królików, w tym młodych królików domowych i królików zaszczepionych przeciwko RHD, ale także u różnych gatunków dzikich królików i różnych gatunków zajęcy, a także innych zwierząt: myszy, norników, ryjówek, piżmowców górskich i borsuków europejskich [3-20].

*L. europaeus* genotyp GI.1, jest wysoce patogenny o współczynniku śmiertelności wynoszącym od 40% do 100% (Tabela 1). Najwyższy wskaźnik śmiertelności w obrębie tego genotypu notuje się w przypadku wariantu GI.1a – 90%-100% [5,21-27]. Okres inkubacji choroby trwa od 1 do 3 dni, a króliki umierają zwykle w ciągu 12–48 godzin od wystąpienia gorączki (> 40°C) lub nieco później w 52-72 godzinie. W zależności od rozwoju klinicznego choroby mogą wystąpić trzy różne przebiegi kliniczne [5]. W postaci nadostrej zakażone zwierzęta nie wykazują żadnych objawów klinicznych i nagle umierają. Ostrym infekcjom towarzyszy brak łaknienia, apatia i przekrwienie spojówek, a także objawy neurologiczne, takie jak pobudzenie, porażenie i ataksja. Mogą występować także objawy ze strony układu oddechowego (ostra niewydolność oddechowa – zapalenie tchawicy, duszność i sinica) oraz pienista i krwawa wydzielina z nosa; może również wystąpić łzawienie oraz krwotoki z oczu. Nieprawidłowości hematologiczne obejmują leukopenie ze spadkiem limfocytów i heterofilii krwi obwodowej oraz spadek liczby płytek krwi. Natomiast zmiany biochemiczne obejmują znaczny wzrost enzymów wątrobowych (aminotransferazy alaninowej (ALT), aminotransferazy asparaginianowej (AST), fosfatazy alkalicznej (ALP) i γ-glutamylotransferazy (γ-GT)), cholesterolu, mocznika i kreatyniny oraz spadek poziomu glukozy [28-34]. Podostra postać choroby daje podobne, ale łagodniejsze objawy kliniczne i nieliczne króliki przeżywają. Ponadto podczas wybuchu RHD u niewielkiego odsetka królików może wystąpić przewlekła postać choroby z objawami obejmującymi ciężką i uogólnioną żółtaczkę, anoreksję i letarg. Zwierzęta te zwykle umierają jeden lub dwa tygodnie później, ale u zwierząt, które przezwyciężyły chorobę, występuje silna serokonwersja [5]. W przypadku *L. europaeus* wariant GI.1a, zwykle występuje ostry przebieg choroby [5,21,22,24,25,35].

*L. europaeus* genotyp GI.2 według różnych danych wywołuje bardzo zmienną śmiertelność u chorych królików od 5% do 80%, do 100% w nadostrej i ostrej postaci choroby [36-38]. W przeciwieństwie do zakażenia *L. europaeus* genotypem GI.1, zakażenie *L. europaeus* genotyp GI.2 częściej skutkuje rozwinięciem się podostrej lub przewlekłej postaci RHD [38,39]. Okres inkubacji wynosi od 3 do 5 dni, a objawy kliniczne mogą trwać do 5 dni, co jest dłuższym okresem w porównaniu z genotypem GI.1. Zakażenie RHDV2 może być trudne do zdiagnozowania we wczesnych stadiach choroby, ponieważ typowe objawy kliniczne związane z zakażeniem *L.europaeus* – GI.1 mogą być nieobecne (np. krwawienie z nosa) [38]. W postaci nadostrej zwierzęta są zwykle znajdowane martwe bez żadnych objawów klinicznych. W postaci ostrej króliki początkowo wykazują oznaki złego samopoczucia i umierają w ciągu 12 do 36 godzin od wystąpienia gorączki (powyżej 40,1°C). Do innych objawów zaliczamy wstrząs krążeniowy z ciężkim niedociśnieniem, krwotokami (krwiomocz,

wydzielina ze sromu przypominająca krew, pieniste i krwawe wydzieliny z nosa), objawy neurologiczne, wokalizacja. Nieprawidłowości hematologiczne i biochemiczne obejmują leukopenię, trombocytopenię, obecność skrzepów fibrynowych i wyraźnie podwyższone enzymy wątrobowe AST, ALT, hormon luteinizujący, γ-GT, ALP i dehydrogenaza mleczanowa (LDH). Śmierć następuje w wyniku rozsianego wykrzepiania wewnątrznaczyniowego (DIC, ang. disseminated intravascular coagulation) wtórnego do ciężkiego ostrego martwiczego zapalenia wątroby, charakteryzującego się rozległym tworzeniem się skrzepów mikronaczyniowych w różnych narządach, prowadząc do niewydolności wielonarządowej – MOF (ang. multi organ failure), często towarzyszącym ciężkim krwotokom z powodu przyspieszonej fibrynolizy. W postaci podostrej lub przewlekłej zwierzęta wykazują przewlekłą chorobę kliniczną z ciężką żółtaczką, anoreksją i letargiem. Śmierć, jeśli wystąpi, zwykle następuje jeden do dwóch tygodni po wystąpieniu objawów klinicznych i jest zazwyczaj wynikiem dysfunkcji wątroby, nerek i śledziony [38,39].

**Tabela 1**. Porównanie genotypów – GI.1 i GI.2 *L. europaeus* [4,5,7,37,38,40-42].

| **Charakterystyka** | **GI.1/RHDV** | **GI.2/RHDV2** |
|---|---|---|
| Infekowane gatunki | *Oryctolagus cuniculus* | *Oryctolagus cuniculus, S. auduboni, S. nuttali, S. floridanus, Romegolagus diazi, Lepus europaeus, L. capensis mediterraneus, L. californicus, L. corsicanus, L. timidus, L. alleni, Apodemus spp., Microtus spp., Crocidura spp., Meles meles* |
| Forma kliniczna | Nadostra<br>Ostra<br>Podostra | Nadostra<br>Ostra<br>Podostra/Przewlekła |
| Wiek podatnych osobników | Dorosłe króliki powyżej 2 miesięcy | Króliki dorosłe oraz młodsze w wieku 11 dni |
| Wskaźnik śmiertelności | 40-100% (90-100%) | 5-80%, do 100% (0-100%) |
| Objawy choroby | Typowe dla RHD | Typowe dla RHD |
| Czas trwania choroby | 2-3 dni form ostra | 12-36h forma nadostra<br>5 dni forma podostra |
| Uszkodzenia narządów w przebiegu choroby | wątroba, płuca, nerki, śledziona, serce, jelita | wątroba, płuca, tchawica, nerki, śledziona, serce |

W przebiegu RHD obserwuje się głównie zmiany w wątrobie, płucach, nerkach i śledzionie, które są tkankami docelowymi wirusa oraz w sercu, układzie nerwowym i przewodzie pokarmowym, zwłaszcza w jelitach [5,38,43-45]. Największe zmiany histopatologiczne stwierdza się w wątrobie, która jest głównym miejscem replikacji wirusa (Tabela 2). Podczas zakażenia dochodzi do jej ostrego zapalenia. Obserwuje się zwiększoną utratę hepatocytów w wyniku apoptozy oraz martwicy wywołanej działaniem wirusa. Wątroba staje się powiększona z wyraźnym wzorem zrazikowym, żółtoszara oraz krucha [38,46,47]. W obrębie układu oddechowego rejestruje się krwawienia, zatory, ogniska zapalne w płucach oraz ich obrzęk. Zmiany te prowadzą do silnej niewydolności płuc, która objawia się występowaniem duszności oraz sinicy [5,38]. W wyniku rozwoju DIC u zakażonych zwierząt obserwuje się krwotoki i przekrwienia w wielu innych narządach, w tym w nerkach i śledzionie. Narządy te stają się powiększone z charakterystycznym ciemnoczerwonym plamistym zabarwieniem. Występowanie zatorów oraz masywnych krwawień, będących następstwem DIC, prowadzi do niewydolności wielonarządowej, która jest zwykle przyczyną śmierci zakażonych królików [48]. U królików zakażonych *L. europaeus* genotypem GI.2 obserwuje się obrzęk płuc z krwotokami pęcherzykowymi i zakrzepicą oraz pienisty wysięk w tchawicy [39,49]. Wątroby zakażonych królików są kruche z uogólnionym, wyraźnym wzorem płatowym. Ponadto wykazano wystąpienie wieloogniskowej i rozproszonej apoptozy oraz martwicę hepatocytów. W obrębie dróg żółciowych występuje łagodna hiperplazja [11,49]. W śledzionie obserwuje się umiarkowane lub silne przekrwienia, splenomegalię. W przebiegu zakażenia dochodzi również do krwawień w nerkach oraz do zakrzepicy kłębuszków nerkowych. Czasami obserwuje się również zmiany występujące w jelicie cienkim, które obejmują stan zapalny kosmków jelitowych jelita cienkiego [11,39,49].

**Tabela 2**. Porównanie zmian patologicznych i histologicznych w czterech głównych narządach królików po zakażeniu *L. europaeus* genotypem GI.1 i *L. europaeus* genotyp GI.2 [5,11,38,39,49].

| **Organ** | ***L. europaeus*/GI.1** | ***L. europaeus*/GI.2** |
|---|---|---|
| Wątroba | • ostre zapalenie wątroby<br>• zmiany zwyrodnieniowe hepatocytów związane z apoptozą (rozległa wakuolizacja, zmiany w strukturze mitochondriów)<br>• martwica hepatocytów<br>• powiększenie narządu z wyraźnym wzorem zrazikowym<br>• naciek granulocytarny<br>• aktywacja komórek Kupffera<br>• leukopenia | • ostre zapalenie wątroby<br>• apoptoza hepatocytów<br>• lityczna martwica hepatocytów<br>• przekrwienia<br>• skrzepy fibrynowe<br>• nacieki zapalne heterofilii |
| Płuca | • przekrwienie<br>• obrzęk<br>• krwotoki wewnątrzpęcherzykowe i okołonaczyniowe<br>• proliferacja limfocytów | • skrzepy fibrynowe<br>• zapalenie płuc<br>• krwotok wewnątrzpęcherzykowy<br>• obrzęk<br>• apoptoza komórek płuc |
| Nerki | • powiększone z plamistym ciemnoczerwonym zabarwieniem<br>• przekrwienie<br>• krwotoki w obrębie pętli kłębuszków nerkowych i rdzenia nerki<br>• skrzepy szkliste<br>• nacieki limfocytarne<br>• zwyrodnienie nabłonka kanalikowego | • skrzepy fibrynowe<br>• wybroczyny<br>• zakrzepica kłębuszków nerkowych |
| Śledziona | • splenomegalia<br>• plamiste ciemnoczerwone zabarwienie<br>• przekrwienie<br>• hemosyredoza<br>• leukopenia | • splenomegalia<br>• limfocytoliza<br>• krwotoki<br>• odkładanie fibryny<br>• nacieki zapalne heterofilii w stadium terminalnym ostrej infekcji |

Choć od pierwszego odnotowanego wybuchu RHD w 1984 roku w Chinach [2] minęło niemal czterdzieści lat, mechanizmy patogenezy RHD nadal nie są w pełni poznane. Jednym z kluczowych elementów patogenezy RHD jest apoptoza [29-31,50-55]. Z uwagi na fakt, że hepatocyty są uważane za główne miejsce replikacji wirusa, jak do tej pory większość badań dotyczących apoptozy koncentrowała się na wątrobie. Badania wykazały, że w przebiegu RHD apoptoza w wątrobie jest aktywowana zarówno szlakiem zewnętrznym, zależnym od

receptorów śmierci: powierzchniowego receptora śmierci komórki Fas (ang. Fas Cell Surface Death Receptor), receptora czynnika martwicy nowotworu (TNFR, ang. tumor necrosis factor receptor), receptora śmierci (DR, ang. death receptor), jak i szlakiem wewnętrznym (mitochondrialnym) [29,32,56,57]. Zaobserwowano, że podczas zakażenia *L. europaeus*/GI.1 dochodzi do wzrostu ekspresji czynnika martwicy nowotworu α (TNF-α, ang. tumor necrosis factor α) oraz ligandu dla receptora Fas (FasL, ang. Fas cell curface death receptor ligand), co wskazuje na aktywację apoptozy szlakiem zewnętrznym [56]. Ponadto wykazano również zwiększoną aktywację kaspazy-3 oraz zwiększony poziom białka polimerazy poli(ADP-rybozy) (PARP, ang. Poly(ADP-ribose) polymerase) [29,31]. Rodzina białek regulatora apoptozy BCL2 (Bcl-2, ang. BCL2 Apoptosis Regulator) odgrywa rolę w regulacji szlaku wewnętrznego. Badania wykazały, że w przebiegu RHD dochodzi do zwiększenia ekspresji proapoptotycznego białka X związanego z BCL2 (Bax, ang. BCL2 Associated X Protein), co prowadzi do indukcji apoptozy poprzez tworzenie porów w mitochondriach umożliwiając uwolnienie cytochromu c, w porównaniu do przeciwapoptotycznego białka Bcl-2 [29,31,56]. Obie ścieżki aktywacji apoptozy w hepatocytach są ze sobą ściśle powiązane. Szlak mitochondrialny jest często wymagany do wzmocnienia stosunkowo słabego sygnału apoptotycznego indukowanego przez receptory śmierci [58]. Podczas infekcji *L. europaeus*/GI.1 obecność komórek apoptotycznych została potwierdzona również w makrofagach brzusznych, komórkach nabłonkowych wątroby, płucach, nerkach, sercu, śledzionie i węzłach chłonnych [46]. Badania nad apoptozą w przebiegu zakażenia *L. europaeus*/GI.2 wykazały, że stosunek poziomu mRNA *Bax*/*Bcl-2*, wskazujący na przebieg lub zahamowanie apoptotycznej śmierci komórki, jest podwyższony w wątrobie, śledzionie i nerkach [50,51]. Kaspaza-3 jest kluczowym elementem wykonawczym apoptozy, zarówno dla szlaku zewnętrznego, jak i wewnętrznego [59]. Badania wykazały, że podczas zakażenia *L.europaeus*/GI.2 poziom białka kaspazy-3 jest podwyższony w wątrobie, śledzionie, nerkach, płucach oraz sercu. Jednak co ciekawe aktywność kaspazy-3, wpływającej na aktywację apoptozy, zaobserwowano tylko w wątrobie, śledzionie i nerkach. Białko PARP uczestnicy w aktywacji apoptozy poprzez ścieżkę wewnętrzną [50,51]. Podczas zakażenia *L. europaeus*/GI.2 poziom białka PARP były podwyższony w sercu, nerkach i śledzionie [51]. Wyniki te wskazują, że niezależnie od genotypu *L. europaeus* zwiększona apoptoza występuje w większości narządów w przebiegu RHD i jest elementem patogenezy tej choroby.

Kolejnym ważnym elementem patogenezy RHD, jest stres oksydacyjny. Podobnie jak w przypadku apoptozy badania nad stresem oksydacyjnym po zakażeniu królików *L. europeus* ograniczały się głównie do wątroby. Podczas zakażenia *L. europaeus*/GI.1 w wątrobie dochodzi

do wzrostu reaktywnych form tlenu (ROS, ang. reactive oxygen species) [31,32,56]. Wzrost ROS w wątrobie związany jest z replikacją wirusa i zależny od czasu infekcji [60]. Obserwuje się również zwiększony stosunek glutationu utlenionego do zredukowanego (GSSG/GSH, ang. oxidised to reduced glutathione ratio), a także zmniejszoną objętość dysmutazy ponadtlenkowej (SOD, ang. superoxide dismutase) [31,32,60,61]. Zmiany te świadczą o zaburzeniu równowagi oksydacyjnej i narastającym stresie oksydacyjnym, który bezpośrednio przyczynia się do uszkodzenia błony mitochondrialnej i aktywacji wewnętrznego szlaku apoptozy w hepatocytach [31]. Podczas przebiegu RHD dochodzi również do zwiększonej ekspresji indukowanej syntazy tlenku azotu (iNOS, ang. inducible nitric oxide synthase). Nadekspresja iNOS prowadzi do zwiększonej produkcji tlenku azotu (NO), który reagując z ROS tworzy silny utleniacz nadtlenoazotynu, który przyczynia się do powstania stresu oksydacyjnego i uszkodzenia wątroby [32]. Jądrowy czynnik transkrypcyjny związany z erytroidem 2 (Nrf-2, ang. nuclear erythroid 2-related factor) jest ważnym czynnikiem chroniącym wątrobę przed uszkodzeniem wywołanym wirusowym zapaleniem wątroby (WZW). Reguluje on ekspresje genów cytoprotekcyjnych, co wskazuje, że jego aktywacja zapewnia ochronę przed stresem oksydacyjnym w przebiegu WZW typu B i C [62]. Badania wykazały, że *L. europaeus*/GI.1 zmniejsza aktywność enzymów antyoksydacyjnych regulowanych przez Nrf-2 w wątrobie za pośrednictwem białka związanego z ECH typu Kelch1 (Keap1 ang. Kelch Like ECH Associated Protein 1). Jednak sam poziom białka Nrf-2 jest również obniżony w przebiegu infekcji, co może przyczyniać się do zwiększonego uszkodzenia narządu w wyniku stresu oksydacyjnego [60]. Opierając się na dostępnej literaturze przedmiotu, można stwierdzić, że obecnie rola stresu oksydacyjnego w przebiegu zakażenia *L. europaeus* w narządach innych niż wątroba nie jest zbadana, co otwiera szerokie pole do badań.

W patogenezie RHD istotną rolę odgrywają również elementy wrodzonej oraz nabytej odpowiedzi immunologicznej i zapalnej. W trakcie zakażenia *L. europaeus* obserwuje się spadek leukocytów krwi obwodowej oraz ogólnoustrojowy stan zapalny. W narządach objętych infekcją (głównie w wątrobie, płucach, nerkach i śledzionie) obserwuje się nacieki bogate w neutrofile oraz limfocyty T i B oraz wzrost biomarkera stanu zapalnego miR-155 [63-65]. Ciężką leukopenię rejestruje się na kilka godzin przed śmiercią królików zakażonych *L. europaeus.* Zarówno neutrofile jaki i limfocyty przyczyniają się do zubożenia liczby krążących leukocytów. Podobnie spada liczba płytek krwi [30,33]. Patogeneza choroby obejmuje również apoptozę komórek T i B w wątrobie, śledzionie i krwi obwodowej [21,23,30,35]. Obserwowano również zmianę aktywności leukocytów krwi obwodowej między innymi w zakresie procesu fagocytozy oraz zmiany w stężeniu i aktywności substancji

produkowanych przez te komórki, takich jak lizozym oraz mieloperokzydaza [7,21,24-26,66-68]. Wykazano również, że podczas zakażenia *L. europaeus* w tkankach (wątroba i śledziona) oraz leukocytach krwi obwodowej wzrasta poziom cytokin pro- i przeciwzapalnych. Cytokiny te obejmują interleukiny (IL-1, IL-6, IL-8, IL-10), TNF-α, TNF-β, interferon gamma (IFN-γ) oraz czynnik stymulujący kolonie granulocytów i makrofagów (GM-CSF) [69-73]. Ponadto O'Toole i wsp. [44] wskazują, że zakażenie *L. europaeus*/GI.2 napędza patogenezę RHD poprzez burzę cytokin. Hepatocyty nadmiernie produkują TNF-α, IL-1β i IL-6, co prowadzi do nadkrzepliwości. Z kolei Yu i wsp. [74] wykazali, że cytokiny prozapalne (IL-1α, IL-6, IL-8, IL-22) i chemokiny (CCL2, CXCL9), biorące udział w zapaleniu, są znacząco zwiększone w śledzionie w późnych stadiach zakażenia *L. europaeus*/GI.2. Dane te sugerują, że zakażenie *L. europaeus*/GI.2 może wywołać deregulację sieci cytokin i osłabić odporność śledziony na zakażenie wirusowe, co prowadzi do zaburzeń zapalnych. W zakresie nabytej odpowiedzi immunologicznej w przebiegu RHD rejestrowano zmienną aktywność limfocytów o różnych fenotypach. Badania wykazały zmiany w liczebności limfocytów T (CD5+) i ich subpopulacji Th (CD4+), Tc (CTLs), Treg (CD25+) oraz limfocytów B (CD19+) we krwi obwodowej [7,21,26,75,76] oraz komórek Treg w śledzionie. Ta zmienność wskazuje na upośledzoną odpowiedź immunologiczną, czasami skutkującą całkowitą utratą komórek efektorowych w krótkim czasie [73].

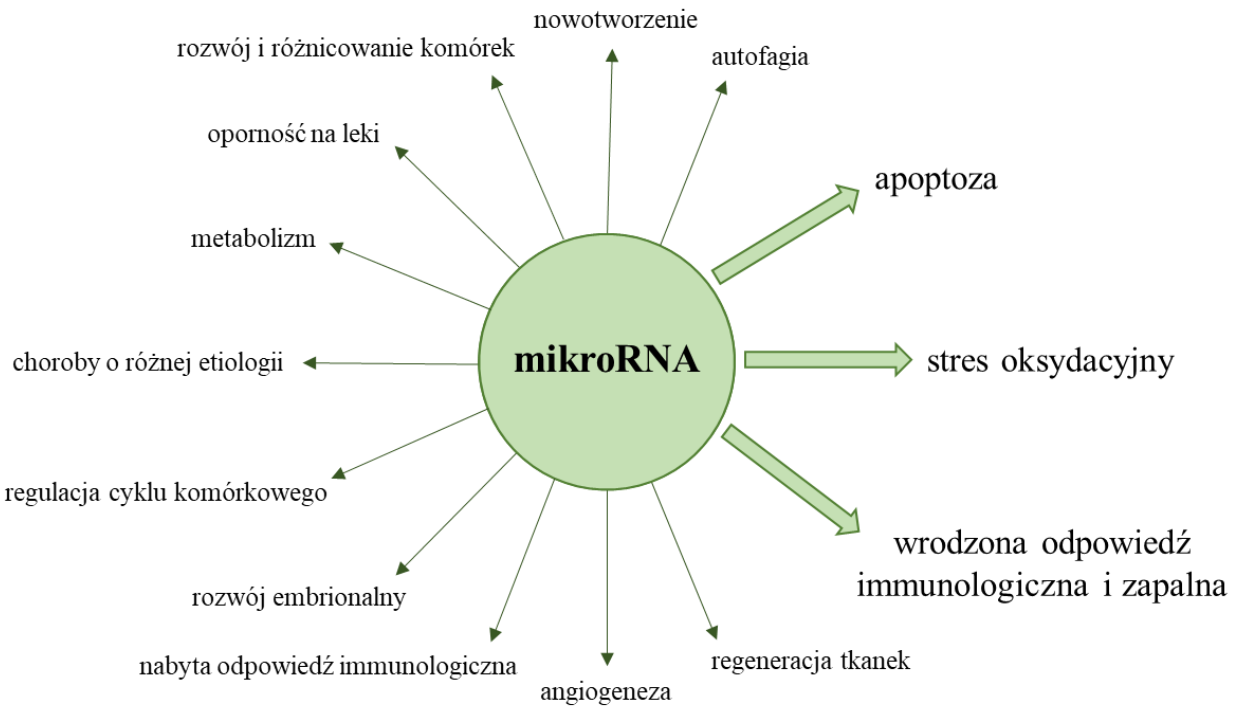
### 1.2. Zakażenie *Lagovirus europaeus* jako model badawczy ostrej niewydolności wątroby (ALF) i niewydolności wielonarządowej (MOF)

Ostra niewydolność wątroby jest poważnym stanem, który prowadzi do gwałtownego pogorszenia funkcji wątroby i charakteryzuje się wysoką śmiertelnością. Ponadto może prowadzić do niewydolności wielonarządowej [77]. Zarówno ALF, jak i MOF są trudnymi jednostkami do badania klinicznego, co sprawia, że modele eksperymentalne, zwłaszcza zwierzęce, stają się kluczowe dla zrozumienia ich mechanizmu i opracowania skutecznych terapii [78]. Przeprowadzone badania wykazały, że objawy uszkodzenia wątroby oraz innych narządów obserwowane u królików zakażonych *L. europaues* są podobne do objawów charakterystycznych dla piorunującej niewydolności wątroby (FHF, ang. fulminant hepatic failure), ALF wywołanej wirusowym zapaleniem wątroby oraz MOF u ludzi [32,79]. Model zakażenia *L. europaues* odtwarza reprezentatywne parametry biochemiczne i histologiczne oraz objawy kliniczne ALF u ludzi. Analizy biochemiczne wykazały wzrost bilirubiny, γ-GT, fosfatazy alkalicznej, LDH oraz aktywności enzymów wątrobowych, przy czym szczególnie wyraźny był wzrost poziomu AST w porównaniu do ALT [56]. Podobną charakterystykę

wzrostu aminotransferaz obserwuje się u ludzi we wczesnym stadium wirusowego zapalenia wątroby, gdzie stosunek AST/ALT wynosi powyżej 2 [31-34]. Zmiany parametrów koagulologicznych takich jak wydłużony czas protrombinowy oraz wyczerpanie czynników krzepnięcia V i VII są charakterystyczne zarówno dla zakażenia *L. europaues*, jak i u pacjentów z ALF i mogą być czynnikiem prognostycznym w postępującej niewydolności wątroby [33,80]. Króliki zakażone *L. europaeus*, ponadto wykazują objawy nadciśnienia wewnątrzczaszkowego oraz encefalopatii, które są charakterystycznymi objawami u pacjentów z FHF [80]. Wystąpienie kwasicy oddechowej, hipoglikemii, hiperfosfatemii oraz zwiększonej aktywności kinazy keratynowej w przebiegu zakażenia *L. europaues* wskazuje na wystąpienie MOF u zakażonych zwierząt [81]. Opierając się na dostępnej literaturze przedmiotu należy stwierdzić, że obecnie nie ma opracowanego dobrego modelu zwierzęcego do badania MOF o etiologii wirusowej. Zakażenie królików *L. europaues* spełnia kryteria dobrego modelu badawczego. Wysoka zgodność cech biochemiczny i klinicznych, możliwość pobrania (oprócz wątroby) także wielu innych narządów zmienionych chorobowo (np. płuc, nerek, śledziony), powtarzalność wyników oraz długie okno terapeutyczne czynią ten model jako jeden z lepszych modeli zwierzęcych do badania ALF oraz MOF, jak również do opracowywania przyszłych terapii leczenia tych stanów chorobowych oraz poszukiwania ich biomarkerów diagnostycznych [80,82].

### 1.3. Funkcja, biogeneza i mechanizm działania mikroRNA

MikroRNA (miR) to małe, niekodujące RNA o długości 17-25 nukleotydów. Występują one u większości eukariontów, w tym u ludzi i zwierząt [83,84]. MiR biorą udział w procesach fundamentalnych dla rozwoju i funkcjonowania organizmu, między innymi takich jak różnicowanie i proliferacja komórek, regulacja cyklu komórkowego, stresu oksydacyjnego czy apoptozy (Rycina 1) [85,86].

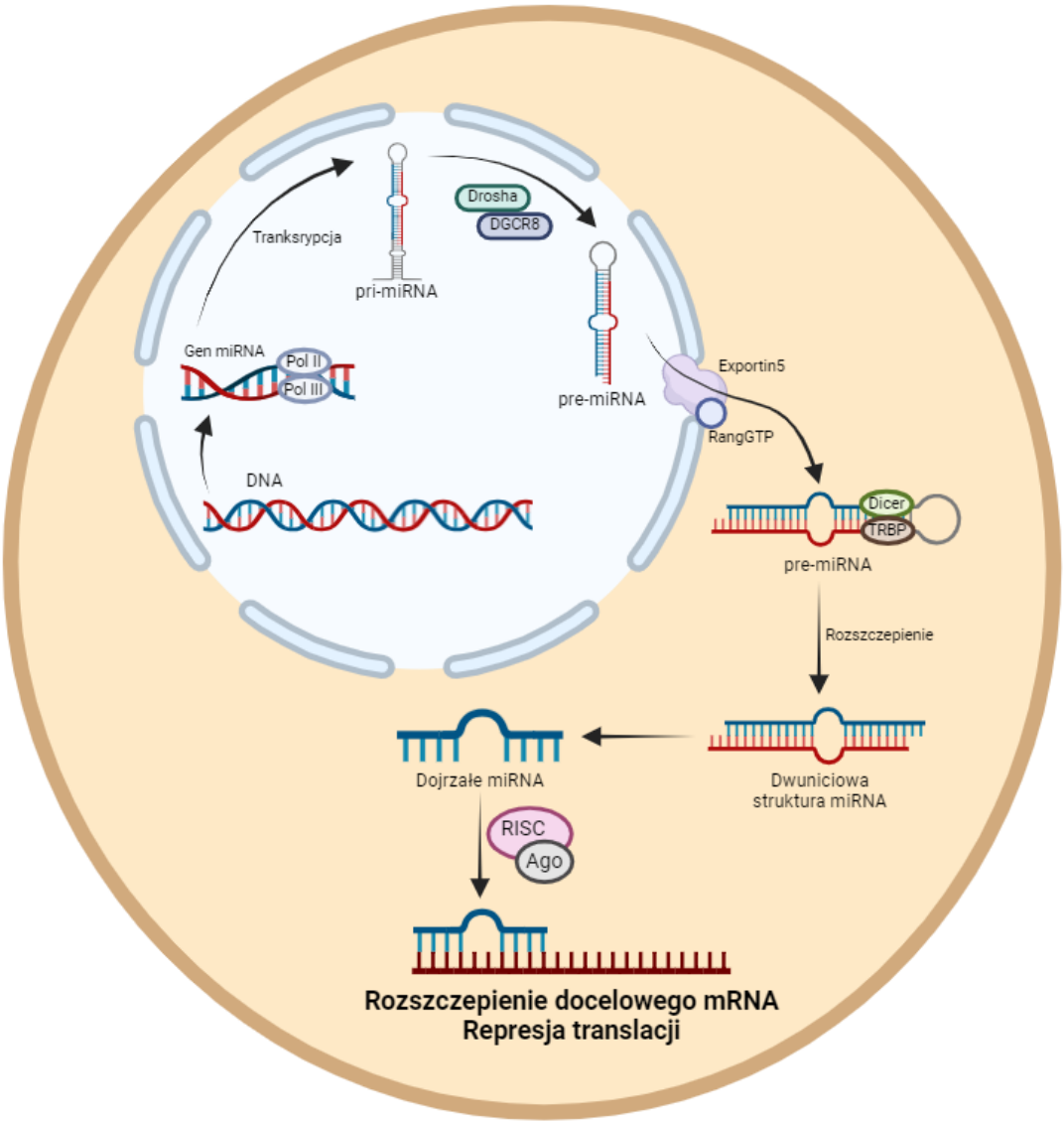



**Rycina 1**. Biologiczna rola mikroRNA.

Ponadto uczestniczą w modulacji genów związanych z układem odpornościowym i odpowiedzią immunologiczną organizmu [87]. MiR odgrywają kluczową rolę w regulacji ekspresji genów na poziomie potranskrypcyjnym. Związanie miR z mRNA prowadzi do destabilizacji transkryptu, co z kolei skutkuje hamowaniem translacji i produkcji białka. MiR mają również zdolność aktywowania ekspresji genów w zależności od typu komórek, panujących warunków oraz obecności różnych kofaktorów [88]. Przewiduje się, że miR stanowią 1-5% ludzkiego genomu i regulują co najmniej 30% genów kodujących białka [89,90]. Ich nieprawidłowa/zmieniona ekspresja jest związana z wieloma chorobami [89,91]. Dodatkowo miR mogą być wydzielane do płynów pozakomórkowych, gdzie mogą służyć jako potencjalne biomarkery wielu stanów patologicznych u ludzi i zwierząt [91]. Ze względu na swoją zdolność do precyzyjnej regulacji genów, miR stanowią obiecujący cel terapeutyczny. Wykorzystanie inhibitorów miR (antagomirów) lub mimików miR (syntetycznych miR) może pomóc w modulacji ekspresji genów w określonych chorobach, w tym w chorobach wirusowych [92].

Biogeneza miR rozpoczyna się w jądrze komórkowym i obejmuje dwuetapowy proces. Pierwszym etapem jest transkrypcja polimerazy RNA II/III (Pol II/Pol III) w celu wytworzenia pierwotnego miRNA (pri-miRNA) (Rycina 2). Kompleks składający się z endonukleaz RNazy III Drosha i genu krytycznego regionu zespołu DiGeorge'a 8 (DGCR8) rozszczepia pri-miRNA

z wytworzeniem prekursorowego miRNA (pre-miRNA) [93]. Pre-miRNA eksportowany jest do cytoplazmy w sposób zależny od Exportin5/RanGTP. W cytoplazmie endonukleaza RNA III Dicer, tworząca kompleks z białkiem TRBP, rozszczepia pre-miRNA w pobliżu pętli tworząc dupleks dojrzałego miRNA. Następnie dupleks rozwija się tworząc jednoniciowy dojrzały miRNA, który łączy się z kompleksem wyciszania indukowanego przez RNA (RISC) oraz białkiem Ago [88]. Powstały kompleks pośrednicy w rozpoznawaniu docelowego mRNA poprzez interakcje parowania zasad. W przypadkach doskonałej lub prawie doskonałej komplementarności do miR, docelowe mRNA mogą zostać rozszczepione (pocięte) i zdegradowane [88,94].




**Rycina 2**. Biogeneza mikroRNA u ludzi i zwierząt.

## 1.4. MikroRNA i jego regulacyjny wpływ na geny zaangażowane w apoptozę, stres oksydacyjny oraz we wrodzoną odpowiedź immunologiczną i zapalną

MiR gospodarza są regulatorami genów uczestniczących w odpowiedzi na infekcje wirusowe. Wiedza na temat miR gospodarza i ich roli w infekcjach wirusowych stanowi niezbędne narzędzie do identyfikacji funkcji biologicznych genów i ścieżek, które są aktywowane w celu wywołania skutecznej odpowiedzi przeciwwirusowej w postaci apoptozy, stresu oksydacyjnego oraz odpowiedzi immunologicznej i zapalnej [95-97].

### 1.4.1. Regulacyjne działanie mikroRNA na geny związane z apoptozą w zakażeniach wirusowych

Jednym z mechanizmów obronnych w przebiegu zakażeń wirusowych oraz chorobach o etiologii wirusowej jest apoptoza. W komórkach zainfekowanych wirusem apoptoza może być inicjowana przez szlak zewnętrzy lub wewnętrzny [98]. W odpowiedzi na infekcję wirusową szlak zewnętrzny jest inicjowany przez receptory śmierci, w tym Fas, ligand TNF – członek rodziny 10 (TRAIL ang, TNF Ligand Superfamily Member 10) i TNFR1, przez białka z rodziny TNF, co prowadzi do aktywacji kaspazy-8, która z kolei aktywuje kaspazy wykonawcze, takie jak kaspaza-3,-6,-7 [99,100]. Zakażenie wirusowe może również indukować apoptozę poprzez aktywacje receptorów Toll-podobnych (TLR, ang. toll-like receptors) oraz produkcję cytokin, takich jak interferon typu I (IFN-I, ang. type-I interferons), które stymulują odpowiedź apoptotyczną w zakażonych komórkach [101]. Z kolei szlak wewnętrzny, mitochondrialny, obejmuje uwolnienie cytochromu c z mitochondriów do cytoplazmy, co prowadzi do aktywacji kaspazy-9 i ostatecznie kaspazy-3 [98]. Szlak ten może być regulowany przez białka z rodziny Bcl-2 [102]. W obrębie tej rodziny występują białka zarówno antyapoptotyczne (m.in. Bcl-2), które wpływają na hamowanie procesu apoptozy, jak również proapoptotyczne (m.in. Bax), które przyczyniają się do jej nasilenia [103].

MiR odgrywają kluczową rolę w regulacji apoptozy podczas infekcji wirusowych wpływając na krytyczne geny zaangażowane w ścieżki apoptozy [97]. Zakażenie wirusem zapalenia wątroby typu B (HBV, ang. hepatitis B virus) może prowadzić do indukcji apoptozy poprzez zwiększenie ekspresji miR-194-5p *in vitro*. Natomiast hamowanie ekspresji genu kaspazopodobnego białka regulującego apoptozę (cFLIP, ang. Caspase-Like Apoptosis Regulatory Protein) przez miR-194-5p silnie uwrażliwia komórki HepG2 na apoptozę w odpowiedzi na bodziec fizjologiczny [97]. HBV może wpływać również na zwiększenie ekspresji miR-21, jak również na zmniejszenie miR-15a/16 w celu zahamowania apoptozy zakażonych komórek [104,105]. Badania wykazały, że białko X wirusa HBV (HBx, ang.

hepatitis B virus X protein) obniża ekspresję genu programowanej śmierci komórki 4 (PDCD4, ang. Programmed Cell Death 4), związanego z apoptozą, poprzez zwiększenie ekspresji miR-21 [106]. Nadekspresja miR-21, indukowana HBx, hamuje również ekspresję proapoptotycznego genu homologu fosfatazy i tensyny (PTEN, ang. Phosphatase And Tensin Homolog) i może aktywować gen kinazy białkowej B (Akt, ang. Protein Kinase B) [107]. Białko to może również hamować apoptozę poprzez hamowanie miR-15a/16 i zwiększenie ekspresji antyapoptotycznego białka Bcl-2 [104]. Innym wirusem hepatotropowym, wywołującym WZW jest wirus zapalenia wątroby typu C (HCV, ang. hepatitis C virus). Jednak w przeciwieństwie do HBV powoduje on zwykle przewlekłe zapalenie wątroby. Zhang i wsp. [108] wykazali, że zakażenie HCV wpływa na zwiększenie poziomu miR-155. Nadmierna ekspresja miR-155 znacząco hamowała apoptozę hepatocytów i promowała proliferację komórek poprzez hamowanie genu regulatora APC szlaku sygnałowego Wnt (APC, ang. APC Regulator Of WNT Signaling Pathway) i aktywację szlaku sygnałowego Wingless/Integrated-1 (Wnt, ang. Wingless/Integrated-1 Pathway) [108]. Podobnie jak w przypadku pacjentów z HBV, u pacjentów zakażonych HCV występuje zwiększona ekspresja miR-21. Zwiększona biodostępność tego miR i jego regulacyjne działanie na gen docelowy – *PTEN* może przyczyniać się do patogenezy choroby powodując stłuszczenie wątroby, które w połączeniu z chronicznym zapaleniem może wpłynąć na rozwój raka wątrobowokomórkowego (HCC, ang. hepatocellular carcinoma) [109]. Wirus grypy typu A (IAV, ang. influenza A virus) indukuje apoptozę w wielu typach komórek. Badania *in vivo* oraz *in vitro* wykazały, że IAV obniża ekspresję miR-34a zarówno w surowicy pacjentów, jak i w linii komórkowej A549 [97]. Dalsze analizy wykazały, że genem docelowym dla miR-34a jest proapoptotyczne białko Bax. Dlatego zmniejszona ekspresja miR-34a podczas zakażenia IAV powoduje nadekspresję białka Bax i indukcję apoptozę podczas infekcji tym wirusem [97]. Zakażenie rotawirusem, który jest najczęstszą przyczyną ostrej biegunki u dzieci, reguluje w górę transformujący czynnik wzrostu β (TGF-β, ang. transforming growth factor β), co może prowadzić do wcześniejszej apoptozy, zapobiegając w ten sposób progresji wirusa. Jednak rotawirus, aby przeciwdziałać apoptozie, indukuje nadeskpresję miR-142 gospodarza, który reguluje poziom genów zaangażowanych w sygnalizację szlaku TGF-β [97]. Sharma i wsp. [110] wykazali, że w zakażonych liniach komórkowych ludzkim wirusem limfotropowym typu 1 (HTLV-1, ang. human T-lymphotropic virus 1) występuje zwiększona ekspresja miR-34a. Nadekspresja miR-34a przyczynia się do zmniejszenia ekspresji sirtuiny 1 (SIRT1, ang. sirtuin 1), a także białka Bax i wpływa na hamowanie apoptozy [110].

### 1.4.2. Regulacyjne działanie mikroRNA na geny związane ze stresem oksydacyjnym w zakażeniach wirusowych

Coraz więcej dowodów wskazuje, że choroby ludzi i zwierząt, w tym infekcje wirusowe zaburzają naturalną równowagę pomiędzy zwiększoną produkcją ROS, a zmniejszoną reakcją gospodarza na przeciwutleniacze, co prowadzi do zwiększonego stresu oksydacyjnego [111-114]. Badania wykazały, że stres oksydacyjny przyczynia się do patogenezy wirusowych infekcji dróg oddechowych i może prowadzić do zwiększonego stanu zapalnego i apoptozy, dysfunkcji komórek śródbłonka oraz zakrzepicy, które mogą prowadzić do niewydolności wielonarządowej [111,112]. Dlatego utrzymanie prawidłowych poziomów ROS jest niezbędne dla prawidłowego funkcjonowania organizmu w trakcie infekcji wirusowej, a miR pełnią ważną rolę w utrzymaniu homeostazy związanej ze stresem oksydacyjnym [115]. Stąd też coraz więcej badań koncentruje się na odkrywaniu sygnatur regulacyjnych interakcji między sygnalizacją redoks, a specyficznymi miR przypisywanymi chorobie [115].

Zakażenie wirusem Dengi (DENV) prowadzi do zmniejszenia ekspresji miR-155 [116]. Natomiast badania *in vitro* wykazały, że nadekspresja miR-155 wywołana egzogennie hamuje replikację wirusa Dengi poprzez hamowanie genu domeny BTB i CNC homolog 1 (Bach1, ang. BTB domain and CNC homolog 1), co skutkowało indukcją oksydazy hemowej I (HO-I, ang. Heme-oxygenase 1) [116]. HO-I jest kluczowym czynnikiem cytoprotekcyjnym, a Bach1 jest jego represorem transkrypcyjnym [117,118]. Wyniki badań wykazały, że HO-I chroni komórki poprzez zmniejszanie stresu oksydacyjnego i stanu zapalnego oraz utrzymywanie integralności mitochondriów, promując w ten sposób przeżycie komórek [117]. Escalera-Cueto i wsp. [119] wykazali, że let-7c może hamować zakażenie wirusem Dengi (DENV2 i DENV4) poprzez hamowanie ekspresji *Bach1* i tym samym zwiększeniu poziomu *HO-I* [119]. Z kolei miR-122 odgrywa ważną rolę w hamowaniu ekspresji *HO-I* w zakażeniu HBV i HCV. W badaniach *in vitro* wykazano, że w zakażeniu HBV nadekspresja miR-122 hamuje *HO-I* i negatywnie przyczynia się do replikacji wirusa [120-122]. Natomiast badania Shan i wsp. [123] wykazały, że miR-122 reguluje ekspresję osi *HO-1*/*Bach1* w hepatocytach. Zmniejszenie ekspresji miR-122 z jednoczesnym zwiększeniem *HO-I* może wpływać na hamowanie replikacji HCV i być ważnym czynnikiem cytoprotekcyjnym w wątrobie podczas infekcji [123]. Innym ważnym genem związanym z regulacją *HO-I* jest *Nrf-2* [124]. Jest on czynnikiem transkrypcyjnym aktywującym geny związane z obroną antyoksydacyjną co chroni komórki przed uszkodzeniami indukowanymi przez ROS. Donoszono, że aktywacja Nrf-2 wpływa zarówno na replikację wirusów układu oddechowego (takich jak IAV, koronawirusy, syncytialny wirus oddechowy, rinowirusy czy wirusy paragrypy) jak i na powiązane z infekcją stany zapalne

[125]. Z kolei miR-132 może hamować ekspresję *Nrf-2*, co może skutkować osłabieniem mechanizmów antyoksydacyjnych i w konsekwencji prowadzić do wzrostu poziomu stresu oksydacyjnego w tkankach [126-128]. Jednak rola miR-132 w regulacji *Nrf-2* podczas infekcji wirusowych nie jest poznana, co wskazuje na obszar do badań.

### 1.4.3. Regulacyjne działanie mikroRNA na geny związane z wrodzoną odpowiedzią immunologiczną i zapalną w zakażeniach wirusowych

W przypadku infekcji wirusowych wrodzona odpowiedź immunologiczna działa jako pierwsza linia obrony, aby zapobiec inwazji wirusa i jego replikacji [129]. Odpowiedź immunologiczna i zapalna to skomplikowany, skoordynowany proces, w który zaangażowane są różne komórki, ich produkty oraz szlaki sygnałowe, wspólnie regulujące poziomy mediatorów zapalnych zarówno w komórkach rezydujących w tkankach, jak i w komórkach zapalnych rekrutowanych z krwi. Choć przebieg reakcji zapalnej zależy od rodzaju początkowego bodźca i jego lokalizacji w organizmie, wszystkie te procesy opierają się na wspólnym mechanizmie. Najpierw receptory powierzchniowe komórek rozpoznają szkodliwy bodziec, co prowadzi do aktywacji szlaków zapalnych. W wyniku tego uwalniane są biomarkery zapalne, które stymulują rekrutację komórek zapalnych z krwi do miejsca uszkodzenia lub infekcji [130]. Odpowiedź zapalna charakteryzuje się skoordynowaną aktywacją różnych szlaków sygnałowych, które regulują ekspresję mediatorów pro- i przeciwzapalnych [131]. Jednym z najważniejszych szlaków wrodzonej odpowiedzi immunologicznej i zapalnej jest szlak czynnika jądrowego κ-łańcucha lekkiego wzmacniacza aktywowanych komórek β (NF-κβ, ang. nuclear factor kappa-light-chain-enhancer of activated B cells) (podjednostki – p50-p65) [131]. Nieaktywny szlak NF-κβ jest aktywowany w odpowiedzi na szereg bodźców, w tym infekcję wirusową, między innymi poprzez receptory TLR [132,133]. Aktywacja pierwotnego białka odpowiedzi na różnicowanie mieloidalne MyD88 (MyD88, ang. Myeloid Differentiation Primary Response Protein MyD88), przez receptory TLR (TLR2, TLR4) skutkuje transdukcją sygnału na inne geny i białka w kaskadzie sygnalizacyjnej szlaku NF-κβ (m. in. na kinazę związaną z receptorem interleukiny 1 (IRAK1, ang. Interleukin-1 Receptor-associated kinase 1), czynnik 6 związany z receptorem TNF (TRAF6, ang. TNF Receptor-associated Factor 6), białko wiążące kinazę aktywowaną przez TGF-β 1 (TAB2, ang. TGF-beta-activated Kinase 1 and MAP3K7-binding Protein 2) i inhibitor podjednostki alfa kinazy czynnika jądrowego kappa-B (IKKα, ang. Inhibitor of Nuclear Factor Kappa-B Kinase Subunit Alpha)), ostatecznie promując translokację heterodimeru NF-κβ (p50-p65) do jądra komórkowego i aktywację transkrypcji genów zapalnych [87,134].

Liczne badania wykazały, że miR odgrywa kluczową rolę w szlaku sygnałowym NF-κβ. MiR-155 negatywnie reguluje sygnalizację szlaku NF-κβ poprzez hamowanie ekspresji genów *MyD88, TAB2* i czynnika transkrypcyjnego p65 (p65, ang. Transcription Factor p65) [87]. Podczas reakcji zapalnych oś NF-κβ-miR-155 współdziała z osią NF-κβ-miR-146a, aby regulować intensywność i czas trwania stanu zapalnego [135]. Podczas zakażenia wirusem japońskiego zapalenia mózgu (JEV, ang. japanese encephalitis virus) wykazano, że miR-155 obniża ekspresję genu *MyD88* i *TAB2*. Dodatkowo zwiększona ekspresja miR-155 w odpowiedzi na zakażenie JEV, poprzez hamowanie swoich genów docelowych, przyczyniła się do zmniejszonej ekspresji genu *IL-6* [136]. Podczas infekcji nadekspresja miR-155 może również hamować podjednostkę *p65* NF-κβ, co może przyczynić się do hamowania odpowiedzi zapalnej [137].

Innym miR regulującym gen *MyD88* jest miR-125b. Podczas stymulacji komórek białkiem rdzeniowym HCV ekspresja miR-125b była hamowana. Dalsze badania wykazały odwrotną korelację pomiędzy miR-125b, a ekspresją *MyD88* oraz ekspresją *IL-6,* co wskazuje na działanie przeciwzapalne miR-125b [138].

Innym ważnym regulatorem szlaku NF-κβ jest miR-146a. Nadmierna ekspresja miR-146a zmniejsza ekspresję *IRAK1* oraz *TRAF6*, które zostały zidentyfikowane jako geny docelowe [87]. Infekcja wirusem Borna (BoDV-1, ang. Borna disease viruses 1), jak i JEV zwiększa ekspresję miR-146a, który poprzez regulację swoich genów docelowych tłumi aktywację NF-κβ oraz negatywnie reguluje produkcję cytokin prozapalnych [139,140]. Ponadto wykazano, że nadekspresja miR-146a poprzez tłumienie *TRAF6* sprzyja replikacji wirusów [139,141]. Wu i wsp. [141] prowadząc badania na liniach komórkowych wykazali, że infekcja DENV2 powoduje zwiększoną ekspresję miR-146a. MiR-146a poprzez tłumienie *TRAF6* upośledzał produkcję IFN-β w zakażeniu i przyczyniał się do łatwiejszej replikacji wirusa [141]. Infekcja wirusem Coxsackie B (CVB) (który jest główną przyczyną zapalenia mięśnia sercowego u ludzi) skutkuje wzrostem ekspresji miR-146a. Podwyższony poziom miR-146a hamował translokację NF-κβ do jądra komórkowego poprzez ukierunkowane działanie na *TRAF6*. Ponadto jego działanie hamujące *TRAF6* zmniejszało produkcję cytokin prozapalnych tj. IL-6 i TNF-α, co świadczy o działaniu przeciwzapalnym miR-146a [142].

miR-223 odgrywa ważną rolę w szlaku NF-κβ poprzez modulację *TLR4* [143]. TLR4 jest silnie aktywowany podczas ostrych infekcji wirusowych. Jego nadmierna stymulacja może prowadzić do nadmiernej odpowiedzi zapalnej, która jest szkodliwa dla narządów [144]. MiR-223 poprzez hamowanie ekspresji *TLR4* działa jako negatywny regulator ścieżki NF-κβ [145,146]. Oprócz regulacji *TLR4,* miR-223 hamuje również ekspresję *IKKα* co zapobiega

translokacji czynnika transkrypcyjnego NF-κβ i aktywacji ekspresji genów prozapalnych, tym samym ograniczając wystąpienie nadmiernego stanu zapalnego [87,145]. W przebiegu choroby koronawirusowej 2019 (COVID-19, ang. Corona-Virus-Disease-2019) wykazano, że nadekspresja miR-223 hamuje ekspresję genów *TLR4* i *IKKα* dzięki czemu łagodzi stan zapalny zapobiegając uszkodzeniu tkanek podczas infekcji [147].

Inflamasomy to wewnątrzkomórkowe kompleksy białkowe, będące elementem wrodzonej odpowiedzi immunologicznej. Inflamasomy są odpowiedzialne za inicjowanie procesów zapalnych w wyniku zakażeń (w tym wirusowych), uszkodzenia komórek, stresu komórkowego oraz produkcję cytokin prozapalnych [145,146,148]. Najlepiej zbadanym i scharakteryzowanym jest inflamasom domeny NACHT, LRR i piryny zawierającej białko 3 (NLRP3, ang. NACHT, LRR, and PYD domains-containing protein 3), który jest regulowany przez różne mechanizmy wpływające na poziomy białka i mRNA, w tym kontrolę transkrypcyjną ekspresji genów oraz modulację posttranskrypcyjną za pomocą miR [148]. miR-155 i miR-223 mogą regulować inflamasom *NLRP3* i tym samym odgrywać znacząca rolę w patogenezie wielu chorób z uszkodzeniem wielonarządowym [147,148]. miR-155 może wykazywać działanie prozapalne poprzez aktywację inflamasomu *NLRP3* i zwiększoną produkcję IL-18 i IL-1β [148,149]. Podczas infekcji wirusem Epsteina-Barr (EBV, ang. Epstein Barr virus) wykazano, że nadekspresja miR-223 hamuje aktywację *NLRP3,* która wpływała na hamowanie produkcji IL-1β co skutkuje osłabieniem odpowiedzi zapalnej [150].

## 1.5. Biomarkery molekularne (mRNA) apoptozy, stresu oksydacyjnego, wrodzonej odpowiedzi immunologicznej i zapalnej oraz profile zapalenia

Biomarkery molekularne to biologiczne cząsteczki znajdujące się we krwi, innych płynach utrojowych i w tkankach, które są oznaką normalnego lub nieprawidłowego procesu, stanu lub choroby [151]**.** Analiza na poziomie mRNA umożliwia określenie specyficznego dla danego schorzenia profilu ekspresji genów. Ponadto analiza mRNA umożliwia ocenę stanu fizjologicznego organizmu czy przebiegu procesu chorobowego. Biomarkery mRNA są bardziej czułe i specyficzne w stosunku do biomarkerów biochemicznych. Dzięki temu umożliwiają wcześniejszą i dokładniejszą diagnozę chorób [152-154].

Ocena poziomów mRNA genów związanych z apoptozą może pomóc we wcześniejszej diagnozie uszkodzenia narządów. Wzrost Bax przyczynia się do uwolnienia cytochromu c z mitochondriów, co aktywuje szlak apoptozy [155]. Ponadto równoczesna ocena stosunku mRNA *Bax*/*Bcl-2* może pomóc w dokładniejszej predykcji wystąpienia apoptozy w tkankach. Wysoki poziom *Bax* oraz obniżony poziom *Bcl-2*, a także zwiększony stosunek *Bax*/*Bcl-2*,

sprzyjają procesowi apoptozy. Stosunek ekspresji *Bax* do *Bcl-2* działa jak kluczowy przełącznik, który decyduje o losie komórki w odpowiedzi na bodźce apoptotyczne [156]. Ocena poziomu mRNA *PARP* może pomóc w predykcji wystąpienia apoptozy. Spadek ekspresji *PARP* może prowadzić do zwiększonej akumulacji uszkodzeń DNA, co może indukować apoptozę. Ocena poziomu mRNA *kaspazy-3*, może wcześniej niż poziom białka, pomóc w ocenie wystąpienia aktywacji szlaku apoptozy i śmierci komórki [157,158].

Analiza poziomów mRNA *HO-I* może być przydatnym biomarkerem do oceny stresu oksydacyjnego i ewentualnych jego następstw. *HO-I* chroni tkanki i narządy przed stresem oksydacyjnym i nadmiernymi reakcjami zapalnymi, uwalniając cząsteczki o działaniu antyoksydacyjnym [117]. Ponadto wykazuje efekt cytoprotekcyjny i chroni komórki przed apoptozą oraz może uczestniczyć w ochronie przed uszkodzeniem narządów takich jak wątroba, płuca, śledziona i nerki. W badaniach osób z niedoborem HO-I wykazano, że ogrywa on kluczową rolę w różnych stanach klinicznych obejmujących występowanie stresu oksydacyjnego [117]. Wielkość indukcji HO-I w następstwie stresu oksydacyjnego i jego szeroka dystrybucja w tkankach układowych w połączeniu z aktywnością biologiczną czynią go bardzo atrakcyjnym i interesującym biomarkerem stresu oksydacyjnego [117,159].

Biomarkery molekularne reakcji zapalnych mogą być przydatnym narzędziem w ocenie intensywności zapalenia, jak również pomocnym narzędziem diagnostycznym. IL-6 jest obiecującym biomarkerem wczesnej fazy stanu zapalnego we względu na dłuższy okres półtrwania niż inne cytokiny, a stężenie we krwi i tkankach może wzrosnąć kilka tysięcy razy w ciągu pierwszych 2–3 godzin po zainicjowaniu procesów zapalnych [160]. Z kolei TNF-α jest jedną z centralnych cytokin odpowiedzi zapalnej o działaniu prozapalnym i przeciwzakaźnym. W zależności od intensywności wydzielania TNF-α może prowadzić do wstrząsu i niewydolności wielonarządowej lub do regeneracji uszkodzonego narządu [161]. IL-18 stanowi ważny element odpowiedzi immunologicznej, ponieważ łączy wrodzoną odpowiedź immunologiczną z produkcją IFN-γ oraz może pośredniczyć w apoptozie zależnej od Fas [162]. Stanowi ona ważny element w infekcjach wirusowych poprzez działanie prozapalne, jednak jej zwiększone wydzielanie może prowadzić do zwiększonej produkcji cytokin prozapalnych i uszkodzenia narządów [163-165]. IL-1β odgrywa kluczową rolę w ostrej fazie zapalenia, indukując IL-6. Dodatkowo wywołuje efekty biologiczne, które ułatwiają rozwój reakcji zapalnej [166]. Określenie profili zapalenia, wynikające z charakterystycznej ekspresji cytokin tkankowych (na poziomie mRNA) w narządach może okazać się elementem arsenału diagnostycznego i terapeutycznego w przypadku chorób wirusowych o ostrym przebiegu jak ALF oraz MOF [162].

### 1.6. Przesłanki do podjęcia badań

Liczne badania wskazują, że w zakażenie *Lagovirus europaeus* oraz w patogenezę RHD zaangażowane są procesy biologiczne takie jak apoptoza, stres oksydacyjny oraz elementy wrodzonej odpowiedzi immunologicznej i zapalnej. Ekspresja miR gospodarza w przebiegu zakażeń wirusowych i ich wpływ na regulację krytycznych genów docelowych zaangażowanych w procesy biologiczne może odgrywać znaczącą rolę w patogenezie chorób o etiologii wirusowej. Jak dotychczas, poza badaniami Hukowska-Szematowicz i wsp. [63,167], brakuje w literaturze naukowej danych dotyczących molekularnych sygnatur regulacyjnych interakcji między miR, a apoptozą, stresem oksydacyjnym oraz wrodzoną odpowiedzią immunologiczną i zapalną w zakażeniu *L. europaeus*, patogenezie RHD. Stąd ten problem naukowy stał się celem badań zaprezentowanych w publikacjach naukowych [publikacja P-1, P-2, P-3] wchodzących w skład osiągniecia naukowego i stanowiących podstawę ubiegania się o stopień naukowy doktora. Poznanie tych zależności z uwzględnieniem mikrośrodowiska tkankowego (wątroby, płuc, nerek, śledziony) może prowadzić do poznania roli cząsteczek miR w zakażeniu *L. europaeus*, a tym samym dostarczyć nowych danych pozwalających lepiej zrozumieć patogenezę RHD w zakresie molekularnej regulacji apoptozy, stresu oksydacyjnego oraz wrodzonej odpowiedzi immunologicznej i zapalnej przez miR. Ponadto zakażenie królików *L. europaeus*, stanowi dobry model badawczy dla ALF oraz MOF o etiologii wirusowej. Model ten może posłużyć do poszukiwania potencjalnych diagnostycznych biomarkerów molekularnych choroby, jak również środków terapeutycznych opartych na regulacji poziomów miR oraz na modulacji szlaków zależnych od miR uczestniczących w patogenezie chorób o etiologii wirusowej.

## 2. Cel badań

Głównym celem badań zaprezentowanych w publikacjach wchodzących w skład rozprawy doktorskiej i stanowiących podstawę ubiegania się o stopień naukowy doktora była ocena ekspresji wybranych cząsteczek mikroRNA i ich genów docelowych związanych z apoptozą, stresem oksydacyjnym oraz wrodzoną odpowiedzią immunologiczną i zapalną w zakażeniu *Lagovirus europaeus* dwoma genotypami – GI.1 i GI.2 w czterech tkankach – wątrobie, płucach, nerkach i śledzionie.

Cel ten został osiągnięty przez:

1. Określenie sygnatury miR w infekcjach wirusowych u ludzi i zwierząt [publikacja P-1].
2. Określenie kluczowych miR oraz ich genów docelowych zaangażowanych w apoptozę, stres oksydacyjny oraz we wrodzoną odpowiedź immunologiczną i zapalną w przebiegu zakażeń wirusowych u ludzi i zwierząt [publikacja P-1] oraz [Tabela 1, publikacja P-2] i [Tabela 2, publikacja P-3].
3. Ocenę poziomu ekspresji wybranych kluczowych miR zaangażowanych w regulację apoptozy (miR-21, miR-16b, miR-34a), stresu oksydacyjnego (miR-122, miR-132) oraz wrodzonej odpowiedzi immunologicznej i zapalnej (miR-155, miR-146a, miR-223, miR-125b) w zakażeniu *L. europaeus* genotypami – GI.1 i GI.2 w czterech tkankach [publikacja P-2, P-3].
4. Ocenę poziomu ekspresji genów docelowych dla wybranych miR w zakażeniu *L. europaeus* genotypami – GI.1 i GI.2 w czterech tkankach. Apoptoza: miR-21 (*PTEN*, *PDCD4*); miR-16b (*Bcl-2, CXCL10*); miR-34a (*p53, SIRT1*). Stres oksydacyjny: miR-122 (*Bach1*); miR-132 (*Nrf-2*) [publikacja P-2]. Wrodzona odpowiedź immunologiczna i zapalna: miR-155 (*MyD88*, *TAB2*, *p65*, *NLRP3*); miR-146a (*IRAK1*, *TRAF6*); miR-223 (*TLR4*, *IKKα*, *NLRP3)*; miR-125b (*MyD88*) [publikacja P-3].
5. Ocenę korelacji poziomu ekspresji miR z poziomem ekspresji ich genów docelowych w celu oceny regulacyjnego wpływu miR oraz określenia ich wpływu na ścieżki apoptozy, stresu oksydacyjnego oraz wrodzonej odpowiedzi immunologicznej i zapalnej w zakażeniu *L. europaeus* genotypami – GI.1 i GI.2 w czterech tkankach [publikacja P-2, P-3].
6. Ocenę poziomu biomarkerów molekularnych (na poziomie mRNA) apoptozy (*Bax*, stosunek *Bax/Bcl-2*, *kaspaza-3*, *PARP*), stresu oksydacyjnego (*HO-I*) oraz wrodzonej odpowiedzi immunologicznej i zapalnej (*IL-6*, *TNF-α, IL-1β i IL-18*) w zakażeniu *L. europaeus* genotypami – GI.1 i GI.2 w czterech tkankach [publikacja P-2, P-3].

Określenie profili stanu zapalnego w tkankach w odpowiedzi na zakażenie *L. europaeus* genotypami – GI.1 i GI.2 [publikacja P-3].

# 3. Materiał i metody badawcze

## 3.1. Materiał badawczy

### 3.1.1. Wirusy

Do wywołania zakażenia u królików wykorzystano dwa genotypy *Lagovirus europaeus* – genotyp GI.1 – o nazwie BBI (wariant GI.1a) (Polska 2017, nr akcesyjny w GenBanku MG602005) oraz genotyp GI.2 – o nazwie PIN (Polska 2018, nr akcesyjny z GenBanku MN853660). Obydwa wirusy zostały przygotowane w Krajowym Laboratorium Referencyjnym ds. Choroby Krwotocznej Królików (RHD) oraz w Zakładzie Pryszczycy, Państwowego Instytutu Weterynaryjnego – Państwowego Instytutu Badawczego w Zduńskiej Woli. Do zakażenia zwierząt użyto 1ml inokulum, zawierającego $0{,}5x10^4$ jednostek hemaglutynacji *L. europaeus* GI.1 oraz $2{,}048x10^4$ jednostek hemaglutynacji *L. europaeus* GI.2 [publikacja P-2, P-3].

### 3.1.2. Model eksperymentalny

Badanie zostało przeprowadzone na 30 królikach europejskich *Oryctolagus cuniculus* – Crl:KBL (NZW) /052. Zwierzęta zostały zakupione od licencjonowanego hodowcy (AnimaLab Sp. z o. o., Poznań, Polska) w wieku 6 miesięcy, o masie ciała 4,0-4,5 kg i równym (50:50) podziale na płeć. Po dostarczeniu zwierząt do Uniwersyteckiej Zwierzętarni Doświadczalnej Pomorskiego Uniwersytetu Medycznego (PUM) w Szczecinie przeszły 3-tygodniowy okres adaptacyjny. W trakcie eksperymentu zwierzęta miały nieograniczony dostęp do jedzenia oraz wody. Autonomiczny system klimatyzacji utrzymywał temperaturę 22 °C (±1 °C) przy poziomie wilgotności 50%-60% oraz zapewniał 15-20 wymian powietrza na godzinę. Zwierzęta przebywały w pomieszczeniach ze sztucznym oświetleniem, sterowanym automatycznie (12 godzin światła/12 godzin ciemności) i czerwonym oświetleniem nocnym. Woda i pożywienie dla zwierząt były dostępne *ad libitum*. Zwierzęta podzielono losowo na trzy grupy. Grupie kontrolnej (n=10) podano domięśniowo 1 ml PBS (buforowanej soli fizjologicznej) jako placebo. Królikom w grupach zakażonych podano domięśniowo 1 ml wirusa. Grupa 2 otrzymywała *L. europaeus* genotyp GI.1, zaś grupa 3 otrzymywała *L. europaeus* genotyp GI.2. Podanie antygenu w grupie królików zakażonych i PBS w grupie kontrolnej oznaczono jako początek eksperymentu. Po tym czasie monitorowano stan zdrowia zwierząt. Po wystąpieniu u zakażonych zwierząt ciężkich objawów choroby, potwierdzonych przez lekarza, poddawano je zabiegowi eutanazji [publikacja P-2, P-3].

### 3.1.3. Pobranie tkanek

Próbki tkanek wątroby, płuc, nerek i śledziony pobrano po eutanazji od królików zdrowych (n = 10) oraz pośmiertnie lub po eutanazji od królików zakażonych *L. europaeus* genotyp GI.1 (n = 10) i GI.2 (n = 10). Wszystkie pobrane tkanki przemyto zimnym PBS, a następnie umieszczono w ciekłym azocie i przechowywano w temperaturze -80$^{o}$C do momentu izolacji całkowitego RNA z frakcją miRNA [publikacja P-2, P-3].

## 3.2. Metody

### 3.2.1. Wybór badanych mikroRNA oraz przewidywanie in silico ich genów docelowych zaangażowanych w apoptozę, stres oksydacyjny oraz we wrodzoną odpowiedź immunologiczną i zapalną u *Oryctolagus cuniculus*

W pierwszym etapie wybrano miR do badań zaangażowane w procesy biologiczne zachodzące podczas infekcji wirusowych u ludzi i zwierząt takie jak apoptoza, stres oksydacyjny oraz wrodzona odpowiedź immunologiczna i zapalna. W celu wyboru miR przeprowadzono dogłębny przegląd literatury [publikacja P-1] oraz [87,126,167-170], a także sugerowano się wcześniejszymi wynikami badań Hukowskiej-Szematowicz i wsp. [167]. Na tym etapie wykorzystano bazę danych miRTarBase do wyselekcjonowania miR, w której zastosowano strategie wyszukiwania według miR, genu docelowego, procesów biologicznych, metod walidacji oraz chorób. Do kryterium wyboru miR wybrano silne dowody oraz dane literaturowe. Wybrano miR *Homo sapiens* o opisanej roli w procesie apoptozy – trzy miR (miR-21, miR-16b, miR-34a), stresie oksydacyjnym – dwa miR (miR-122, miR-132) oraz cztery miR biorące udział w regulacji wrodzonej odpowiedzi immunologicznej i zapalnej (miR-155, miR-146a, miR-223, miR-125b) [publikacja P-2, P-3]. W kolejnym etapie z wykorzystaniem baz miRTarBase oraz miRDB dokonano wyboru genów docelowych dla wyselekcjonowanych miR. Wybrany zestaw genów posłużył do analizy ontologii genów (GO) poprzez analizę wzbogacenia GO z wykorzystaniem narzędzia PANTHER. Po wyborze genów docelowych zaangażowanych w badane procesy oceniono sekwencje 3′-UTR genów *Oryctolagus cuniculus* pod kątem obecności miejsc wiązania dla wybranych miR używając bazy TargetScan. Następnie w celu weryfikacji znaczenia wybranych miR w infekcji *L. europaeus*, przeprowadzono analizę *in silico* potencjalnych genów docelowych. Ze względu na brak jednej bazy danych do wykazania interakcji miR-mRNA u *Oryctolagus cuniculus* porównano sekwencje miRNA u *Oryctolagus cuniculus* i *Homo sapiens*, nie stwierdzając różnic. Użyto bazy miRTarBase do identyfikacji genów z potwierdzonymi interakcjami miR-mRNA u *Homo sapiens*, tworząc listy genów docelowych dla każdego miRs. Następnie przeprowadzono

analizę GO, aby zidentyfikować procesy związane z patogenezą RHD, ALF i MOF. Analizy wykazały, że wiele z tych regulacji zachodzi również u *Oryctolagus cuniculus*, co potwierdzono przy użyciu bazy TargetScan. Stwierdzono, że geny wybranych miR posiadają miejsca wiązania w 3′-UTR u *Oryctolagus cuniculus*. Wybrane miRs i ich geny docelowe przedstawiono w Tabeli 3 [publikacja P-2, P-3].

**Tabela 3**. Wybrane do badań mikroRNA i geny docelowe zaangażowane w apoptozę, stres oksydacyjny oraz wrodzą odpowiedź immunologiczną i zapalną.

| miR | Gen docelowy | Produkt genu | Piśmiennictwo |
|---|---|---|---|
| **Apoptoza** [publikacja P-2] | | | |
| miR-21 | *PTEN* | Phosphatase and tensin homolog/Homolog fosfatazy i tensyny | [168,171-173] |
| | *PDCD4* | Programmed cell death factor 4/Gen programowanej śmierci komórki 4 | [106,168] |
| miR-16b | *Bcl-2* | B-cell lymphoma 2/Regulator apoptozy Bcl-2 | [167,169,174,175] |
| | *CXCL10* | C-X-C motif chemokine ligand 10/Ligand chemokiny z rodziny C-X-C motyw 10 | [169,176] |
| miR-34a | *SIRT1* | Silent information regulator 1/ Cichy regulator informacji 1 | [110,170,177,178] |
| | *p53* | Protein p53/Białko p53 | |
| **Stres oksydacyjny** [publikacja P-2] | | | |
| miR-122 | *Bach1* | BTB domain and CNC homolog 1/Domena BTB i CNC homolog 1 | [123,167,179] |
| miR-132 | *Nrf-2* | Nuclear factor erythroid 2-related factor 2/Czynnik jądrowy związany z erytroidem 2 | [124,126-128,180] |
| **Wrodzona odpowiedź immunologiczna i zapalna** [publikacja P-3] | | | |
| miR-155 | *MyD88* | Myeloid differentiation primary response protein MyD88/Pierwotne białko odpowiedzi na różnicowanie mieloidalne MyD88 | [87,181,182] |
| | *TAB2* | TGF-beta-activated kinase 1 and MAP3K7-binding protein 2/Białko wiążące kinazę aktywowaną przez TGF-beta 1 | [87,183,184] |
| | *p65* (subunit of NF-κβ) | Transcription factor p65/Czynnik transkrypcyjny p65 | [87,185,186] |
| | *NLRP3 inflammasome* | NOD, LRR, and pyrin domains containing protein 3/Domena NOD, LRR i piryny zawierające białko 3 | [87] |
| miR-146a | *IRAK1* | Interleukin-1 receptor-associated kinase 1/Kinaza związana z receptorem interleukiny 1 | [87,95,139] |
| | *TRAF6* | TNF receptor-associated factor 6/Czynnik 6 związany z receptorem TNF | [87,95,139] |
| miR-223 | *TLR4* | Toll-like receptor 4/Receptor Toll-podobny 4 | [87,187,188] |
| | *IKKα* | Inhibitor of nuclear factor kappa-B kinase subunit alpha/Inhibitor podjednostki alfa kinazy czynnika jądrowego kappa-B | [87,189] |
| | *NLRP3 inflammasome* | NACHT, LRR, and PYD domains-containing protein 3/Domena NACHT, LRR i piryny zawierające białko 3 | [87,147,190] |
| miR-125b | *MyD88* | Myeloid differentiation primary response protein MyD88/Pierwotne białko odpowiedzi na różnicowanie mieloidalne MyD88 | [138,191] |

### 3.2.2. Izolacja mikroRNA oraz mRNA z tkanek królików zakażonych oraz kontrolnych

Całkowite RNA, wraz z frakcją miR, izolowano z 50 mg tkanki wątroby, płuc, nerek i śledziony od królików kontrolnych oraz zakażonych *L. europaeus* przy użyciu zestawu miRNeasy Mini Kit (Qiagen, Hilden, Niemcy) zgodnie z protokołem producenta. Próbki tkanek poddano mechanicznej homogenizacji w 700µl odczynnika do lizy (QIAzol), a następnie inkubowano przez 5 minut w temp. pokojowej. Następnie do homogenatu dodano 140µl chloroformu, wytrząsano i inkubowano 3 minuty. Po inkubacji próbki wirowano przy 12 000xg przez 15 minut w temp. 4°C. Uzyskaną górna fazę wodną ostrożnie przeniesiono do nowej próbówki i dodano 1,5 objętości 100% etanolu w celu wytrącenia RNA. Uzyskaną mieszaninę przeniesiono na kolumnę RNeasy Mini i wirowano przy prędkości 8000xg przez 15 sekund. Etap ten powtórzono dla całej objętości próbki. Następnie kolumnę przemyto 700µl buforu RWT i dwukrotnie 500µl buforu RPE. W celu wypłukania buforów, próbkę wirowano przy prędkości 900xg przez 1 minutę, przy czym ostatnie wirowanie wydłużono do 2 minut w celu zapewnienia suchości membrany. W ostatnim etapie na kolumnę dodano 50µl wody wolnej od nukleaz i wirowano przez 1 minutę z prędkością 9000xg. Stężenie i jakość RNA określono przy użyciu spektrofotometru NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) [publikacja P-2, P-3].

### 3.2.3. Reakcja poliadenylacji mikroRNA i odwrotnej transkrypcji

Reakcję odwrotnej transkrypcji przeprowadzono przy użyciu zestawu miRCURY LNA RT Kit (Qiagen, Hilden, Niemcy) zgodnie z instrukcją producenta. Całkowita objętość mieszaniny reakcyjnej wynosiła 10µl i składała się z: 2µl buforu reakcyjnego 5× miRCURY RT Reaction Buffer, 1µl 10x mieszaniny enzymów miRCURY RT, 5µl wody wolnej od nukleaz i 2µl matrycy RNA. Do reakcji syntezy cDNA użyto 5 ng/µl całkowitego RNA. Reakcja oraz profil temperaturowy był zgodny z zaleceniami producenta i obejmował następujące kroki: inkubacja w temperaturze 42°C przez 60 minut; inaktywacja cieplna odwrotnej transkryptazy w temperaturze 95°C przez 5 minut oraz schłodzenie mieszaniny reakcyjnej do 4°C. Uzyskane w reakcji cDNA przechowywano w temperaturze -20°C do czasu dalszych analiz [publikacja P-2, P-3].

### 3.2.4. Reakcja odwrotnej transkrypcji mRNA

Syntezę cDNA przeprowadzono przy użyciu zestawu RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) zgodnie z protokołem producenta. Do przeprowadzenia reakcji w celu zwiększenia jej wydajności użyto oligo(dT) i losowych heksametrów. Reakcję odwrotnej transkrypcji przeprowadzono w mieszaninie o łącznej

objętości 20µl zawierającej: po 1µl Oligo(dT) Primer i Random Hexamer primer, 4 µl buforu reakcyjnego o stężeniu 5x, 1µl inhibitora RNas RiboLock RNase Inhibitor, 1µl enzymu RevertAid M-MuL V RT (200 U/µl), 5µl wody wolnej od nukleaz (Sartorius, Izrael) i 4µl matrycy RNA o stężeniu 200 ng/µl. Warunki reakcji i profil temperaturowy określono na podstawie instrukcji producenta i obejmował następujące kroki: inkubacja w temperaturze 25$^{o}$C przez 5 minut; inkubacja w temperaturze 42$^{o}$C przez 60 minut; terminacja reakcji w temperaturze 70$^{o}$C przez 5 minut; schłodzenie mieszaniny reakcyjnej do 4$^{o}$C. Uzyskane w reakcji cDNA przechowywano w temperaturze -20$^{o}$C do czasu dalszych analiz [publikacja P-2, P-3].

### 3.2.5. Ocena poziomu ekspresji mikroRNA w tkankach przy użyciu ilościowej reakcji PCR w czasie rzeczywistym (Real-Time PCR) i analiza danych

Poziom ekspresji badanych miR w próbkach tkanek królików kontrolnych oraz zakażonych *L. europaeus* genotypami GI.1 i GI.2 określono przy zastosowaniu łańcuchowej reakcji polimerazy w czasie rzeczywistym (Real-Time PCR) przy użyciu staterów miRCURY LNA miRNA PCR Assay (Qiagen, Hilden, Niemcy) oraz zestawu miRCURY LNA SYBR Green PCR Kit, zgodnie z instrukcją producenta. Mieszanina reakcyjna składała się z 5µl miRCURY SYBR Green Master Mix, 0,05µl barwnika referencyjnego ROX, 1µl mieszaniny specyficznych starterów (dla badanych miR były to: ocu- miR-21-5p, ocu-miR-16b-5p, ocu-miR-34a-5p, ocu-miR-122-5p, ocu-miR-132-5p, ocu-miR-155-5p, ocu-miR-146a-5p, ocu-miR-223-5p, ocu-miR-125b-5p oraz miR referencyjne-ocu-miR-103a-3p) i 1µl wody wolnej od nukleaz (Sartorius, Izrael) [publikacja P-2, P-3]. Sekwencje badanych miR *Oryctolagus cuniculus* przedstawiono w Tabeli 4.

**Tabela 4**. Sekwencja badanych miR *Oryctolagus cuniculus* (ocu).

| miRNA | Sekwencja |
|---|---|
| **Apoptoza** [publikacja P-2] | |
| ocu-miR-21-5p | 5′-UAGCUUAUCAGACUGAUGUUGACU-3′ |
| ocu-miR-16b-5p | 5′-UAGCAGCACGUAAAUAUUGGCGU-3′ |
| ocu-miR-34a-5p | 5′-UGGCAGUGUCUUAGCUGGUUGU-3′ |
| **Stres oksydacyjny** [publikacja P-2] | |
| ocu-miR-122-5p | 5′-UGGAGUGUGACAAUGGUGUUUG-3′ |
| ocu-miR-132-5p | 5′-ACCAUGGCUUUCGAUUGUUACU-3′ |
| **Wrodzona odpowiedź immunologiczna i zapalna** [publikacja P-3] | |
| ocu-miR-155-5p | 5′UUAAUGCUAAUCGUGAUAGGGGUU3′ |
| ocu-miR-146a-5p | 5′UGAGAACUGAAUUCCAUGGGUUG3′ |
| ocu-miR-223-5p | 5′CGUGUAUUUUGACAAGCUGAGUUG3′ |
| ocu-miR-125b-5p | 5′UCCCCUGAGACCCUAACUUGUGA3′ |
| **miR referencyjny** | |
| ocu-miR-103a-3p | 5′AGCAGCAUUGUACAGGGCUAUGA3′ |

Matryce cDNA przed reakcją zostały 60-krotnie rozcieńczone w wodzie wolnej od nukleaz (Sartorius, Izrael). Końcowa objętość reakcji wynosiła 10µl. Warunki reakcji i profil temperaturowy określono na podstawie instrukcji producenta i obejmował następujące kroki: początkowa aktywacja w temperaturze 95$^{o}$C przez 2 minuty; 40 cykli: denaturacji w temperaturze 95$^{o}$C przez 10 sekund oraz przyłączania starterów i elongacji w temperaturze 56$^{o}$C przez 1 minutę. Po zakończeniu reakcji amplifikacji, w celu weryfikacji niespecyficznych produktów, przeprowadzono analizę krzywej topnienia w zakresie temperatur 60-95°C. Dane Real-Time PCR znormalizowano przy użyciu stabilnego genu referencyjnego ocu-miR-103a-3p, który został wybrany na podstawie wcześniejszych badań Hukowskiej-Szematowicz i wsp. [166] oraz oceny stabilności innych genów referencyjnych w warunkach eksperymentalnych przy użyciu algorytmów geNorm, NormFinder i BestKeeper [166].

Dane fluorescencji analizowano przy użyciu systemu Real-Time qPCR (QuantStudio5 Real-Time PCR System, Applied Biosystems, USA). Uzyskane wyniki znormalizowano w odniesieniu do miR referencyjnego, a poziom względnej ekspresji został określony przy użyciu metody $2^{-\Delta\Delta Ct}$ [publikacja P-2, P-3].

### 3.2.6. Ocena poziomu ekspresji mRNA w tkankach przy użyciu ilościowej reakcji PCR w czasie rzeczywistym (Real-Time PCR) i analiza danych

Poziom ekspresji badanych genów docelowych (mRNA) dla miR oraz badanych biomarkerów (apoptozy, stresu oksydacyjnego oraz wrodzonej odpowiedzi immunologicznej i zapalnej) na poziomie mRNA w próbkach tkanek królików kontrolnych oraz zakażonych *L. europaeus* genotypami GI.1 i GI.2 określono przy zastosowaniu ilościowej reakcji PCR w

czasie rzeczywistym (Real-Time PCR) [publikacja P-2, P-3]. W celu zaprojektowania starterów dla badanych genów docelowych (mRNA) oraz biomarkerów wykorzystano narzędzia bioinformatyczne dostępne online (Primer-BLAST i Becon Designer). Sekwencje starterów użytych do badania ekspresji genów docelowych dla miR, biomarkerów oraz sekwencję genu referencyjnego przedstawiono w Tabeli 5. Syntezę starterów wykonała firma Genomed (Warszawa, Polska). W celu weryfikacji zaprojektowanych starterów przeprowadzono reakcję PCR z zastosowaniem gradientu temperaturowego przy użyciu zestawu Color OptiTaq PCR Master Mix (2x) (Euryx, Polska), a następnie wykonano elektroforezę w żelu agarozowym. Mieszanina reakcyjna PCR o objętości 50µl składała się z 25µl Color OptiTaq PCR Master Mix (2×), po 0,25µl starterów forward i revers badanego genu o stężeniu 10mM, 23,5µl wody wolnej od nukleaz (Sartorius, Izrael) oraz 1µl matrycy cDNA o stężeniu 0,2 µg/µl. Reakcję przeprowadzono zgodnie z następującym profilem temperaturowo-czasowym: wstępna denaturacja w 94°C przez 2 minuty, 30 cykli denaturacji w 94°C przez 15 sekund, przyłączania staterów w gradiencie temperaturowym (w zakresie temperatur 52-65°C) przez 30 sekund i elongacji w 72°C przez 1 minutę. Na koniec reakcji przeprowadzono wydłużanie końcowe w temperaturze 72°C przez 7 minut. Po zakończeniu reakcji próbki schłodzono do 4°C. Czynności te pozwoliły na określenie temperatury przyłączenia dla każdej pary specyficznych starterów oraz kontrolę czy w trakcie reakcji nie powstają produkty niespecyficzne.

Po przeprowadzeniu walidacji starterów, dokonano optymalizacji reakcji Real-Time PCR [publikacja P-2, P-3]. Proces ten obejmował sekwencyjną modyfikację parametrów, takich jak, temperatury przyłączania, stężenia starterów oraz zakresy stężeń cDNA, dostosowane indywidualnie dla każdego badanego genu. W celu sporządzenia krzywej standardowej zastosowano metodę kalibracji. Optymalizację przeprowadzono z wykorzystaniem najlepiej dopasowanych temperatur przyłaczania oraz stężeń starterów dla każdej pary, przeprowadzając seryjne rozcieńczenia tego samego cDNA (1:2, 1:4, 1:8, 1:16 i 1:32) z wyjściowego stężenia 10 ng/µl, zgodnie z zaleceniami producenta (HOT FIREPol® EvaGreen® qPCR Supermix, 5×; Solis BioDyne, Estonia). Zaobserwowano, że różne pary starterów wymagały różnych optymalnych zakresów stężeń cDNA dla każdego genu, aby osiągnąć najwyższy współczynnik determinacji ($R^2$) oraz wydajność na poziomie 100% ± 5%. Ponadto swoistość starterów została potwierdzona za pomocą analizy krzywej topnienia produktów PCR po zakończeniu właściwej reakcji amplifikacji.

Reakcja została przeprowadzona przy użyciu zestawu HOT FIREPol® EvaGreen® qPCR Supermix, 5× (Solis BioDyne, Estonia) zgodnie z instrukcją producenta. Mieszanina reakcyjna o objętości 20 µl zawierała 4 µl zestawu HOT FIREPol® EvaGreen® qPCR Supermix 5×, po

0,4 µl starterów forward i reverse o stężeniu 10 µM, zmienną objętość matrycy cDNA (o stężeniu 10 ng/µl) oraz wodę wolną od nukleaz (Sartorius, Izrael), której ilość dostosowywano w zależności od wymagań poszczególnych starterów. Reakcję PCR przeprowadzono zgodnie z następującym profilem temperaturowo-czasowym: początkowa aktywacja enzymu poprzez inkubację w 95°C przez 12 minut, następnie 40 cykli denaturacji w 95°C przez 15 sekund, przyłączanie starterów w temperaturze specyficznej dla pary starterów od 20 do 30 sekund (w zależności od długości amplifikowanego produktu) oraz wydłużanie w 72°C od 20 do 30 sekund.

Dane fluorescencji analizowano przy użyciu systemu Real-Time qPCR (QuantStudio5 Real-Time PCR System, Applied Biosystems, USA). Uzyskane wyniki znormalizowano w odniesieniu do genu referencyjnego 18S, a poziom względnej ekspresji został określony przy użyciu metody $2^{-\Delta\Delta Ct}$ [publikacja P-2, P-3].

**Tabela 5**. Sekwencje starterów użytych do badania ekspresji genów docelowych dla miR, biomarkerów apoptozy, stresu oksydacyjnego i stanu zapalnego oraz sekwencja genu referencyjnego.

| Gen | Numer dostępu w GenBanku | | Sekwencja starterów | Ta (°C) | Długość amplikonu (bp) | Tm poduktu (°C) |
|---|---|---|---|---|---|---|
| **Apotoza** [publikacja P-2] | | | | | | |
| *PTEN* | XM_008270133.3 | Forward<br>Revers | 5'- GCGGAACTTGCAATCCTCAG-3'<br>5′-TCGTGTGGGTCCTGAATTGG-3′ | 60 | 70 | 81 |
| *PDCD4* | XM_017348553.2 | Forward<br>Revers | 5′-GAATAACCGTGCCAACCAGTCC-3′<br>5′-CTTTCCCTCCTGCACCACCTTTC-3′ | 60 | 102 | 85 |
| *Bcl-2* | XM_008261439.2 | Forward<br>Revers | 5′-TGTGTGTGGAGAGCGTCAAC-3′<br>5′-AGTTCCACGAAGGCATCCCAG-3′ | 62 | 133 | 87.5 |
| *CXCL10* | XM_002717106.4 | Forward<br>Revers | 5′-AGCATTTAGCAAGGAAAGGTCCAG-3′<br>5′-AGAAGGGAAGTGTGGCAGAGG-3′ | 60 | 110 | 83.8 |
| *p53* | XM_008270660.3 | Forward<br>Revers | 5′-TGACGGAAGTTGTCAGACGC-3'<br>5′-TACAGTCAGAGCCAACCTCGG-3′ | 60 | 183 | 89.8 |
| *SIRT1* | XM_002718460.4 | Forward<br>Revers | 5′-AGTAAGCGGCTCGATGGTAATCAG-3′<br>5′-TCCAGTTCCTCCAGGTCTCTCTG-3′ | 63 | 249 | 85.5 |
| **Biomarkery apoptozy** [publikacja P-2] | | | | | | |
| *Bax* | XM_008252361.3 | Forward<br>Revers | 5′-ACATGGAGCTACAGAGGATGATCG-3′<br>5′-AGCGTCCAGCCCATAATAGTCC-3′ | 61 | 205 | 86 |
| *Kaspaza-3* | NM_001082117.1 | Forward<br>Revers | 5′-AACTTTTCATTATTCAGGCTTGCCG-3′<br>5′-TCAACCCCACTGTCTGTCTCG-3 | 58 | 70 | 84.2 |
| *PARP* | XM_008268352.3 | Forward<br>Revers | 5′-CGGACAAGCTCTACCGAGTG-3′<br>5′-CATCGAACATGGGCGACTGC-3′ | 60 | 123 | 88 |
| **Stres oksydacyjny** [publikacja P-2] | | | | | | |
| *Bach1* | XM_002716782.4 | Forward<br>Revers | 5′-ACTCTACCAGAAGAGGTGACAG-3<br>5′-TGAGAAACTGAAAGCAGGACTC-3′ | 60 | 160 | 78 |
| *Nrf-2* | XM_051849401.1 | Forward<br>Revers | 5′-AGAAACAGAACACAAGGACATGG-3′<br>5′-TTGGGCTGGCTGAATTGGG-3′ | 60 | 241 | 85.7 |
| **Biomarker stresu oksydacyjnego** [publikacja P-2] | | | | | | |
| *HO-I* | XM_051846030.1 | Forward<br>Revers | 5′-ACTGCCGAGGGTTTTAAGCTGG-3′<br>5′-ACCGGGTTCTCCTTGTTGTGC-3′ | 60 | 92 | 85 |
| **Wrodzona odpowiedź immunologiczna i zapalana** [publikacja P-3] | | | | | | |
| *MyD88* | XM_002723869.4 | Forward<br>Revers | 5'-CCCCAGCGACATGCAGTTTG-3'<br>5'-TTCTGATGGGCACCTGGAGAG-3' | 61 | 227 | 90,8 |
| *TAB2* | XM_051836813.1 | Forward<br>Revers | 5'-ACCTCCAGCAGTTCCTCTTC-3'<br>5'-TCATCTCCTGTGGTGGCATT-3' | 60 | 152 | 83,5 |
| *p65* | XM_051827970.1 | Forward<br>Revers | 5'-CCCTTCCAAGTGCCCATAGA-3'<br>5'-CCTCTTTCTGCACCTTGTCG-3' | 60 | 250 | 91,5 |
| *NLRP3* | MK829787.1 | Forward<br>Revers | 5'- TGTCTCACGTCCAGCTTTTG-3'<br>5'- AGCCAGAGTCTGCGAATGTT-3' | 60 | 161 | 87,5 |
| *IRAK1* | XM_051837494.1 | Forward<br>Revers | 5'-GGACTTTGCTGGCTACTGTG-3'<br>5'-CAGGAGGACGTTGGAACTCT-3' | 60 | 229 | 89,9 |
| *TRAF6* | XM_002709054.4 | Forward<br>Revers | 5'-ACGGGGAACCTTTCTGGCTC-3'<br>5'-TGTGGCCTGCATCCCTTATTG-3' | 61 | 187 | 86,4 |
| *TLR4* | NM_001082732.2 | Forward<br>Revers | 5'-TTTCACACGGCCACTGCTG-3'<br>5'-ATTGGGAACGACCTCCACAC-3' | 61 | 142 | 81,4 |
| *IKKα* | XM_002718612.4 | Forward<br>Revers | 5'-GGTAACTCCTCAAGATGGGGAC-3'<br>5'-TGCCCTGTTCCTCATTTGCT-3' | 60 | 107 | 78,7 |
| **Biomarkery stanu zapalnego** [publikacja P-3] | | | | | | |
| *IL-1β* | NM_001082201.1 | Forward<br>Revers | 5'-GGTGTTGTCTGGCACGTATG-3'<br>5'- TTGGGGTCTACACTCTCCAG-3' | 60 | 210 | 84 |
| *IL-6* | NM_001082064.2 | Forward<br>Revers | 5'- GGCGGTGAATAATGAGACCTG-3'<br>5'- ATGAAGTGGATCGTGGTCGT-3' | 60 | 276 | 87,3 |
| *TNF-α* | NM_001082263.1 | Forward<br>Revers | 5'- CGTAGTAGCAAACCCGCAAG-3'<br>5'- TGATGGCAGAGAGGAGGTTG-3' | 60 | 245 | 91,3 |
| *IL-18* | NM_001122940.1 | Forward<br>Revers | 5'-TGTATAGAAAATGCACCCCAGAC-3'<br>5'- TCTTTCTGTCCTGCGAGATGT-3' | 60 | 221 | 80 |
| **Gen referencyjny** | | | | | | |
| *18S* | NR_033238.1 | Forward<br>Revers | 5'ATCAGATACCGTCGTAGTTC-3'<br>5'-TTCCGTCAATTCCTTTAAG-3' | 60 | 167 | 88 |

### 3.2.7. Badania kliniczne

W trakcie eksperymentu oceniano stan kliniczny królików zakażonych i królików zdrowych stanowiących grupę kontrolną, a także rejestrowano objawy kliniczne i śmiertelność zakażonych zwierząt [publikacja P-2, P-3].

### 3.2.8. Analiza statystyczna

Wszystkie uzyskane wyniki [publikacja P-2, P-3] zostały poddane analizie statystycznej przy użyciu pakietu STATISTICA PL Wersja 13 (StatSoft, Palo Alto, CA, USA). W celu określenia zgodności rozkładu uzyskanych wartości z rozkładem normlanym przeprowadzono test Shapiro-Wilka. W zależności od uzyskanego rozkładu danych wykorzystano test t-Studenta dla prób niezależnych dla danych o rozkładzie normalnym oraz test U Manna-Whitneya dla danych nieparametrycznych. W celu określenia istotności różnic w poziomie ekspresji miRs i mRNA we wszystkich badanych grupach przeprowadzono jednokierunkową analizę wariancji (ANOVA) dla danych parametrycznych oraz test Kruskala-Wallisa dla danych nieparametrycznych. Wszystkie uzyskane dane przedstawiono na wykresach jako wartości średnie ± błąd standardowy średniej (SEM). Aby określić zależność statystyczną pomiędzy ekspresją miR, a ekspresją mRNA genów docelowych przeprowadzono analizę korelacji przy użyciu nieparametrycznego testu rang Spearmana. Dodatkowo obliczono współczynnik korelacji rang Spearmana (R), aby określić siłę oraz kierunek korelacji . Wszystkie wyniki były uznawane za istotne statystycznie, gdy wartość p-value była równa lub mniejsza niż 5% ($p \leq 0,05$) [publikacja P-2, P-3].

### 3.2.9. Deklaracja etyczna

Eksperyment z udziałem zwierząt przeprowadzono w Uniwersyteckiej Zwierzętarni Doświadczalnej Pomorskiego Uniwersytetu Medycznego (PUM) w Szczecinie. Na przeprowadzenie eksperymentu dr hab. Beata Hukowska-Szematowicz uzyskała zgodę Lokalnej Komisji Etycznej ds. Doświadczeń na Zwierzętach w Poznaniu (nr 51/2022) [publikacja P-2, P-3].

## 4. Omówienie publikacji naukowych wchodzących w skład rozprawy doktorskiej

### 4.1. Sygnatura mikroRNA gospodarza w infekcjach wirusowych u ludzi i zwierząt

(na podstawie publikacji [P-1]: Ostrycharz E., Hukowska-Szematowicz B. (2022). Micro-players of great significance-host microRNA signature in viral infections in humans and animals. *International Journal of Molecular Science* 23 (18), 10536. DOI: 10.3390/ijms231810536)

Publikacja pt.: „Micro-players of great significance—host microRNA signature in viral infections in humans and animals" jest pracą przeglądową przedstawiającą aktualny stan wiedzy na temat kluczowych mikroRNA zaangażowanych w infekcje wirusowe u ludzi i zwierząt. W pracy skupiono się na miR ludzi i zwierząt aby wyróżnić cząsteczki wspólne dla obu grup, a także aby zidentyfikować miR, które nie były do tej pory przedmiotem badań w infekcjach wirusowych u zwierząt.

W pierwszym rozdziale pracy opisano cząsteczki miR, zaprezentowano ich biogenezę oraz rolę w infekcjach wirusowych u ludzi i zwierząt. MiR to małe, niekodujące cząsteczki RNA, zazwyczaj o długości 17–25 nukleotydów, które odgrywają kluczową rolę w regulacji ekspresji genów. Nieprawidłowa ekspresja miR została powiązana z różnymi chorobami co podkreśla ich znaczenie w zdrowiu i chorobie. Biogeneza miR zachodzi poprzez dwuetapowy proces inicjowany w jądrze gdzie wytwarzane jest pri-miRNA. Pri-miRNA ulega rozszczepieniu do pre-miRNA, który jest następnie eksportowany do cytoplazmy i dalej przetwarzany przez kompleks Dicer w dojrzałe miR. Dojrzałe miR wraz z kompleksem RISC pośredniczy w wyciszaniu genów poprzez degradację mRNA lub zahamowanie translacji [94]. W kontekście infekcji wirusowych Barbu i wsp. [192] podzielili miR na miR gospodarza i miR wirusowe. Znaczenie miR w patogenezie infekcji wirusowych podkreśla ich potencjał jako celów terapeutycznych i biomarkerów w leczeniu chorób wirusowych. Zrozumienie biologicznej roli miR zaangażowanych w różne infekcje wirusowe może pomóc w zrozumieniu patogenezy chorób wirusowych, a także ułatwić w przyszłości rozwój środków terapeutycznych dla ludzi i zwierząt [192].

W drugim rozdziale opisano sygnaturę miR w infekcjach wirusowych u ludzi. Na podstawie przeglądu literatury wyróżniono 12 kluczowych miR (miR-155, miR-223, miR-146a, miR-122, miR-125b, miR-132, miR-34a, miR-21, miR-16, rodzina miR-181, rodzina let-7 i miR-10a) zaangażowanych w procesy biologiczne zachodzące w infekcjach wirusowych u ludzi takimi wirusami jak – wirusy zapalenia wątroby, krwotoczne, oddechowe, niedoboru

odporności ludzkiej, neurotropowe, opryszczki ludzkiej, limfotropowe, kardiotropowe oraz wirusy przenoszone przez wektory.

MiR-155 to wielofunkcyjna cząsteczka, która ulega ekspresji w komórkach odpornościowych i jest zaangażowana w modulację odpowiedzi immunologicznych i zapalnych podczas infekcji wirusowych [135]. Ekspresja miR-155 szybko wzrasta w odpowiedzi na infekcję i uraz. Cząsteczka ta jest wysoce wrażliwa na szereg bodźców zapalnych, takich jak TNF-α i IL-1β, a także wzorce molekularne związane z patogenami (PAMP, ang. pathogen-associated molecular patterns) i wzorce molekularne związane z uszkodzeniami (DAMP, ang. damage associated molecular patterns) [135]. Należy jednak zauważyć, że niektóre wirusy mogą hamować ekspresję miR-155, wpływając tym samym na odpowiedź immunologiczną gospodarza. Ekspresja miR-155 jest obniżona w zdrowych hepatocytach, ale może być zwiększona w różnych stanach patologicznych wątroby. Biorąc pod uwagę jego krytyczną rolę w regulacji odpowiedzi immunologicznych i zapalnych, miR-155 jest szczególnie ważny w zakażeniach wywołanych przez wirusy hepatotropowe, w tym HBV i HCV [193]. Badania wykazały, że miR-155 zwiększa ekspresję indukowanych interferonem genów przeciwwirusowych i promuje odporność przeciwwirusową poprzez ukierunkowanie na supresor sygnalizacji cytokin 1 (SOCS1, ang. Suppressor Of Cytokine Signaling 1), wzmacniając w ten sposób szlak sygnałowy kinazy JAK/STAT [194]. Co ciekawe, gdy poziomy miR-155 są obniżone w komórkach jednojądrzastych krwi obwodowej (PBMC, ang. peripheral blood mononuclear cell) od pacjentów zakażonych HBV, osoby z przewlekłym zapaleniem wątroby typu B (CHB, ang. chronić hepatitis B) i podwyższonym poziomem ALT wykazują wyższe poziomy miR-155, co sugeruje korelację z aktywacją odpowiedzi immunologicznej [195]. Stwierdzono, że stymulacja TLR7 indukuje syntezę miR-155 poprzez szlak NF-κβ podczas zakażenia HBV [196]. Ponadto wykazano, że antygen HBV (HBeAg, ang. hepatitis B virus antigen) zwiększa ekspresję miR-155 w makrofagach poprzez szlaki sygnałowe fosfatydyloinozytolu 3-kinazy (PI3K, ang. phosphoinositide 3-kinase) i NF-κβ, promując produkcję cytokin zapalnych poprzez hamowanie ekspresji genu chłoniaka komórkowego 6 (Bcl-6, ang. B-cell Lymphoma 6), genu inozytolu polifosforanu-5-fosfazaty 1 (SHIP-1, ang. Inositol Polyphosphate 5-phosphatase 1) i *SOCS-1* [197]. W kontekście HCV, ekspresja miR-155 jest podwyższona w surowicy, monocytach i tkance wątroby osób zakażonych. Zwiększenie ekspresji miR-155 w odpowiedzi na zakażenie HCV jest związane ze zwiększoną produkcją TNF-α i może odgrywać rolę w regulacji odpowiedzi immunologicznej w komórkach NK (ang. natural killer) [198]. Wykazano, że transfekcja miR-155 do komórek NK moduluje ekspresję genu T-box w komórkach T (T-bet, ang. T-box

Expression in T-cells) i genu domeny immunoglobuliny limfocytów T i mucyny 3 (TIM-3, ang. T-cell Immunoglobulin and domain Mucin-3) , co prowadzi do zwiększonej produkcji IFN-γ i może pomóc zachować równowagę immunologiczną podczas zakażenia [199]. Co godne uwagi, niższe poziomy miR-155 w komórkach NK od osób przewlekle zakażonych korelują z dysfunkcją immunologiczną. W nieparenchymalnych komórkach wątroby (NPC, ang. non-parenchymal liver cells) ekspresja miR-155 może być indukowana przez ligandy TLR3, podczas gdy czynniki takie jak TGF-β i IL-10 mogą hamować odpowiedź przeciwwirusową zależną od TLR3 poprzez zmniejszenie ekspresji NF- κβ i czynnika regulacyjnego interferonu 3 (IRF3, ang. interferon regulatory factor 3) [200]. MiR-155 odgrywa znaczącą rolę w zakażeniu DENV i pełni on główną funkcję w regulacji szlaków sygnałowych indukowanych przez TLR/NF- κβ w makrofagach [201]. Badacze wykazali, że nadekspresja miR-155 może ograniczać replikację wirusa. Mechanizm ten obejmuje ukierunkowanie na gen Bach1, który następnie indukuje HO-1 i wzmacnia reakcję interferonu [116]. Podobnie, zakażenie wirusem Zachodniego Nilu (WNV, ang. West Nile virus) prowadzi do zwiększenia ekspresji miR-155 w mózgu, co jest związane z reakcjami neurozapalnymi i może modulować odpowiedź cytokin [202]. MiR-155 może działać jako pozytywny regulator prozapalny w komórkach zakażonych wirusem grypy H1N1. Wzrost ekspresji miR-155, wpływa na obniżenie poziomu receptora 1 sfingozyno-1-fosforanu (S1PR1, ang. sphingosine-1-phosphate receptor 1), co prowadzi do aktywacji NF-κβ i zwiększonej produkcji cytokin prozapalnych [186]. Podobnie jak w przypadku grypy, zakażenie syncytialnym wirusem oddechowym (RSV, ang. respiratory syncytial virus) prowadzi do zwiększonej ekspresji miR-155. Wysokie poziomy miR-155 w drogach oddechowych podczas zakażenia RSV korelują ze zwiększoną odpornością przeciwwirusową i zwiększoną produkcją IFN-γ, a także korzystnym profilem cytokin Th1 [203]. Aktywacja NF-κβ po rozpoznaniu antygenu RSV jest kluczowym etapem odpowiedzi immunologicznej i zapalnej, która prowadzi do zwiększonej ekspresji miR-155 [204]. W zakażeniu ludzkim adenowirusem miR-155 może wzmacniać odpowiedzi IFN-I poprzez hamowanie *SOCS1*, promując w ten sposób ekspresję genów stymulowanych interferonem (ISG, ang. interferon-stimulated gene) [205].

MiR-223 jest kluczowym regulatorem rozwoju i homeostazy układu odpornościowego. Ekspresja miR-223 jest wyraźnie zmieniona podczas reakcji zapalnych w wielu typach komórek. Zmiany w ekspresji miR-223 mogą modulować stan zapalny tkanek, wpływając w ten sposób na odpowiedź immunologiczną podczas infekcji wirusowych [187]. Podczas wirusowego zapalenia wątroby miR-223 ulega znacząco zwiększonej ekspresji w wątrobie i jest związany z patologią WZW. Podwyższone poziomy miR-223 obserwowano w surowicy

pacjentów z HCC i CHB. Jednak obniżone poziomy miR-223 odnotowano w tkance wątroby u pacjentów z HCC, co sugeruje, że uszkodzenie hepatocytów spowodowane stanem zapalnym lub rakiem może prowadzić do uwolnienia miR-223 do krwiobiegu [206]. Z drugiej strony, niektóre badania wykazały niższe poziomy miR-223 w surowicy u pacjentów z dodatnim HBV w porównaniu ze zdrowymi kontrolami [207]. Podczas zakażenia HCV poziomy miR-223 w surowicy są również obniżone, a niższe poziomy w biopsjach wątroby od pacjentów z przewlekłym WZW typu C mogą przyczyniać się do przewlekłego stanu zapalnego i powikłań poprzez ukierunkowanie na szlak NF-κβ [208]. Co godne uwagi, wysokie poziomy miR-223 wykryto u pacjentów osiągających trwałą odpowiedź przeciwwirusową po leczeniu, co wskazuje na jego potencjał jako nieinwazyjnego biomarkera skuteczności terapeutycznej i patologii chorób wątroby [207]. Podobnie jak podczas WZW, w zakażeniu DENV poziom miR-223 został obniżony w zakażonych komórkach. Regulacja miR-223 przez czynniki transkrypcyjne białka wiążące wzmacniacze CAAT α (C/EBPα, ang. CCAAT-enhancer-binding Proteins α) i E2F1 (ang. E2F Transcription Factor 1) sugeruje pętlę sprzężenia zwrotnego, która może wpływać na bodźce zapalne i podkreśla potencjalną rolę miR-223 jako czynnika przeciwwirusowego przeciwko DENV [209]. Wykazano, ze podczas zakażenia wirusem grypy ma miejsce wzrost ekspresji miR-223 w płucach [210]. Badania *in vitro* wykazały, że wprowadzenie syntetycznego miR-223 do komórek zakażonych wirusem grypy H1N1 zmniejsza wydzielanie cytokin prozapalnych (TNF-α, IL-1β, IL-6, and IL-18) oraz ekspresję inflamasomu NLRP3 [211]. Ponadto miR-223 poprzez regulację *TLR4* i *IKKα* uczestniczy w modulacji procesu zapalnego w przebiegu COVID-19 [147]. W zakażeniu wirusem HIV miR-223 hamuje replikację wirusa, jednak jego działanie może być dwojakie. Z jednej strony, miR-223 może działać jako czynnik negatywny, zmniejszając aktywację szlaku AKT–NF-κβ za pośrednictwem homologu Ras członka rodziny B (RhoB, ang. Ras Homolog Family Member B). Z drugiej strony, może pełnić rolę pozytywną, wpływając na supresorowe białka HIV takie jak Sp3 i LIF [212].

MiR-146a jest krytycznym modulatorem zarówno wrodzonych, jak i adaptacyjnych odpowiedzi immunologicznych. miR-146a jest szybko indukowany w ludzkich monocytach po aktywacji i jest regulowany przez stan zapalny poprzez mechanizm zależny od NF-κβ, ukierunkowany na geny *TRAF6* i *IRAK1* [213]. Podwyższone poziomy miR-146a obserwowano u pacjentów z przewlekłym HBV i ostrą/przewlekłą niewydolnością wątroby. Przewlekłe zakażenie HBV prowadzi do wzrostu ekspresji miR-146a. miR-146a przyczynia się do zmniejszenia cytotoksyczności limfocytów T, a także produkcji cytokin min. IFN-γ, IL-2 i TNF-α. Badania *in vitro* wykazały, że blokowanie miR-146a zwiększa aktywność komórek T

specyficznych dla wirusa [214]. Białko HBx wirusa HBV promuje ekspresję miR-146a poprzez sygnalizację NF-κβ, która z kolei obniża poziom czynnika H dopełniacza (CFH, ang. Complement Factor H), negatywnego regulatora szlaku dopełniacza, potencjalnie zaostrzając stan zapalny wątroby [215]. W zakażeniu HCV, białko rdzeniowe wirusa może zwiększać ekspresję miR-146a poprzez szlak TLR2-MyD88. Jednak stymulacja TLR może prowadzić do zmniejszenia poziomów miR-146a w monocytach zakażonych HCV. Wykazano, że hamowanie miR-146a w tych komórkach zmniejsza ekspresję *IL-23, IL-10* i *TGF-β* poprzez regulację szlaku SOCS1/STAT3, co wskazuje na jego rolę w promowaniu produkcji cytokin, co może przyczyniać się do uszkodzenia układu odpornościowego wątroby [216,217]. Badania wykazały, że ekspresja miR-146a jest znacząco zwiększona w ludzkich monocytach pierwotnych i komórkach linii monocytowych białaczki ludzkiej (THP-1, ang. human monocytic cell line) po zakażeniu DENV-2. Nadmierna ekspresja miR-146a hamuje produkcję IFN-β i IL-28A/B, przyczyniając się do zwiększonej replikacji DENV [141]. Poprzez ukierunkowanie na gen *TRAF6*, miR-146a negatywnie reguluje również procesy autofagii [218]. W zakażeniu wirusem grypy nadmierna ekspresja miR-146a w komórkach A549 zmniejsza odpowiedź IFN-I, promując w ten sposób replikację wirusa [219]. Hamowanie miR-146a *in vivo* wiązało się ze zmniejszeniem uszkodzenia płuc i poprawą wskaźników przeżycia podczas zakażenia. Ponadto miR-146a może negatywnie regulować *IRAK1* i wpływać na szlaki sygnałowe (m.in. NF-κβ) zaangażowane w odpowiedzi immunologiczne i zapalne [220]. Natomiast podczas zakażenia SARS-CoV-2 poziomy miR-146a są obniżone. miR-146a jest ukierunkowany na regulację *TRAF6, IRAK1* i *IRAK2*, które są zaangażowane w prozapalny szlak sygnałowy NF-κβ. Dlatego niedobór miR-146a u pacjentów z COVID-19 może prowadzić do nadmiernej aktywacji odpowiedzi immunologicznej i burzy cytokinowej [221].

MiR-122 to specyficzny dla wątroby miR, który odgrywa kluczową rolę w biologii wątroby [222]. Zmiany w ekspresji miR-122 są związane z różnymi chorobami wątroby, w tym WZW typu B i C. U pacjentów zakażonych HBV ekspresja miR-122 jest znacząco zmniejszona w wątrobie porównaniu z osobami zdrowymi. Badania wykazały, że nadekspresja miR-122 hamuje replikację HBV [223]. Hamowanie *HO-I* przez miR-122 negatywnie wpływała na zmniejszenie replikacji wirusa HBV, podczas gdy hamowanie miR-122 zwiększa ekspresję *HO-I* poprzez zmniejszenie poziomu *Bach1* i znacząco hamuje replikację HCV [123,224].

MiR-125b jest kluczowym regulatorem zaangażowanym w różne szlaki sygnałowe, w tym NF-κβ, białka p53 (p53, ang. protein p53), PI3K/Akt/mTOR, receptor Erb-B2 kinazy tyrozynowej (ErbB2, ang. Erb-B2 Receptor Tyrosine Kinase 2) i Wnt, które łącznie wpływają na proliferację, różnicowanie, metabolizm i apoptozę komórek [191]. miR-125b odgrywa

znaczącą rolę w modulacji odpowiedzi zapalnej poprzez ukierunkowanie na prozapalne cytokiny, takie jak TNF-α i jest szybko indukowany w ludzkich makrofagach [225]. Nadmierna ekspresja miR-125b-5p została powiązana ze zwiększoną replikacją HBV w komórkach wątrobiaka, a dowody sugerują, że stymuluje on replikację HBV poprzez oś LIN28B/let-7 bez zmiany transkrypcji HBV [226]. W zakażeniu HCV ekspresja miR-125b jest zwiększona w odpowiedzi na białko rdzeniowe wirusa, a jej poziom koreluje ze wzmożoną produkcją cytokin poprzez szlak sygnalizacyjny TLR2/MyD88 w monocytach [138]. miR-125b jest powiązany z promowaniem replikacji HCV poprzez ukierunkowanie szlaków sygnałowych, w tym szlaku IL-6/STAT3 i może służyć jako biomarker postępu choroby [227]. MiR-125b bierze również udział w infekcjach wirusowych układu oddechowego. W przypadku zakażenia wirusem RSV jego ekspresja jest zmniejszona u niemowląt, podczas gdy ciężkie przypadki zapalenia płuc związanego z RSV wykazują zwiększone poziomy miR-125b. Sugeruje to złożoną rolę w modulacji odpowiedzi immunologicznej podczas zakażenia RSV [228]. W przypadku zakażeń wirusem grypy zmniejszenie ekspresji miR-125b wiązało się ze zwiększoną replikacją wirusa i aktywacją szlaków sygnałowych NF-κβ i szlak aktywowany mitogenem (MAPK, ang. mitogen activated protein kinases), które regulują odpowiedzi immunologiczne [229]. Natomiast zwiększona ekspresja miR-125b wpływa na hamowanie replikacji HIV w limfocytach T [230]. W przypadku wirusa JEV miR-125b-5p jest regulowany w górę podczas infekcji i wykazano, że ogranicza replikację wirusa, celując w kluczowe cząsteczki sygnałowe. Sugeruje to, że miR-125b może pełnić rolę regulatora, który równoważy replikację wirusa z odpowiedzią przeciwwirusową gospodarza [231].

MiR-132 jest kluczowym regulatorem różnych procesów komórkowych w różnych typach tkanek, w szczególności w modulacji stresu oksydacyjnego [127]. Podwyższony poziom ekspresji miR-132 może hamować ekspresję niektórych białek wirusowych prowadząc tym samym do zmniejszenia poziomu białek przeciwwirusowych [232]. Nadekspresja miR-132 w zakażeniu wirusem grypy hamuje produkcję interferonów typu I (IFN-α i IFN-β) oraz ekspresję ISG [233]. Dodatkowo badania na liniach komórkowych dowiodły, że po zakażeniu wirusem grypy dochodzi do zwiększenia ekspresji miR-132, który może regulować wrodzone szlaki sygnałowe układu odpornościowego poprzez ukierunkowanie na MAPK3 [234]. Zwiększoną ekspresję miR-132 obserwuje się również podczas infekcji wirusem opryszczki pospolitej [235].

MiR-34a ulega ekspresji w komórkach układu odpornościowego. Poprzez regulację *Bcl-2, SITR1*, kinazy zależnej od cyklin (CDK) 4 i cykliny D1, między innymi, indukuje apoptozę i odgrywa znaczącą rolę w zakażeniach wirusowych [178]. Zakażenie wirusem HBV zmniejsza

ekspresję miR-34a [236]. Natomiast podczas zakażenia wirusami DENV i WNV miR-34a działa jako silny aktywator odpowiedzi IFN-I poprzez zmniejszenie ekspresji genów szlaku Wnt/β-kateniny (ang. Wnt/β-catenin pathway), wzmacniając w ten sposób odpowiedź przeciwwirusową [237]. Podczas infekcji wirusami układu oddechowego, takimi jak wirus grypy i SARS-CoV-2, poziom miR-34a ulega obniżeniu. Natomiast inne obserwacje wskazują, że w przebiegu zakażenia wirusem grypy, jak i SARS-CoV-2 badania *in vitro* i analizy in silico wykazały, że nadekspresja miR-34a, poprzez hamowanie genu *Bax*, może hamować apoptozę indukowaną wirusem [238,239]. Ponadto podczas zakażenia SARS-CoV-2 miR-34a może zmniejszać stan zapalny za pośrednictwem receptora IL-6 [239]. Badania wykazały, że transfekcja syntetycznym miR-34a komórek zakażonych wirusem grypy przyczynia się do zwiększonej ekspresji *STAT3*, co odgrywa kluczową rolę w odpowiedzi przeciwzapalnej [240]. Badania wykazały, że linie komórkowe zakażone ludzkim wirusem HTLV-1 wyrażają wyższe poziomy miR-34a w porównaniu z komórkami niezainfekowanymi. miR-34a zawiera miejsca wiążące dla NF-κβ i p53, a aktywacja p53 w zakażonych komórkach dodatkowo zwiększa poziomy miR-34a. Wzrost ten przyczyniał się do zmniejszonej ekspresji *SIRT1* i proapoptotycznego czynnika *Bax*, co wskazuje na rolę miR-34a w dostrajaniu ekspresji genów w komórkach zakażonych HTLV-1 [110].

miR-21 odgrywa znaczącą rolę w różnych funkcjach biologicznych, ale jego działanie regulacyjne jest szczególnie zauważalne w kontekście przeżycia komórek i apoptozy [241]. Zmniejszenie ekspresji miR-21 wiąże się ze zwiększoną śmiercią komórek, prawdopodobnie z powodu ukierunkowania jej na kluczowe białka regulacyjne, takie jak czynnik indukowany niedotlenieniem 1-alfa (HIF-1α, ang. Hypoxia-inducible Factor 1), PTEN oraz PDCD4. Z drugiej strony, zwiększenie ekspresji miR-21 podczas infekcji wirusowych, często wywoływane przez cytokiny, sugeruje jego udział w reakcjach zapalnych i potencjalny wkład w dysfunkcję układu odpornościowego [242]. Badania wskazują, że podczas przewlekłego WZW typu B miR-21 aktywuje komórki gwiaździste wątroby (HSC, ang. hepatic stellate cells) poprzez szlak sygnałowy PTEN/Akt oraz promuje ekspresję α-aktyny mięśni gładkich (α-SMA, ang. Alpha Smooth Muscle Actin) i kolagenu I w HSC poprzez szlak sygnałowy Smad7 [172,243]. Wykazano również, że HBx zmniejsza ekspresję *PDCD4* poprzez zwiększenie ekspresji miR-21, co prowadzi do zwiększonej proliferacji komórek i zmniejszonej ekspresji białek proapoptotycznych [107]. Dodatkowo ustalono, że w przebiegu infekcji HCV miR-21 hamuje produkcji IFN-I [244]. W przypadku DENV ustalono, że poziomy miR-21 korelują dodatnio z aktywnością AST i ALT w surowicy, podczas gdy korelują ujemnie z liczbą białych krwinek i płytek krwi [245]. W przypadku zakażeń wirusem grypy typu A miR-21 jest

regulowany w dół, co ma wpływ na replikację wirusa i odpowiedź immunologiczną. Jednak inni badacze zaobserwowali wzrost miR-21 w późnych fazach zakażenia i stwierdzono, że miR-21 może przyśpieszać replikację H5N1 poprzez hamowanie odpowiedzi IFN-I [246]. Natomiast w zakażeniu adenowirusem wykazano zmniejszoną ekspresji miR-21, a u pacjentów zakażonych SARS-CoV-2 wykazano zwiększone poziomy ekspresji miR-21 [247,248]. W infekcji HIV wykazano, że ekspresja miR-21 w komórkach Jurkat jest powiązana z obniżeniem ekspresji białek związanych z apoptozą [249]. W komórkach HeLa zakażonych wirusem Coxsackie B3 ekspresja miR-21 była znacząco zwiększona, co korelowało ze zmniejszonym uwalnianiem wirusa i apoptozą miocytów [250]. W przypadku wirusa Chandipura ustalono, że nadmierna ekspresja miR-21 w ludzkich komórkach mikroglejowych prowadzi do obniżenia ekspresji *PTEN* i aktywacji transkrypcji cytokin prozapalnych [251].

MiR-16 odgrywa kluczową rolę w modulacji cyklu komórkowego, hamowaniu proliferacji komórek i promowaniu apoptozy [252]. Jego działanie jest przede wszystkim pośredniczone przez ukierunkowanie kilku kluczowych genów, w tym antyapoptotycznego genu *Bcl-2* [253]. W badaniach na linii komórkowej HepG2 ustalono, że białko HBx zwiększało ekspresję miR-16, który hamował proliferację, klonogenność i wzrost komórek poprzez indukowanie zatrzymania cyklu komórkowego poprzez cyklinę D1 (CCND1, ang. Cyclin D1) oraz indukował apoptozę poprzez ukierunkowanie na Bcl-2 [175]. Natomiast w zakażeniu HCV stwierdzono, że miR-16 ulegał zwiększonej ekspresji i negatywnie korelował z ekspresją czynnika wzrostu hepatocytów (HGF, ang. Hepatocyte Growth Factor) i Smad7 [254]. W badaniach nad wirusem grypy ustalono, że poziom miR-16 wzrasta na skutek infekcji i hamuje IFN-β, co wskazuje na jego potencjalną rolę w modulacji odpowiedzi przeciwwirusowej [234]. Natomiast u dzieci z zakażeniem RSV ekspresja miR-16 znacząco wzrosła, co potencjalnie wpływa na szlak NF-κβ, aktywowany podczas odpowiedzi immunologicznej na RSV [204].

Rodzina miR-181 pełni różnorodne role w zakresie regulacji kluczowych aspektów wzrostu, rozwoju i aktywacji komórek. W zakażeniu HBV wykazano, że miR-181a jest regulowany w górę co prowadzi do zmniejszenia ekspresji *PTEN* [255]. Ponadto miR-181a hamuje ekspresję *Fas*, kluczowego regulatora apoptozy, promując w ten sposób wzrost raka wątrobowokomórkowego [256]. W przypadku zakażenia wirusem HCV poziomy miR-181a są podwyższone w surowicy, ale obniżone w tkankach wątroby, a jego ekspresja jest odwrotnie skorelowana z wiremią i poziomami enzymów wątrobowych [257]. Ponadto stwierdzono, że spadek poziomu miR-181a wraz z nadekspresją fosfatazy 6 o podwójnej specyficzności (DUSP6, ang. Dual Specificity Phosphatase 6) w limfocytach T (CD4+) upośledza odpowiedzi

immunologiczne [258]. MiR-181c znacząco hamowało ekspresję fosfo-CDK2 i cykliny-A, zatrzymując postęp cyklu komórkowego, a jednocześnie promując apoptozę hepatocytów zakażonych HCV [259]. Rodzina miR-181 odgrywa również rolę w odpowiedzi na zakażenie wirusami układu oddechowego. Na przykład, miR-181c ulega zwiększonej ekspresji w komórkach A549 zakażonych różnymi podtypami wirusa grypy A (H5N1, H3N2, H1N1), hamując geny zaangażowane w obronę immunologiczną, takie jak IL-2 i TNF-α [229]. Natomiast ekspresja miR-181a jest zmniejszana w zakażeniu wirusem grypy [260]. Natomiast przeciwnie do zakażenia wirusem grypy, podczas zakażenia wirusem RSV poziomy miR-181a są podwyższone, co sugeruje jego udział w odpowiedzi immunologicznej przeciw RSV [261].

Rodzina miR let-7 odgrywa kluczową rolę w różnych procesach biologicznych, w tym regulacji cyklu komórkowego, proliferacji komórkowej i apoptozie [262]. Ponadto członkowie let-7 są zaangażowani w potranskrypcyjną regulację wrodzonych odpowiedzi immunologicznych na infekcje wirusowe [263]. Wykazano, że białko Hbx HBV obniża poziom let-7, promując w ten sposób proliferację komórkową i przyczyniając się do rozwoju HCC. Ponadto wskazano, że let-7a negatywnie reguluje proliferację komórkową, częściowo poprzez ukierunkowanie na *STAT3*, który bierze udział w wielu procesach komórkowych [264]. Ustalono również, że zmniejszenie ekspresji let-7a prowadzi do zmniejszenia replikacji HBV [265]. Podczas infekcji HCV, let-7a i let-7b, podobnie jak w HBV, wykazują zmniejszona ekspresję w tkance [266]. W zakażeniu wirusem grypy H7N9 let-7e hamuje ekspresję *IL-6* [267]. Po zakażeniu RSV let-7f ulega zwiększonej ekspresji i prawdopodobnie reguluje takie geny jak geny ligant chemokiny C-C motyw 7 (CCL7, ang. C-C Motif Chemokine Ligand 7) i *SOCS3*, które są zaangażowane w odpowiedź przeciwwirusową [268]. Co ciekawe, ekspresja let-7b jest podwyższona w PBMC podczas COVID-19 i tłumi sygnalizację szlaku TLR4/NF-κβ oraz zmniejsza wydzielanie cytokin prozapalnych, co sugeruje jego rolę w modulacji odpowiedzi immunologicznej i zmniejszaniu stanu zapalnego [269].

MiR-10a może być powiązany ze zmianami homeostazy immunologicznej i jest regulowany w dół przez wiele czynników, takich jak TNF-α, IL-1β i IL-6. Zmniejszony poziom miR-10a może przyczynić się do aktywacji NF-κβ [270]. W kontekście wirusów zapalenia wątroby stwierdzono podwyższone poziomy miR-10a w surowicy pacjentów z CHB, co sugeruje jego potencjał jako biomarkera diagnostycznego, zwłaszcza w przypadkach utrzymania prawidłowych wartości ALT [271]. Podczas zakażenia HCV miR-10a jest znacząco regulowany w górę i bierze udział w regulacji różnych genów metabolizmu wątroby. Wykazano, że nadmierna ekspresja miR-10a w hepatocytach hamuje syntezę lipidów i geny związane z glukoneogenezą, przyczyniając się do uszkodzenia wątroby i postępu włóknienia

[272]. W przypadku wirusowych infekcji układu oddechowego, w szczególności wirusem RSV, obserwowano zwiększone poziomy ekspresji miR-10a. Badania wskazują, że miR-10a-3p jest podwyższony u dzieci z zapaleniem płuc związanym z RSV, przy czym wyższe poziomy korelują z ciężkością choroby. Analiza ontologii genów (GO) wykazała, że geny docelowe miR-10a są zaangażowane w krytyczne szlaki odpowiedzi immunologicznej, w tym szlaki sygnałowe NF-κβ i MAPK [228].

W trzecim rozdziale opisano sygnaturę miR w infekcjach wirusowych u zwierząt. Na podstawie przeglądu literatury opisano 10 kluczowych miR-miR-155, miR-223, miR-146a, miR-145, miR-21, grupa miR-15a/miR-16, rodzina miR-181, rodzina let-7, miR-122) zaangażowanych w procesy biologiczne zachodzące w przebiegu infekcji wirusowych u zwierząt takimi wirusami jak wirusy krwotoczne, oddechowe, limfotropowe, neurotropowe, przenoszone przez wektory, wirus pryszczycy oraz wirus zespołu rozrodczo-oddechowego świń. Wśród miR opisanych w infekcjach wirusowych u zwierząt 8 było wspólnych z miR u ludzi.

MiR-155. U królików zakażonych *L. europaeus* zaobsewowano, że poziom ekspresji ocu-miR-155-5p w wątrobie jest prawie sześciokrotnie wyższy niż w grupie kontrolnej, co sugeruje związek pomiędzy zakażeniem, a odpowiedzią zapalną w wątrobie [167]. Zwiększoną ekspresje miR-155 wykazano również u kurcząt w przebiegu zakażenia ptasią grypą. Jego zwiększona ekspresja może odgrywać rolę w interakcji gospodarz-wirus oraz aktywować szlak kinazy c-Jun N-końcowej (JNK, ang. c-Jun N-terminal kinases) prowadząc do apoptozy zakażonych komórek [273]. Natomiast podczas zakażenia wirusem choroby Mareka (MDV, ang. Marek's disease virus) poziom ekspresji miR-155 jest obniżony w guzach śledziony oraz w limfocytach T [274].

miR-223. U świń, podczas zakażenia wirusem grypy A/H1N2, wykazano, że poziom miR-223 wzrasta w pierwszym dniu po infekcji, po czym wraca do poziomu wyjściowego [275]. Natomiast podczas infekcji MDV, zarówno w splenocytach, jak i limfocytach T obserwowano obniżenie poziomu miR-223. Z kolei w zakażeniu wirusem pęcherzykowego zapalenia jamy ustnej (VSV, ang. vesicular stomatitis virus) wykazano wzrost ekspresji miR-223 w makrofagach myszy, co skutkowało wzrostem poziomu IFN-I poprzez regulację czynnika transkrypcyjnego *FOXO3* [276].

miR-146a. Wykazano, że podczas zakażenia wirusem grypy H9N2 występuje zwiększona ekspresja miR-146a w płucach u drobiu [273]. Natomiast w tkance płuc świń zakażonych wirusem grypy H1N2 wykazano spadek ekspresji miR-146a, co mogło wpływać na zwiększoną ekspresję krytycznych mediatorów odpornościowych (*IRAK1, STAT1, TLR4*) [275]. Z kolei

zakażenie wirusem Hendra (HeV), (wywołującym ciężkie choroby układu oddechowego i neurologicznego u koni), wywołuje wzrost ekspresji miR-146a poprzez regulacje genu *RIG-1* [277]. Zakażenie wirusem VSV indukowało ekspresję miR-146a, zależną od szlaku sygnałowego RIG-I/NF-κβ. Ponadto ustalono, że miR-146a negatywne reguluje wytwarzanie IFN-I i reguluje odpowiedź immunologiczną poprzez oddziaływanie z genami *IRAK1* i *IRAK2* [278]. W zakażeniu wirusem pryszczycy (FMDV, ang. foot and mouth disease virus) wykazano zwiększoną ekspresję miR-146a, która hamuje *IRAK1* i *TRAF6* tym samym tłumiąc odpowiedzi prozapalne indukowane przez sygnalizację TLR [279]. Podczas zakażenia wirusem zespołu rozrodczo-odddechowego świń (PRRSV, ang. porcine reproductive and respiratory syndrome) miR-146a reguluje geny – białko 3 związane z czynnikiem martwicy nowotworu i C1q (C1QTNF3, ang. C1q And TNF Related 3) oraz czynnik transkrypcyjny MafB (MAFB, ang. Transcription Factor MafB), co może wzmacniać odpowiedź immunologiczną skierowaną przeciwko wirusowi [280].

MiR-145. Dotychczas nie było szeroko badane u ludzi, a jego rola w odpowiedzi immunologicznej pozostaje w dużej mierze nieznana. Podczas infekcji wirusem grypy A/H1N1 stwierdzono spadek poziomu miR-145. Hipoteza zakłada, że miR-145 jest ukierunkowany na gen hemaglutyniny (HA, ang. hemagglutinin) i może przyczyniać się do immunosupresji [281]. Podobnie jak w infekcji wirusem grypy, podczas zakażenia wirusem wścieklizny ustalono zmniejszoną ekspresję miR-145. Za pomocą analiz in silico wykazano, że miR-145 może regulować szlak sygnałowy Jak/STAT oraz wytwarzanie cytokin [282].

MiR-21. W infekcjach wirusowych u zwierząt wykazano, że miR-21 jest podwyższone u świń i kurcząt podczas infekcji wirusem świńskiej grypy H1N2. Ponadto stwierdzono, że miR-21 odgrywa kluczową rolę w rozwoju i modulacji limfocytów, a także może regulować ekspresję chemokiny CXCL10 [275]. Natomiast w zakażeniu wirusem wścieklizny (RABV, ang. rabies virus) ekspresja miR-21 zmienia się w czasie, osiągając najwyższy poziom w 144 h od zakażenia, co może świadczyć o zaangażowaniu tego miR w dysfunkcję neuronalną związaną z zakażeniem tym wirusem [283].

miR-16/miR-15a. Podczas zakażenia *L. europaeus* zaobserwowano znaczący wzrost ekspresji ocu-miR-16b w wątrobie zakażonych królików. Ustalono, że miR-16b w przebiegu RHD promuje apoptozę komórek poprzez celowanie w *Bcl-2* oraz reguluje procesy regeneracji wątroby poprzez regulację *HGF* [167]. W kontekście zakażenia wirusem grypy A/H1N2 zaobserwowano znaczący wzrost poziomu miR-15a w tkance płuc zakażonych świń, wraz z równoczesnym zwiększeniem ekspresji jego genów docelowych [275].

Rodzina miR-181. Podczas zakażenia wirusem grypy H9N2 wykazano obniżone poziomu miR-181a oraz miR-181b [284]. W guzach śledziony kurcząt zakażonych MDV zaobserwowano również obniżone poziomy miR-181a i miR-181b, co może odgrywać kluczową rolę w unikaniu odpowiedzi immunologicznej przez wirusa [285]. Natomiast obniżony poziom miR-181b w infekcji FMDV może regulować proliferację komórkową poprzez ukierunkowanie na RASSF1A i NF-κβ oraz może wywierać działanie immunomodulacyjne poprzez cyklazę adenylową 9 (AC9, ang. adenylate cyclase 9) i hamowanie ekspresji IFNα [279].

Rodzina let-7. Infekcja MDV wpływa na obniżenie ekspresji let-7i. Analizy bioinformatyczne wskazują, że let-7i może oddziaływać z mRNA aktywującego czynnika transkrypcyjnego 2 (ATF2, ang. Activating Transcription Factor 2), regulatora proliferacji i apoptozy komórek [286]. Wykazano również, że ekspresja let-7i oraz let-7b ulega obniżeniu podczas zakażenia wirusem białaczki ptaków (ALV, ang. avian leukosis virus) [287]. Natomiast w zakażeniu FMDV obniżeniu ulega let-7g, który może brać udział w proliferacji komórek poprzez regulację receptora utlenionych LDL typu 1 podobnego do lektyny (LOX-1, ang. oxidized low density lipoprotein receptor 1) i kaspazy-3 [274].

miR-122. W badaniach nad ocu-miR-122 ustalono, że podczas infekcji *L.europaeus* jego poziom nie ulega zmianie w tkance wątroby, natomiast wzrasta w surowicy. Dalsze analizy wykazały, że ocu-miR-122-5p może odgrywać rolę w odpowiedzi na zakażenie *L. europaeus* poprzez regulację genów zaangażowanych w homeostazę wątroby i apoptozę [167]. Z kolei w płucach kurcząt zakażonych AIV stwierdzono wzrost ekspresji miR-122. Zasugerowano, że może on regulować ekspresję genów *MX1*, *IL-8*, *IRF-7* i *TNFRS19* i tym samym wpływać na regulację odpowiedzi przeciwzakaźnej [273].

Na podstawie przeglądu literatury ustalono, że sygnatura miR w zakażeniach wirusowych obejmuje 12 miR u ludzi (miR-155, miR-223, miR-146a, miR-122, miR-125b, miR-132, miR-34a, miR-21, miR-16, rodzina miR-181, rodzina let-7, miR-10a) oraz 10 miR u zwierząt (miR-155, miR-223, miR-146a, miR-145, miR-21, miR-15a, miR-16, rodzina miR-181, rodzina let-7, miR-122). Wyróżniono 8 miR wspólnych dla ludzi i zwierząt (miR-155, miR-146a, miR-223, miR-122, miR-21, miR-16, rodzinę miR-181, rodzinę let-7). Ponadto miR-34a, miR-132 oraz miR-125b nie zostały dotychczas zbadane u zwierząt w kontekście zakażeń wirusowych, co uczyniono, a wyniki badań zaprezentowano w publikacjach [publikacja P-2, P-3]. Ustalono, że w zakażeniach wirusowych miR odgrywają kluczową rolę w regulacji procesów biologicznych, takich jak apoptoza, stres oksydacyjny oraz odpowiedź immunologiczna i zapalna. Opisano miR oraz ich potencjalne geny docelowe zaangażowane w procesy

biologiczne w tym w apoptozę, stres oksydacyjny oraz odpowiedź immunologiczną i zapalną w zakażeniach wirusowych u ludzi i zwierząt [Tabela 1 i 2, publikacja P-1]. Nie wszystkie geny docelowe dla miR, zaprezentowano w [Tabeli 1 i 2, publikacja P-1]. Zostało to uzupełnione i podane w [Tabeli 1, publikacja P-2] i [Tabeli 2, publikacja P-3]. Kompleksowy przegląd literatury pozwolił na lepsze zrozumienie roli miR w infekcjach wirusowych zarówno u ludzi, jak i zwierząt. Wiedza na temat miR i ich roli w infekcjach wirusowych stanowi niezwykle przydatne narzędzie do identyfikacji funkcji biologicznych genów i ścieżek, które są aktywowane w celu wywołania skutecznej odpowiedzi immunologicznej, a także innych procesów biologicznych zaangażowanych w odpowiedź przeciwwirusową, takich jak apoptoza i stres oksydacyjny. Określenie sygnatury miR w zakażeniach wirusowych u ludzi i zwierząt, może stanowić podstawę do opracowania modeli badawczych, umożliwiających lepsze poznanie patogenezy chorób wirusowych oraz rozwój przyszłych strategii terapeutycznych dla ludzi i zwierząt.

## 4.2. MikroRNA uczestniczą w regulacji ekspresji genów związanych z apoptozą i stresem oksydacyjnym u królików zakażonych *Lagovirus europaeus* genotypami – GI.1 i GI.2

(na podstawie publikacji [P-2]: Ostrycharz E., Fitzner A., Kęsy A., Siennicka A., Hukowska-Szematowicz B. (2024). MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *Frontiers in Microbiology* 15:1349535. DOI: 10.3389/fmicb.2024.1349535)

Badania wskazują, że miR odgrywają kluczową rolę w regulacji procesów biologicznych w przebiegu infekcji wirusowych. Cząsteczki miR mogą regulować ekspresję genów związanych z apoptozą oraz stresem oksydacyjnym i tym samym wpływać na aktywność szlaków sygnałowych [97]. Apoptoza i równowaga oksydacyjna utrzymują homeostazę komórkową i odgrywają zasadniczą rolę w zakażeniach wirusowych [288,289]. Uszkodzenie tkanek związane z apoptozą i stresem oksydacyjnym stanowi istotny element patogenezy zakażeń wieloma wirusami [112,290-292]. Jak dotąd niewiele wiadomo na temat molekularnych sygnatur interakcji regulacyjnych miedzy specyficznymi miR, a apoptoza i stresem oksydacyjnym w chorobach wirusowych. Jak do tej pory brak badań podejmujących ten problemem naukowy w zakażeniu *L. europaeus.*

W omawianej publikacji pt.: „MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes" podjęto badania mające na celu ocenę ekspresji miR i ich genów docelowych związanych z apoptozą i stresem oksydacyjnym oraz ocenę ich wpływu na ścieżki sygnałowe związane z tymi procesami w zakażeniu *L. europaeus* dwoma genotypami – GI.1 i GI.2 w czterech tkankach (wątrobie, płucach, nerkach, śledzionie).

W celu wyboru miR oraz selekcji i przewidywania docelowych genów dla miR zaangażowanych w apoptozę i stres oksydacyjny posłużono się publikacją [publikacja P-1] oraz metodami in silico z wykorzystaniem baz danych dedykowanych do pracy z miR. W celu zbadania miR i ich genów docelowych zaangażowanych w apoptozę i stres oksydacyjny w zakażeniu *L. europaeus* genotypami – GI.1 i GI.2 w czterech tkankach, wykorzystano metody z zakresu biologii molekularnej w postaci pomiaru ekspresji miR i genów docelowych. Takie samo podejście metodyczne wykorzystano do zbadania biomarkerów apoptozy i stresu oksydacyjnego. Dla apoptozy wybrano: miR-21 (geny docelowe *PTEN* i *PDCD4*), miR-16b (*Bcl-2* i *CXCL10)* oraz miR-34a (*p53* i *SIRT1*). Natomiast dla stresu oksydacyjnego wybrano: miR-132 (*Nrf-2*) i miR-122 (*Bach1*) [Tabela 1, publikacja P-2]. Dodatkowo zbadano na

poziomie mRNA biomarkery apoptozy (*Bax*, stosunek *Bax/Bcl-2*, *PARP* i *kaspazę-3*) i stresu oksydacyjnego (*HO-I*) w wątrobie, płucach, nerkach i śledzionie po zakażeniu *L. europaeus* genotypami -GI.1 i GI.2.

W wyniku przeprowadzonych badań po raz pierwszy, wykazano, że podczas zakażenia *L.europaeus*/GI.1 i GI.2 dochodzi do zmian ekspresji miR-21, miR-16b i miR-34a zaangażowanych w proces apoptozy [Rycina 1-3, publikacja P-2]. Wykazano, że miR-21, zaangażowany w regulację zewnętrznej ścieżki apoptozy [293], ma działanie antyapoptotyczne w zakażeniu *L. europaeus* oboma genotypami. MiR-21 wykazuje działanie hamujące na badane geny docelowe *PTEN* i *PDCD4* w badanych tkankach z wyjątkiem płuc. W płucach nie obserwowano hamującego działania miR-21 na geny docelowe, co może mieć związek z innymi czynnikami indukującymi ekspresję *PTEN* i *PDCD4*, które wymagają dalszych badań. W prezentowanych badaniach po raz pierwszy opisano działanie proapototyczne miR-16b i miR-34a w zakażeniu *L. europaeus*/GI.1 i GI.2. Podwyższony poziom miR-16b wykazano w wątrobie, nerkach i śledzionie po zakażeniu *L. europaeus*/GI.1 i GI.2. Zwiększona ekspresja miR-16b korelowała ze spadkiem poziomu ekspresji antyapoptycznego genu *Bcl-2* w tych tkankach. Tylko w płucach nie obserwowano zmiany ekspresji miR-16b i genu docelowego *Bcl-2*. Ponadto wykazano, że miR-16b wpływa na wzrost chemotraktanta *CXCL10* w wątrobie, płucach i śledzionie, co może skutkować zwiększoną apoptozą i uszkodzeniem tkanek w zakażeniu *L. europaeus*/GI.1 i GI.2. W płucach pomimo braku zmiany ekspresji miR-16b zarejestrowano wzrost *CXCL10* w zakażeniu *L.europaeus*/GI.1 i GI.2. Co warte podkreślenia po zakażeniu *L. europaeus*/GI.2 wzrost ekspresji *CXCL10* był prawie czterokrotnie wyższy w porównaniu z odnotowanym po zakażeniu *L. europaeus*/GI.1. W przeciwieństwie do danych literaturowych, w prezentowanych badaniach nie wykazano hamującego działania miR-16b na *CXCL10* [169]. Możliwe, że podczas zakażenia *L. europaeus* miR-16b wykazuje działanie aktywujące ekspresję genu *CXCL10* poprzez łączenie się z promotorem i zwiększenie transkrypcji. MiR-34a w apoptozie wykazuje mechanizm sprzężenia zwrotnego. MiR-34a hamuje *SIRT1*, zwiększając tym samym ekspresję *p53*, który jest aktywatorem transkrypcyjnym miR-34a [170,294]. W wyniku przeprowadzonych badań po raz pierwszy wykazano, że miR-34a ma działanie proapoptotyczne w wątrobie podczas zakażenia *L. europaeus*/GI.1 i w śledzionie po zakażeniu *L. europaeus* oboma genotypami. Jednak zwiększony poziom miR-34a nie wpływał na poziom ekspresji *SIRT1*, ale wykazywał działanie indukcyjne na gen *p53* w wątrobie i śledzionie. Zwiększenie poziomu ekspresji genu *p53* prowadzi do zwiększenia apoptozy. Dodatkowo wykazano, że w wątrobie i śledzionie występuje dodatnia pętla sprzężenia zwrotnego pomiędzy miR-34a i *p53*. Regulacyjne

działanie miR-34a na *SIRT1* zaobserwowano tylko w płucach, gdzie zmniejszona ekspresja miR-34a korelowała ze wzrostem *SIRT1* w zakażeniu *L. europaeus* oboma genotypami. Powyższe badania są unikalne, ponieważ po raz pierwszy opisują rolę miR-34a w regulacji apoptozy w infekcji wirusowej u zwierząt.

Aby zbadać apoptozę jako odpowiedź na bodziec wirusowy, zbadano biomarkery apoptozy (*Bax*, stosunek *Bax/Bcl-2, kaspaza-3, PARP*) na poziomie mRNA [Rycina 4, publikacja P-2]. Białka z rodziny Bcl-2 są ważnymi cząsteczkami regulującymi apoptozę. Można je podzielić na dwie grupy: antyapoptotyczne – Bcl-2 oraz proapoptotyczne – Bax. Wzrost stosunku Bax/Bcl-2 wskazuje na zwiększoną podatność komórki na apoptozę [156]. W omawianej publikacji opisano nadekspresję *Bax* w wątrobie po zakażeniu *L. europaeus*/GI.2, zmniejszoną ekspresję *Bax* w płucach i śledzionie oraz brak zmian tego biomarkera w nerkach. Być może w przypadku nerek taki wynik jest efektem negatywnej regulacji przez *p53*. *P53* promuje apoptotyczną zdolność *Bax* jako czynnika transkrypcyjnego, a obserwowany spadek poziomu *p53* w nerkach może być tego przyczyną [295]. Wykazano również wzrost stosunku *Bax/Bcl-2* we wszystkich czterech tkankach po zakażenia *L. europaeus*/GI.1 i GI.2 i był on 2-3 krotnie wyższy po zakażeniu *L. europaeus*/GI.2 w wątrobie, śledzionie i płucach. Natomiast w nerkach wyższy stosunek *Bax/Bcl-2* występował po zakażeniu *L. europaeus*/GI.1. Kaspaza-3 jest główną kaspazą wykonawczą w zewnętrznym i wewnętrznym szlaku apoptozy [296]. We wcześniejsze badania nad patogenezą RHD wykazano, że aktywność kaspazy-3 wzrasta w wątrobie zakażonych królików w 36h i 48h [29]. Ponadto obserwowano wzrost rozszczepionej kaspazy-3 w płucach, sercu, nerkach i śledzionie [51]. Z kolei w badaniach Vallejo i wsp. [55] wykazano, że w wątrobie apoptoza indukowana zakażeniem *L. europaeus*/GI.1 przebiegała bez istotnej zmiany aktywności kaspazy-3 po 12h, 18h i 24h od zakażenia [55]. W wyniku przeprowadzonych badań wykazano, że podczas zakażenia *L. europaeus*/GI.1 i GI.2 w płucach i śledzionie dochodzi do wzrostu ekspresji genu *kaspazy-3* na podobnym poziomie, co wskazuje na aktywację apoptozy, natomiast w nerkach po zakażeniu *L. europaeus*/GI.1 oraz w wątrobie po zakażeniu *L.europaeus*/GI.2 dochodzi do spadku poziomu ekspresji *kaspazy-3*. PARP odgrywa rolę w wykrywaniu i naprawie uszkodzeń DNA. Jego aktywacja wpływa na systemy naprawcze. Natomiast hamowanie PARP prowadzi do akumulacji uszkodzeń DNA i do apoptozy [297]. Wykazano, że w zakażeniu *L. europaeus* ekspresja genu *PARP* była istotnie zmniejszona w wątrobie, nerkach i śledzionie, co wskazuje na akumulację uszkodzeń DNA i indukcję apoptozy. Uzyskane wyniki badań dotyczące biomarkerów apoptozy skłaniają do wysunięcia wniosku, że w zakażeniu królików *L. europaeus* genotypami GI.1 i GI.2 — apoptoza zachodzi w czterech tkankach (wątrobie, płucach, nerkach i śledzionie), a proces ten

był silniej wyrażony po zakażeniu królików *L. europaeus*/GI.2. Apoptozie towarzyszyła zwiększona ekspresja lub redukcja *Bax*, stosunku *Bax/Bcl-2*, *kaspazy-3* i *PARP*.

Szczegółowy opis korelacji rang Spearmana dla badanych miR, mRNA i biomarkerów apoptozy w czterech tkankach królików zakażonych *L. europaeus*/GI.1 i GI.2 został zaprezentowany na [Rycinie 8, publikacja P-2].

Przeprowadzony przegląd literatury wyłonił miR-122, jako cząsteczkę zaangażowaną w regulację genów związanych ze stresem oksydacyjnym [123] [Rycina 5, publikacja P-2]. W przeprowadzonych badaniach, wykazano nadekspresję miR-122 we wszystkich badanych tkankach po zakażenia *L. europaeus/GI.1* oraz w płucach, nerkach i śledzionie po zakażenia *L. europaeus/GI.2*. Co ciekawe, poziomy miR-122 były zdecydowanie wyższe w zakażeniu *L. europaeus*/GI.2. Uzyskane wyniki wskazują, że miR-122 uczestniczy w uszkodzeniu tkanek podczas zakażenia *L.europaeus*/GI.1 i GI.2, a jego nadekspresja wpływa hamująco na gen docelowy *Bach1* we wszystkich badanych tkankach z wyjątkiem płuc. Nadekspresja miR-122 wpływa na hamowanie ekspresji genu *HO-I* (w wątrobie i śledzionie po zakażeniu *L.europaeu*/GI.1 i GI.2 oraz w nerkach po zakażeniu *L.europaeus*/GI.1), co prowadzi do zwiększonego uszkodzenia tych tkanek w wyniku stresu oksydacyjnego. Uzyskane wynik pokazują, że HO-I w zakażeniu *L. europaeus* nie ma działania ochronnego w wątrobie, nerkach i śledzionie. Jedynie w płucach w przebiegu zakażenia *L. europaeus* GI.2 zarejestrowano wzrost ekspresji *HO-I*, co może świadczyć o działaniu ochronnym przed stresem oksydacyjnym.

W omawianej publikacji po raz pierwszy opisano regulacyjny wpływ miR-132 na szlak stresu oksydacyjnego w zakażeniu wirusowym u zwierząt [Rycina 6, publikacja P-2]**.** Wyniki badań wskazują, że miR-132 może być zaangażowany w uszkodzenie tkanek podczas zakażenia *L. europaeus* i może stanowić element patogenezy RHD. W wyniku przeprowadzonych badań wykazano nadekspresję miR-132 podczas zakażenia *L. europaeus*/GI.1 i GI.2 w wątrobie i śledzionie oraz nerkach po zakażeniu *L. europaeus*/GI.1. W wątrobie i nerkach wzrost ekspresji miR-132 korelował ze spadkiem poziomu mRNA *Nrf-2*. W wątrobie i nerkach obserwowano spadek ekspresji biomarkera stresu oksydacyjnego *HO-I*, co może prowadzić do zwiększonego uszkodzeniach tych narządów w przebiegu RHD. W badaniach nie wykazano hamującego działania miR-132 na *Nrf-2* w śledzionie. Pomimo wzrostu ekspresji miR-132, ekspresja *Nrf-2* również wzrosła, podczas gdy poziom ekspresji *HO-I* zmniejszył się. Niezbędne są dalsze badania w celu wyjaśnienia tego mechanizmu. Dane dotyczące sygnalizacji Nrf-2 (krytycznego czynnika obrony oksydacyjnej) w zakażeniach wirusowych są ograniczone [124]. Dowody sugerują, że aktywacja Nfr-2 w komórkach

gospodarza ma działanie ochronne podczas zakażeń wirusowych. Ochrona może polegać na działaniu przeciwwirusowym, hamowaniu śmierci komórek w celu ochrony przed nadmiernym uszkodzeniem tkanek lub na obu tych działaniach. Nie odnotowano zmian ekspresji miR-132 w płucach po zakażeniu *L. europaeus*/GI.1 i GI.2. W płucach po zakażeniu *L. europaeus GI.1* nie odnotowano zmian *HO-I*. Można zasugerować, że wynikiem tych reakcji jest mniejsze uszkodzenie oksydacyjne w płucach i większe w wątrobie, śledzionie i nerkach.

HO-I chroni wiele tkanek i narządów przed stresem oksydacyjnym i nadmiernymi reakcjami zapalnymi, uwalniając wiele cząsteczek o właściwościach antyoksydacyjnych [117]**.** W wyniku przeprowadzonych badań [Rycina 7, publikacja P-2] wykazano, że zmniejszona ekspresja *HO-I* w wątrobie, nerkach i śledzionie w przebiegu zakażenia *L. europaeus*/GI.1 i GI.2 nie chroni komórek przed występującym podczas infekcji stresem oksydacyjnym. Wyniki innych badaczy [117,159], wskazują, że spadek *HO-I* może dodatkowo nasilać uszkodzenie tkanek. Dodatkowo brak ochrony przed stresem oksydacyjnym może prowadzić do nasilonej apoptozy komórek.

Szczegółowy opis korelacji rang Spearmana dla badanych miR, mRNA i *HO-I* w czterech tkankach królików zakażonych *L. europaeus*/GI.1 i GI.2 został zaprezentowany na [Rycinie 9, publikacja P-2].

Zwierzęta zakażone *L. europaeus*-genotypami GI.1 i GI.2 wykazywały objawy kliniczne zgodne z RHD. Śmiertelność po zakażeniu *L. europaeus* oboma genotypami wyniosła 90% do 60 godziny badania. *L. europaeus*-genotyp GI.2 okazał się bardziej wirulentny, powodując 90% śmiertelność u królików w ciągu 32h od zakażenia i piorunujący przebieg choroby w formie nadostrej i ostrej. Natomiast po zakażeniu *L. europaeus* /GI.1 wskaźnik śmiertelności wynosił – 10% do 32h, 40% do 36h i 40% między 56 a 60h po zakażeniu, a przebieg choroby u przeważającej większości zwierząt był ostry.

Na podstawie przeprowadzonych badań wykazano, że miR poprzez regulację genów docelowych zaangażowanych w apoptozę i stres oksydacyjny, mogą przyczynić się do patogenezy RHD, wywołanej zakażeniem *L.europaeus*/GI.1 i GI.2. Cząsteczki miR – miR-16b i miR-34a mogą wpływać na nasilenie apoptozy w badanych narządach w odpowiedzi na zakażenie, a z drugiej strony hamować, co obserwowano w przypadku miR-21. Zaburzenie równowagi pomiędzy miR klasyfikowanymi jako proapototyczne, a antyapoptotyczne, jak również deregulacja poziomów tych miR może być wyznacznikiem patogenezy RHD i uszkodzenia tkanek. Ponadto na podstawie przeprowadzonych badań stwierdzono, że miR-122 i miR-132 regulują dwa szlaki stresu oksydacyjnego w patogenezie RHD, wywołanej zakażeniem *L.europaeus*/GI.1 i GI.2 związane z uszkodzeniem tkanek. Biomarker *Bax* i

stosunek *Bax/Bcl-2* promują intensywniejszą apoptozę w zakażeniu *L. europaeus*/GI.2. Biomarker *HO-I* w przebiegu RHD wskazuje na oksydacyjne uszkodzenie tkanek. Wyniki zaprezentowane w omawianej publikacji mogą mieć także potencjał diagnostyczny (poszukiwanie potencjalnych biomarkerów choroby), jak i terapeutyczny (modulacja szlaków zależnych od miR) w ALF i MOF o etiologii wirusowej, które występują podczas zakażenia *L. europaeus*.

### 4.3. MikroRNA uczestniczą w regulacji ekspresji genów związanych z wrodzoną odpowiedzią immunologiczną i zapalną u królików zakażonych *Lagovirus europaeus* genotypami – GI.1 i GI.2

(na podstawie publikacji [P-3]: Ostrycharz-Jasek E., Fitzner A., Siennicka A., Budkowska M., Hukowska-Szematowicz B. (2024). MicroRNAs regulate the expression of genes related to the innate immune and inflammatory response in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *International Journal of Molecular Science* 25 (17), 9531. DOI: 10.3390/ijms25179531)

MiR są zaangażowane w regulację wrodzonej odpowiedzi immunologicznej i zapalnej w odpowiedzi na bodziec zapalny, jakim jest infekcja wirusowa [87]. W kontekście wrodzonej odpowiedzi immunologicznej i zapalnej rola miR może być dwojaka [87,135,298]. Z jednej strony ekspresja miR może być regulowana przez elementy wrodzonej odpowiedzi immunologicznej. Z drugiej strony miR mogą regulować kluczowe geny odporności wrodzonej (odgrywając rolę prozapalną lub przeciwzapalną/lub nawet obie) [87]. Wrodzona odpowiedź immunologiczna i zapalna jest kluczowym elementem w walce z patogenami, zwłaszcza z wirusami wywołującymi ostry przebieg infekcji [299]. Jednak jej rozregulowanie oraz zwiększona produkcja i uwalnianie cytokin prozapalnych tzw. burza cytokinowa, może przyczynić się do zwiększonego uszkodzenia tkanek w przebiegu infekcji oraz może stanowić ważny element patogenezy chorób wirusowych [300]. Deregulacja ekspresji lub funkcji miR może przyczynić się do nieprawidłowej ekspresji kluczowych genów odporności wrodzonej i szlaków sygnałowych, prowadząc między innymi do nasilenia reakcji zapalnych, a w konsekwencji do patologii choroby [87]. Jak dotąd brakuje badań na temat molekularnej regulacji wrodzonej odpowiedzi immunologicznej i zapalnej przez miR w zakażeniu *L. europaeus*. Dlatego też ten problem naukowy podjęto w omawianej publikacji.

W omawianej publikacji pt.: „MicroRNAs Regulate the Expression of Genes Related to the Innate Immune and Inflammatory Response in Rabbits Infected with *Lagovirus europaeus* GI.1 and GI.2 Genotypes" podjęto badania mające na celu ocenę ekspresji miR i ich genów docelowych związanych z wrodzoną odpowiedzią immunologiczną i zapalną oraz ocenę ich wpływu na szlak sygnałowy NF-κβ i NLRP3 w zakażeniu *L. europaeus* dwoma genotypami – GI.1 i GI.2 w czterech tkankach (wątrobie, płucach, nerkach, śledzionie).

W celu wyboru miR oraz selekcji i przewidywania docelowych genów dla miR zaangażowanych we wrodzoną odpowiedź immunologiczną i zapalną (kluczowych w regulacji szlaku TLR4–MyD88– NF-κβ oraz inflamasomu NLRP3) posłużono się publikacją [publikacja

P-1] oraz metodami in silico z wykorzystaniem baz danych dedykowanych do pracy z miR. W celu zbadania miR i ich genów docelowych zaangażowanych we wrodzoną odpowiedź immunologiczną i zapalną w zakażeniu *L. europaeus* genotypami – GI.1 i GI.2 w czterech tkankach wykorzystano metody z zakresu biologii molekularnej w postaci pomiaru ekspresji miR i genów docelowych. Takie samo podejście metodyczne wykorzystano do zbadania biomarkerów stanu zapalnego. Do badań wybrano: miR-155 (geny docelowe *MyD88, TAB2, p65, NLRP3*), miR-146a (*IRAK1, TRAF6*), miR-223 (*TLR4, IKKα, NLRP3*), miR-125b (*NLRP3*) [Tabela 2, publikacja P-3]. Dodatkowo zbadano na poziomie mRNA biomarkery stanu zapalnego (*IL-1β, IL-6, TNF-α i IL-18*), aby ocenić intensywność odpowiedzi zapalnej w czterech badanych tkankach w zakażeniu *L. europaeus* genotypami – GI.1 i GI.2.

Z uwagi na fakt, że działanie regulacyjne miR na geny docelowe było zróżnicowane w zależności od mikrośrodowiska tkankowego wyniki badań przedstawiono oddzielnie dla każdej badanej tkanki –wątroby, płuc, nerek i śledziony, jak również dla każdej tkanki przedstawiono osobny szlak regulacyjny [Rycina 1-4, Rycina 7-10, publikacja P-3].

W wyniku przeprowadzonych badań, wykazano, że w zakażeniu *L. europaeus*/GI.1 i GI.2 miR są kluczowe w regulacji wrodzonych odpowiedzi immunologicznych i zapalnych. W badaniu po raz pierwszy opisano regulacyjne działanie miR-155, miR-146a, miR-223 i miR-125b na geny zaangażowane w sygnalizację szlaku NF-κB oraz inflamasomu NLRP3 w zakażeniu *L. europaeus* dwoma genotypami-GI.1 i GI.2. Wykazano, że miR-155 w wątrobie podczas zakażenia *L. europaeus* oboma genotypami wykazuje działanie zarówno prozapalne, jak i przeciwzapalne. Zwiększona ekspresja miR-155 podczas zakażenia *L. europaeus*/GI.1 i GI.2 znacząco hamowała ekspresję *MyD88* i podjednostki *p65*, genów kluczowych do aktywacji czynnika transkrypcyjnego NF-κβ i produkcji cytokin prozapalnych. Jednak z drugiej strony, nadekspresja miR-155 przyczyniła się do zwiększenia ekspresji inflamasomu *NLRP3*, który jest kluczowym składnikiem odpowiedzi immunologicznej i pośredniczy w nasileniu stanu zapalnego poprzez wydzielanie cytokin prozapalnych takich jak IL-1β i IL-18, w odpowiedzi na zakażenie wirusowe i uszkodzenie komórek [301,302]. Badania wykazały, że gen *TAB2*, który jest niezbędny do aktywacji szlaku NF-κβ, jest również genem docelowym miR-155 [87]. Jednak w badaniach nie wykazano regulacyjnego wpływu miR-155 na gen *TAB2*. Zarejestrowano jedynie zwiększoną ekspresję *TAB2* w wątrobie podczas zakażenia *L. europaeus*/GI.1. Niezbędne są dalsze badania w celu wyjaśnienia wzrostu ekspresji tego genu w zakażeniu *L. europaeus*. W badaniach wykazano również nadekspresję miR-223 w wątrobie po zakażeniu *L. europaeus* oboma genotypami. Zwiększony poziom miR-223 wpływał na zmniejszenie ekspresji *IKKα*. Dalsza analiza wykazała również ujemną korelację pomiędzy

miR-223, a *TLR4*, którego ekspresja nie była istotnie statystycznie obniżona podczas zakażenia. Jednak możliwe, że w wątrobie, jako miejscu replikacji wirusa, ekspresja *TLR4* jest na tyle mocno indukowana przez wirus, że ekspresja miR-223 nie jest wystraczająca do zmniejszenia ekspresji *TLR4*. W przypadku wątroby podczas zakażenia nie zarejestrowano hamującego działania miR-223 na poziom mRNA *NLRP3*. Jednak wyniki te sugerują, że miR-223 podczas zakażenia *L. europaeus* oboma genotypami wykazuje działanie przeciwzapalne. Takie samo działanie przeciwzapalne w wątrobie wykazują również miR-125b i miR-146a. Nadekspresja miR-125b w wątrobie przyczyniła się do zmniejszenia ekspresji *MyD88,* podobnie jak miR-155. W wątrobie królików po zakażeniu *L .europaeus*/GI.1 i Gi.2 wykazano również zwiększoną ekspresję miR-146a, która wykazuje działanie hamujące na gen docelowy *TRAF6*. Jednak co ciekawe, pomimo podobnego poziomu ekspresji miR-146a w wątrobie królików zakażonych oboma genotypami, spadek *TRAF6* podczas zakażenia *L. europaeus*/GI.2 był znacznie większy niż podczas zakażenia *L. europaeus*/GI.1. Zaobserwowana w badaniach zwiększona ekspresja *IRAK1* w obu zakażonych grupach królików wymaga dalszych badań i wyjaśnienia.

W prezentowanych badaniach wykazano, że w płucach (w przeciwieństwie do wątroby) zmienioną ekspresję wykazują jedynie miR-146a i miR-223. Zwiększony poziom miR-146a podczas zakażenia *L. europaeus*/GI.1 i GI.2 hamuje ekspresję kluczowych genów odporności *IRAK1* i *TRAF6*, co świadczy o jego działaniu przeciwzapalnym. W przeciwieństwie do miR-146a, ekspresja miR-223 jest obniżona w płucach królików zakażonych *L. europaeus* oboma genotypami. Zmniejszonemu poziomowi miR-223 towarzyszył wzrost jego genów docelowych *TLR4*, *IKKα* oraz *NLRP3*. Dodatkowo wykazano silną ujemną korelację pomiędzy miR-223, a jego genami docelowymi, co pozwala wysnuć wniosek, że w płucach miR-223 wykazuje działanie przeciwzapalne. Jednak w tym przypadku zmniejszona ekspresja miR-223 może prowadzić o zaostrzenia odpowiedzi zapalnej. W płucach u królików zakażonych *L. europaeus* /GI.1 i GI.2, nie wykazano zmian w poziomie ekspresji miR-155 i miR-125b. Jednak zaobserwowano zwiększoną ekspresję *MyD88* w obu grupach królików. Wzrost ten może być spowodowany zwiększoną ekspresją *TLR4*, która może wpływać na szybkość inicjacji transkrypcji i tym samym zwiększać ekspresję *MyD88* [303].

Na podstawie przeprowadzonych badań wykazano, że w nerkach podczas zakażenia *L. europaeus*/GI.1 i GI.2 tendencje zmian poziomów ekspresji miR, jak również ich genów docelowych są takie same. Ponadto wykazano, że w przebiegu zakażenia *L. europaeus* miR-223, miR-155 i miR-146a wykazują działanie przeciwzapalne w nerkach zakażonych zwierząt. Podczas zakażenia *L. europaeus*/GI.1 i GI.2 zaobserwowano wzrost miR-223, który korelował

ujemnie z poziomem ekspresji *TLR4, IKKα* oraz *NLRP3*. Z kolei wzrost ekspresji miR-155 wpływa na hamowanie genu *MyD88* oraz spadek ekspresji podjednostki *p65* głównego czynnika transkrypcyjnego NF-κβ. Jednak w naszych badaniach nie wykazaliśmy regulacyjnego wpływu miR-155 na ekspresję *TAB2*, która pozostała niezmienna w obu grupach królików zakażonych *L. europaeus*. Podobnie jak w płucach, w nerkach wykazano wzrost ekspresji miR-146a, który hamuje geny docelowe *IRAK1 i TRAF6*. Podczas zakażenia *L. europaeus* oboma genotypami nie wykazano zmian ekspresji miR-125b w nerkach.

Na podstawie przeprowadzonych badań wykazano, że w śledzionie podczas zakażenia *L. europaeus*/GI.1 i GI.2 mają miejsce najbardziej zróżnicowane zmiany poziomów ekspresji miR. Ponadto wykazano, że w przebiegu zakażenia *L. europaeus* miR-155 i miR-223 wykazują działanie przeciwzapalne w śledzionie chorych królików. W odpowiedzi na infekcję wirusową u chorych królików dochodzi do wzrostu ekspresji miR-155 (po zakażeniu *L. europaeus* oboma genotypami) oraz spadku miR-223, miR-146a i miR-125b (tylko po zakażeniu *L. europaeus*/GI.1). Wzrost ekspresji miR-155, zmniejszał ekspresję genów docelowych *MyD88 i p65*, co podkreśla jego działanie przeciwzapalne w śledzionie. Pomimo wzrostu ekspresji miR-155 w obu badanych zakażonych grupach królików, nie zaobserwowano zmniejszonego poziomu *TAB2*. Co więcej podczas infekcji *L. europaeus*/GI.1 ekspresja *TAB2* wzrosła. Potrzebne są dalsze badania w celu zidentyfikowania czynników, które mogą wpływać na zwiększenie ekspresji genu *TAB2* w zakażeniu *L. europaeus*. W przeprowadzonych badaniach nie wykazano regulacyjnego działania miR-146a na geny docelowe w śledzionie. Wykazano spadek ekspresji miR-146a tylko podczas zakażenia *L. europaeus*/GI.1. Nie zaobserwowano zmiany ekspresji *IRAK1* w zakażeniu *L. europaeus* oboma genotypami, natomiast dla obu genotypów opisano spadek ekspresji *TRAF6*. Podobnie jak w przypadku miR-146a, spadek miR-223 zaobserwowano jedynie podczas zakażenia *L.europaeus*/GI.1. Zmniejszona ekspresja miR-223 korelowała ze wzrostem ekspresji genu *NLRP3*, co może świadczyć o jego działaniu przeciwzapalnym w śledzionie w zakażeniu *L. europaeus*/GI.1. Trudnym do interpretacji w środowisku śledziony jest fakt spadku ekspresji genu *IKKα* podczas zakażenia oboma genotypami. Podobnie jak spadek miR-125b w zakażeniu *L. europaeus*/GI.1. Na podstawie uzyskanych wyników badań trudno jest określić rolę miR-146a i miR-125b podczas zakażenia *L. europaeus* w śledzionie. Wiadomo, że środowisko śledziony, jako centralnego organu systemowej odpowiedzi immunologicznej, jest bardzo złożone, co potwierdzają ostatnie badania Yu i wsp. [74]. Biorąc pod uwagę wyniki Yu i wsp. [74], uzyskane rezultaty badań w omawianej pracy wydają się potwierdzać złożoność profili ekspresji miR w mikrośrodowisku śledziony i ich wpływ na regulację genów zaangażowanych

we wrodzoną odpowiedź immunologiczną w zakażeniu *L. europaeus*/GI.1 i GI.2. Konieczne są dalsze badania w tym obszarze.

Szczegółowy opis korelacji rang Spearmana dla badanych miR i mRNA w czterech tkankach królików zakażonych *L. europaeus*/GI.1 i GI.2 został zaprezentowany na [Rycinach 7-10, publikacja P-3].

Ponadto po raz pierwszy zbadano biomarkery stanu zapalnego w czterech tkankach- wątrobie, płucach, nerkach i śledzionie u królików zakażonych *L. europaeus* dwoma genotypami – GI.1 i GI.2 [Tabela 1, Rycina 6, publikacja P-3]. Analiza poziomów mRNA cytokin prozapalnych *IL-1β* , *IL-6* , *TNF-α* i *IL-18* w czterech tkankach wykazała, że profil ekspresji biomarkerów stanu zapalnego u królików zakażonych *L. europaeus*/GI.1 i GI.2 jest podobny pod względem tendencji zmian (wzrost/spadek lub brak zmiany w ekspresji), ale różny dla poszczególnych tkanek [Rycina 11, publikacja P-3]. Po raz pierwszy wyróżniono trzy profile zapalenia w badanych tkankach. Profil płucny, charakteryzujący się wzrost trzech kluczowych cytokin fazy ostrej IL-1β, IL-6, TNF-α, bez zmian w poziomie ekspresji *IL-18*. Ponadto podobny wzrost ekspresji *TNF-α* po zakażeniu królików *L. europaeus*/GI.1 i GI.2 wskazuje na podobny stopień uszkodzenia płuc (nieco bardziej nasilony u zwierząt zakażonych *L.europaeus*/GI.1), być może ze względu na dłuższy okres przeżycia zwierząt. Profil nerkowy, charakteryzujący się jedynie wzrost ekspresji *IL-6*, który był trzydziestokrotnie wyższy w przebiegu zakażenia *L. europaeus*/GI.2 niż GI.1. W profilu nerkowym zaobserwowano również spadek ekspresji *IL-1β* i *IL-18,* przy czym spadek *IL-18* był ośmiokrotnie niższy podczas infekcji *L. europaeus*/GI.1 w porównaniu z GI.2. Nie zaobserwowano zmiany w poziomie ekspresji TNF-α (chociaż pomiędzy GI.1 i GI.2 odnotowano dwukrotny spadek tego biomarkera). Ostatni profil wątrobowo-śledzionowy charakteryzował się wzrostem ekspresji *IL-6* w zakażeniu *L. europaeus* obydwoma genotypami. Przy czym w śledzionie zaobserwowano największy wzrost ekspresji *IL-6*, który w przypadku zakażenia *L. europaeus*/GI.1 okazał się ponad dziewięciuset krotnie wyższy niż w śledzionie u królików zdrowych. Wzrost ekspresji *TNF-α* w wątrobie wykazano tylko podczas infekcji *L. europaeus*/GI.1 i okazał się sześciokrotnie wyższy w porównaniu z GI.2. Fakt ten może sugerować rozwój odpowiedzi zapalnej i uszkodzenie wątroby. Dodatkowo w profilu tym wykazano spadek *IL-1β i IL-18*. Powyższe fakty wskazują na silne i szybkie zaangażowanie lokalnej wrodzonej odpowiedzi immunologicznej i zapalnej w zakażeniu *L. europaeus*/GI.1 i GI.2 oraz na aspekt patogenezy RHD. Znaczący wzrost cytokin prozapalnych w tkankach może napędzać patogenezę RHD, co może prowadzić do nadkrzepliwości, powstawania

mikrozakrzepów (obserwowanych *post mortem*), ostrej niewydolności wątroby, niewydolności wielonarządowej, koagulopatii z krwotokiem i ostatecznie śmierci zwierząt.

Zwierzęta zakażone *L. europaeus*-genotypami GI.1 i GI.2 wykazywały objawy kliniczne zgodne z RHD. Śmiertelność po zakażeniu *L. europaeus* oboma genotypami wyniosła 90% do 60 godziny badania. *L. europaeus*-genotyp GI.2 okazał się bardziej wirulentny, powodując 90% śmiertelność u królików w ciągu 32h od zakażenia i piorunujący przebieg choroby w formie nadostrej i ostrej. Natomiast po zakażeniu *L. europaeus* /GI.1 wskaźnik śmiertelności wynosił – 10% do 32h, 40% do 36h i 40% między 56 a 60h po zakażeniu, a przebieg choroby u przeważającej większości zwierząt był ostry.

Na podstawie przeprowadzonych badań po raz pierwszy wykazano regulacyjny wpływ miR na geny wrodzonej odpowiedzi immunologicznej i zapalnej u królików zakażonych *L. europaeus* genotypami – GI.1 i GI.2 w czterech tkankach (wątroba, płuca, nerki i śledziona). Uzyskane wyniki pozwalają lepiej zrozumieć patogenezę RHD wywołaną zakażeniem *L. europaeus* oraz molekularną regulację wrodzonej odpowiedzi immunologicznej i zapalnej przez miR. Głównymi regulatorami wrodzonej odpowiedzi immunologicznej i zapalnej w zakażeniu *L. europaeus*/GI.1 i GI.2, a także RHD są miR-155, miR-223 i miR-146a. Ekspresja miR-125b była ograniczona i dotyczyła tylko wątroby i śledziony. Wykazano, że podczas zakażenia *L. europaeus*/GI.1 i GI.2 miR-155 – ma działanie pro- i przeciwzapalne w wątrobie, jak i działanie przeciwzapalne w nerkach i śledzionie; miR-146a ma działanie przeciwzapalne w wątrobie, płucach i nerkach; miR-223 ma działanie przeciwzapalne we wszystkich tkankach; miR-125b ma działanie przeciwzapalne tylko w wątrobie. W każdym przypadku taki efekt może być wyznacznikiem patogenezy RHD. Badania sugerują, że miR mogą regulować trzy wrodzone szlaki odpowiedzi immunologicznej i zapalnej w zakażeniu *L. europaeus*: (1) szlak sygnałowy TLR4-MyD88, (2) szlak sygnałowy NF-κβ (p65) i (3) szlak inflamasomu NRLP3. Jednakże, jak zaobserwowano w badaniach, na wynik tej regulacji może mieć wpływ mikrośrodowisko tkankowe. W omawianej publikacji po raz pierwszy przedstawiono profil ekspresji biomarkerów stanu zapalnego (*IL-1β, IL-6, TNF-α, IL-18*) na poziomie mRNA w czterech tkankach po zakażeniu *L. europaeus* dwoma genotypami. W efekcie czego zaproponowano trzy profile zapalenia (płucny, nerkowy oraz wątrobowo-śledzionowy) w odpowiedzi na zakażenie *L.europaeus*/GI.1 i GI.2. Wyniki zaprezentowane w omawianej publikacji mogą mieć także potencjał diagnostyczny (poszukiwanie potencjalnych biomarkerów choroby), jak i terapeutyczny (modulacja szlaków zależnych od miR) w ALF i MOF o etiologii wirusowej, które występują podczas zakażenia *L. europaeus*.

## 5. Stwierdzenia i wnioski

Wyniki uzyskane w trakcie realizacji niniejszej rozprawy doktorskiej istotnie pogłębiły wiedzę dotyczącą patogenezy RHD (wywołanej zakażeniem *L. europaeus* dwoma genotypami – GI.1 i GI.2) z uwzględnieniem mikrośrodowiska tkankowego wątroby, płuc, nerek i śledziony. Przyczyniły się do częściowego poznania roli cząsteczek miR w zakażeniu *L. europaeus* oraz dostarczyły nowych danych pozwalających lepiej zrozumieć patogenezę RHD w zakresie molekularnej regulacji apoptozy, stresu oksydacyjnego oraz wrodzonej odpowiedzi immunologicznej i zapalnej przez miR.

Na podstawie wyników uzyskanych podczas badań oraz przeglądu literatury prowadzonych w ramach rozprawy doktorskiej sformułowano następujące stwierdzenia i wnioski:

1. Sygnatura miR w zakażeniach wirusowych obejmuje 12 miR u ludzi (miR-155, miR-223, miR-146a, miR-122, miR-125b, miR-132, miR-34a, miR-21, miR-16, rodzina miR-181, rodzina let-7, miR-10a) oraz 10 miR u zwierząt (miR-155, miR-223, miR-146a, miR-145, miR-21, miR-15a, miR-16, rodzina miR-181, rodzina let-7, miR-122). Wyróżniono 8 miR wspólnych dla ludzi i zwierząt (miR-155, miR-146a, miR-223, miR-122, miR-21, miR-16, rodzina miR-181, rodzina let-7) [publikacja P-1].
2. W zakażeniach wirusowych kluczowe cząsteczki miR i ich geny docelowe zaangażowane w apoptozę to: miR-21 (*PTEN*, *PDCD4*), miR-16b (*Bcl-2*, *CXCL10*) oraz miR-34a (*p53*, *SIRT1*) w stres oksydacyjny: miR-132 (*Nrf-2*) i miR-122 (*Bach1)* oraz we wrodzoną odpowiedź immunologiczną i zapalną: miR-155 (*MyD88*, *TAB2*, *p65*, *NLRP3*), miR-146a (*IRAK1*, *TRAF6*), miR-223 (*TLR4*, *IKKα*, *NLRP3*) oraz miR-125b (*NLRP3*) [publikacja P-1] oraz [Tabela 1, publikacja P-2] i [Tabela 2, publikacja P-3].
3. Podczas zakażenia *L. europaeus*/GI.1 i GI.2 dochodzi do zmian ekspresji miR-21, miR-16b i miR-34a zaangażowanych w proces apoptozy. MiR-21 wzrasta we wszystkich narządach podczas zakażenia *L. europaeus*/GI.1 i GI.2, ale tylko w wątrobie, nerkach i śledzionie hamuje geny docelowe (*PTEN* i *PDCD4*) wpływając na zmniejszenie ich ekspresji. MiR-21 pełni rolę antyapoptotyczną w zakażeniu *L. europaeus* (w wątrobie, nerkach i śledzionie). Poziom ekspresji miR-16b wzrasta w wątrobie, nerkach i śledzionie w zakażeniu *L. europaeus*/GI.1 i GI.2 i korelował ze zmniejszoną ekspresją genu docelowego *Bcl-2* oraz ze wzrostem ekspresji *CXCL10* w tych tkankach, co świadczy o proapoptotycznym działaniu tego miR. Po raz pierwszy opisano rolę miR-34a w regulacji apoptozy w przebiegu infekcji wirusowej u zwierząt. Poziom ekspresji miR-34a wzrasta w wątrobie podczas zakażenia *L. europaeus*/GI.I oraz w

śledzionie w zakażeniu *L. europaeus*/GI.1 i GI.2. Wzrost ekspresji miR-34a pozytywnie koreluje ze wzrostem poziomu genu *p53*, co sugeruje, że *p53* na zasadzie pozytywnego sprzężenia zwrotnego może wpływać na wzrost ekspresji miR-34a i jego działanie proapoptotyczne. Natomiast w płucach podczas zakażenia *L. europaeus*/GI.1 i GI.2 stwierdzono zmniejszenie ekspresji miR-34a, który korelował negatywnie z poziomem *SIRT1*, co może wpływać hamująco na apoptozę w tym narządzie [publikacja P-2].

4. W odniesieniu do molekularnej regulacji apoptozy w zakażeniu *L. europaeus*/GI.1 i GI.2 stwierdzono, że badane miR mogą regulować trzy szlaki apoptozy. Wzrost ekspresji miR-21 (działanie antyapoptotyczne) wydaje się być niewystarczający do ochrony tkanek przed zwiększoną apoptozą indukowaną przez miR-16b i miR-34a [publikacja P-2].
5. Na podstawie biomarkerów *Bax* i stosunku *Bax*/*Bcl-2*, wykazano, że proces apoptozy był silniej wyrażony po zakażeniu królików *L. europaeus*/GI.2 [publikacja P-2].
6. Wzrost ekspresji miR-122 i miR-132 zaangażowanych w regulację stresu oksydacyjnego w zakażeniu *L. europaeus* wskazuje na hamowanie procesów ochronnych i antyoksydacyjnych w badanych tkankach i może przyczynić się do uszkodzenia tkanek oraz stanowić element patogenezy RHD. Poziom ekspresji miR-122 wzrasta we wszystkich badanych tkankach po zakażenia *L. europaeus*/GI.1 oraz w płucach, nerkach i śledzionie po zakażenia *L. europaeus*/GI.2, a jego nadekspresja wpływa hamująco na gen docelowy *Bach1* we wszystkich badanych tkankach z wyjątkiem płuc. Nadekspresja miR-122 wpływa na hamowanie ekspresji genu *HO-I* (w wątrobie i śledzionie po zakażeniu *L. europaeu*/GI.1 i GI.2 oraz w nerkach po zakażeniu *L. europaeus*/GI.1), co prowadzi do zwiększonego uszkodzenia tych tkanek w wyniku stresu oksydacyjnego. Po raz pierwszy opisano regulacyjny wpływ miR-132 na gen *Nrf-2* w zakażeniu wirusowym u zwierząt. Poziom ekspresji miR-132 wzrasta w wątrobie i śledzionie w zakażeniu *L. europaeus*/GI.1 i GI.2 i nerkach w przypadku GI.1. Wykazano hamujące działanie miR-132 na ekspresję *Nrf-2* w wątrobie podczas zakażenia *L. europaeus*/GI.1 i GI.2 oraz w nerkach, ale tylko w zakażeniu *L. europeus*/GI.1, co może skutkować brakiem działania antyoksydacyjnego i przyczyniać się do zwiększonego uszkodzenia tkanek w wyniku stresu oksydacyjnego podczas zakażenia *L. europaeus* [publikacja P-2].
7. MiR-122 i miR-132 regulują dwa szlaki stresu oksydacyjnego w patogenezie RHD, wywołanej zakażeniem *L.europaeus*/GI.1 i GI.2 związane z uszkodzeniem tkanek [publikacja P-2].

8. Zmniejszona ekspresja biomarkera *HO-I* w wątrobie, nerkach i śledzionie w przebiegu zakażenia *L. europaeus*/GI.1 i GI.2 nie chroni komórek przed stresem oksydacyjnym [publikacja P-2].
9. Po raz pierwszy wykazano, że tendencja zmian poziomów ekspresji miR zaangażowanych w regulację genów szlaku NF-κβ i NLRP3 jest podobna w przebiegu zakażenia *L. europaeus* dwoma genotypami – GI.1 i GI.2, ale różni się w zależności od mikrośrodowiska tkankowego [publikacja P-3].
10. W wątrobie miR-155 wykazuje działanie przeciwzapalne poprzez hamowanie ekspresji genów docelowych *MyD88* i *p65* oraz działanie prozapalne poprzez aktywację *NLRP3*. Natomiast w nerkach i śledzionie miR-155 wykazuje jedynie działanie przeciwzapalne negatywnie korelując z poziomem ekspresji *MyD88* i *p65*. MiR-155 ma większe działanie prozapalne w wątrobie w przebiegu zakażenia *L. europaeus*/GI.1 niż GI.2 [publikacja P-3].
11. W wątrobie nadekspresja miR-146a koreluje ze spadkiem poziomu ekspresji *TRAF6*. Natomiast w płucach i nerkach wykazano, że wzrost ekspresji miR-146a hamuje ekspresję dwóch krytycznych genów wrodzonej odpowiedzi immunologicznej i zapalnej *IRAK1* i *TRAF6*. Sugeruje to, że miR-146a w wątrobie, płucach i nerkach ma działanie przeciwzapalne w zakażeniu *L. europaeus*/GI.1 i GI.2 [publikacja P-3].
12. Wzrost ekspresji miR-223 wykazano w wątrobie gdzie korelował negatywnie z poziomem ekspresji genów *IKKα* i *TLR4* oraz w nerkach, w których hamował ekspresję *TLR4*, *IKKα* oraz *NLRP3*. Brak hamującego efektu miR-223 na *TLR4* w wątrobie może wynikać z faktu silnej indukcji tego receptora przez wirus, którego głównym miejscem replikacji jest wątroba. W płucach i śledzionie wykazano spadek poziomu ekspresji miR-223. W płucach obniżona ekspresja miR-223 wpływa na zwiększony poziom mRNA krytycznych genów wrodzonej odpowiedzi immunologicznej i zapalnej *IKKα* oraz *NLRP3* co może skutkować zwiększonym procesem zapalnym w tkance płuc. Natomiast w śledzionie zmniejszony poziom miR-223 korelował jedynie ze wzrostem ekspresji *NLRP3*. We wszystkich badanych tkankach podczas zakażenia *L. europaeus*/GI.1 i GI.2 miR-223 wykazuje działanie przeciwzapalne [publikacja P-3].
13. Wzrost ekspresji miR-125b był bardzo ograniczony i występował jedynie w wątrobie gdzie wykazywał negatywną korelację z poziomem ekspresji genu *MyD88*. Badania sugerują, że w wątrobie miR-125b wykazuje działanie przeciwzapalne poprzez hamowanie *MyD88*, jednak jest ono mniejsze niż działanie hamujące miR-155. [publikacja P-3].

14. W odniesieniu do molekularnej regulacji wrodzonej odpowiedzi immunologicznej i zapalanej w zakażeniu *L. europaeus*/GI.1 i GI.2 stwierdzono, że miR-155, miR-223, miR-146a i miR-125b mogą regulować trzy wrodzone szlaki odpowiedzi immunologicznej i zapalnej w zakażeniu *L. europaeus*: szlak sygnałowy TLR4-MyD88; szlak sygnałowy NF-κβ (p65) oraz szlak inflamasomu NRLP3 [publikacja P-3].
15. Zakażenie królików *L. europaeus*/GI.1 i GI.2 powoduje nadmierną ekspresję dwóch krytycznych cytokin fazy ostrej: *IL-6* we wszystkich badanych tkankach i *TNF-α* (w wątrobie, płucach i śledzionie). Wysoki poziom ekspresji *IL-1β* stwierdzono tylko w płucach po zakażeniu *L. europaeus* oboma genotypami. Wskazuje to na szybkie i silne zaangażowanie lokalnej wrodzonej odpowiedzi immunologicznej i zapalnej w zakażeniu *L. europaeus* oraz na udział w patogenezie RHD. Zmiany w ekspresji biomarkerów stanu zapalnego mogą różnicować profile zapalenia w zależności od mikrośrodowiska tkankowego. W zakażeniu *L. europaeus*/GI.1 i GI.2 wyróżniono trzy profile zapalenia płucny, nerkowy i wątrobowo-śledzionowy [publikacja P-3].

## 6. Piśmiennictwo


1. Le Pendu, J.; Abrantes, J.; Bertagnoli, S.; Guitton, J.S.; Le Gall-Recule, G.; Lopes, A.M.; Marchandeau, S.; Alda, F.; Almeida, T.; Celio, A.P.; et al. Proposal for a unified classification system and nomenclature of lagoviruses. *J Gen Virol* **2017**, *98*, 1658-1666, doi:10.1099/jgv.0.000840.
2. Liu, S.J.; Xue, H.P.; Pu, B.Q.; Qian, N.H. A new viral disease in rabbits. *Animal Husbandry and Veterinary Medicine* **1984**, *16*, 253-255.
3. Capucci, L.; Fallacara, F.; Grazioli, S.; Lavazza, A.; Pacciarini, M.L.; Brocchi, E. A further step in the evolution of rabbit hemorrhagic disease virus: the appearance of the first consistent antigenic variant. *Virus Res* **1998**, *58*, 115-126, doi:10.1016/s0168-1702(98)00106-3.
4. Le Gall-Recule, G.; Zwingelstein, F.; Boucher, S.; Le Normand, B.; Plassiart, G.; Portejoie, Y.; Decors, A.; Bertagnoli, S.; Guerin, J.L.; Marchandeau, S. Detection of a new variant of rabbit haemorrhagic disease virus in France. *Vet Rec* **2011**, *168*, 137-138, doi:10.1136/vr.d697.
5. Abrantes, J.; van der Loo, W.; Le Pendu, J.; Esteves, P.J. Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. *Vet Res* **2012**, *43*, 12, doi:10.1186/1297-9716-43-12.
6. Kennedy, A.; Britton, L.; Byrne, A.W.; Byrne, C.; Casey, M.; Flynn, O.; Lozano, J.M.; Marnell, F.; McElroy, M.; Reid, N.; et al. First detected case of rabbit Haemorrhagic disease virus 2 (RHDV2) in the Irish hare (Lepus timidus hibernicus). *Ir Vet J* **2021**, *74*, 25, doi:10.1186/s13620-021-00205-2.
7. Tokarz-Deptula, B.; Kulus, J.; Baraniecki, L.; Stosik, M.; Deptula, W. Characterisation of Lagovirus europaeus GI-RHDVs (Rabbit Haemorrhagic Disease Viruses) in Terms of Their Pathogenicity and Immunogenicity. *Int J Mol Sci* **2024**, *25*, doi:10.3390/ijms25105342.
8. Calvete, C.; Mendoza, M.; Sarto, M.P.; Bagues, M.P.J.; Lujan, L.; Molin, J.; Calvo, A.J.; Monroy, F.; Calvo, J.H. Detection of Rabbit Hemorrhagic Disease Virus GI.2/RHDV2/b in the Mediterranean Pine Vole ( Microtus duodecimcostatus) and White-Toothed Shrew ( Crocidura russula). *J Wildl Dis* **2019**, *55*, 467-472, doi:10.7589/2018-05-124.
9. Abade Dos Santos, F.A.; Pinto, A.; Burgoyne, T.; Dalton, K.P.; Carvalho, C.L.; Ramilo, D.W.; Carneiro, C.; Carvalho, T.; Peleteiro, M.C.; Parra, F.; et al. Spillover events of rabbit haemorrhagic disease virus 2 (recombinant GI.4P-GI.2) from Lagomorpha to Eurasian badger. *Transbound Emerg Dis* **2022**, *69*, 1030-1045, doi:10.1111/tbed.14059.
10. Buehler, M.; Jesse, S.T.; Kueck, H.; Lange, B.; Koenig, P.; Jo, W.K.; Osterhaus, A.; Beineke, A. Lagovirus europeus GI.2 (rabbit hemorrhagic disease virus 2) infection in captive mountain hares (Lepus timidus) in Germany. *BMC Vet Res* **2020**, *16*, 166, doi:10.1186/s12917-020-02386-4.
11. Neimanis, A.; Larsson Pettersson, U.; Huang, N.; Gavier-Widen, D.; Strive, T. Elucidation of the pathology and tissue distribution of Lagovirus europaeus GI.2/RHDV2 (rabbit haemorrhagic disease virus 2) in young and adult rabbits (Oryctolagus cuniculus). *Vet Res* **2018**, *49*, 46, doi:10.1186/s13567-018-0540-z.
12. Dalton, K.P.; Nicieza, I.; Abrantes, J.; Esteves, P.J.; Parra, F. Spread of new variant RHDV in domestic rabbits on the Iberian Peninsula. *Vet Microbiol* **2014**, *169*, 67-73, doi:10.1016/j.vetmic.2013.12.015.
13. Puggioni, G.; Cavadini, P.; Maestrale, C.; Scivoli, R.; Botti, G.; Ligios, C.; Le Gall-Recule, G.; Lavazza, A.; Capucci, L. The new French 2010 Rabbit Hemorrhagic Disease Virus causes an RHD-like disease in the Sardinian Cape hare (Lepus capensis mediterraneus). *Vet Res* **2013**, *44*, 96, doi:10.1186/1297-9716-44-96.
14. Kerr, P.J.; Hall, R.N.; Strive, T. Viruses for Landscape-Scale Therapy: Biological Control of Rabbits in Australia. *Methods Mol Biol* **2021**, *2225*, 1-23, doi:10.1007/978-1-0716-1012-1_1.
15. Peacock, D.; Kovaliski, J.; Sinclair, R.; Mutze, G.; Iannella, A.; Capucci, L. RHDV2 overcoming RHDV immunity in wild rabbits (Oryctolagus cuniculus) in Australia. *Vet Rec* **2017**, *180*, 280, doi:10.1136/vr.104135.
16. Hall, R.N.; Peacock, D.E.; Kovaliski, J.; Mahar, J.E.; Mourant, R.; Piper, M.; Strive, T. Detection of RHDV2 in European brown hares (Lepus europaeus) in Australia. *Vet Rec* **2017**, *180*, 121, doi:10.1136/vr.104034.

17. Camarda, A.; Pugliese, N.; Cavadini, P.; Circella, E.; Capucci, L.; Caroli, A.; Legretto, M.; Mallia, E.; Lavazza, A. Detection of the new emerging rabbit haemorrhagic disease type 2 virus (RHDV2) in Sicily from rabbit (Oryctolagus cuniculus) and Italian hare (Lepus corsicanus). *Res Vet Sci* **2014**, *97*, 642-645, doi:10.1016/j.rvsc.2014.10.008.
18. Mahar, J.E.; Hall, R.N.; Peacock, D.; Kovaliski, J.; Piper, M.; Mourant, R.; Huang, N.; Campbell, S.; Gu, X.; Read, A.; et al. Rabbit Hemorrhagic Disease Virus 2 (RHDV2; GI.2) Is Replacing Endemic Strains of RHDV in the Australian Landscape within 18 Months of Its Arrival. *J Virol* **2018**, *92*, doi:10.1128/JVI.01374-17.
19. Velarde, R.; Cavadini, P.; Neimanis, A.; Cabezon, O.; Chiari, M.; Gaffuri, A.; Lavin, S.; Grilli, G.; Gavier-Widen, D.; Lavazza, A.; et al. Spillover Events of Infection of Brown Hares (Lepus europaeus) with Rabbit Haemorrhagic Disease Type 2 Virus (RHDV2) Caused Sporadic Cases of an European Brown Hare Syndrome-Like Disease in Italy and Spain. *Transbound Emerg Dis* **2017**, *64*, 1750-1761, doi:10.1111/tbed.12562.
20. Capucci, L.; Frigoli, G.; Ronshold, L.; Lavazza, A.; Brocchi, E.; Rossi, C. Antigenicity of the rabbit hemorrhagic disease virus studied by its reactivity with monoclonal antibodies. *Virus Res* **1995**, *37*, 221-238, doi:10.1016/0168-1702(95)00033-m.
21. Hukowska-Szematowicz, B. Genetic and immunological characteristic of European strains of RHD (rabbit heamorrhagic disease) virus. *Pol. J. Environ. Stud.* **2013**, *2*, 1-114.
22. Rocha, G.; Alda, F.; Pages, A.; Merchan, T. Experimental transmission of rabbit haemorrhagic disease virus (RHDV) from rabbit to wild mice (Mus spretus and Apodemus sylvaticus) under laboratory conditions. *Infect Genet Evol* **2017**, *47*, 94-98, doi:10.1016/j.meegid.2016.11.016.
23. Niedzwiedzka-Rystwej, P.; Hukowska-Szematowicz, B.; Tokarz-Deptula, B.; Trzeciak-Ryczek, A.; Dzialo, J.; Deptula, W. Apoptosis of peripheral blood leucocytes in rabbits infected with different strains of rabbit haemorrhagic disease virus. *Acta Biochim Pol* **2013**, *60*, 65-69.
24. Niedźwiedzka-Rystwej, P.; Deptuła, W. Non-specific immunity in rabbits infected with 10 strains of the rabbit haemorrhagic disease virus with different biological properties. *Cent. eur. j. biol.* **2010**, *5*, 613-362, doi:10.2478/s11535-010-0049-6.
25. Niedzwiedzka-Rystwej, P.; Tokarz-Deptula, B.; Deptula, W. Reactivity of selected markers of innate and adaptive immunity in rabbits experimentally infected with antigenic variants of RHD (Lagovirus europaeus/GI.1a). *Vet Res Commun* **2022**, *46*, 233-242, doi:10.1007/s11259-021-09851-x.
26. Tokarz-Deptula, B. Immunity phenomena in rabbits infected with the RHD virus (rabbit haemorrhagic disease). *Pol. J. Environ. Sci.* **2009**, *7*, 1-81.
27. Fitzner, A.; Niedbalski, W. Różnorodność wirusa RHD-znaczenie epidemiologiczne, diagnostyczne, i immunologiczne. *Med. Weter.* **2017**, *7*,, 811-818, doi:10.21521/mw.5815.
28. Ferreira, P.G.; Costa, E.S.A.; Monteiro, E.; Oliveira, M.J.; Aguas, A.P. Liver enzymes and ultrastructure in rabbit haemorrhagic disease (RHD). *Vet Res Commun* **2006**, *30*, 393-401, doi:10.1007/s11259-006-3297-1.
29. Garcia-Lastra, R.; San-Miguel, B.; Crespo, I.; Jorquera, F.; Alvarez, M.; Gonzalez-Gallego, J.; Tunon, M.J. Signaling pathways involved in liver injury and regeneration in rabbit hemorrhagic disease, an animal model of virally-induced fulminant hepatic failure. *Vet Res* **2010**, *41*, 2, doi:10.1051/vetres/2009050.
30. Marques, R.M.; Costa, E.S.A.; Aguas, A.P.; Teixeira, L.; Ferreira, P.G. Early acute depletion of lymphocytes in calicivirus-infected adult rabbits. *Vet Res Commun* **2010**, *34*, 659-668, doi:10.1007/s11259-010-9437-7.
31. San-Miguel, B.; Alvarez, M.; Culebras, J.M.; Gonzalez-Gallego, J.; Tunon, M.J. N-acetyl-cysteine protects liver from apoptotic death in an animal model of fulminant hepatic failure. *Apoptosis* **2006**, *11*, 1945-1957, doi:10.1007/s10495-006-0090-0.
32. Sanchez-Campos, S.; Alvarez, M.; Culebras, J.M.; Gonzalez-Gallego, J.; Tunon, M.J. Pathogenic molecular mechanisms in an animal model of fulminant hepatic failure: rabbit hemorrhagic viral disease. *J Lab Clin Med* **2004**, *144*, 215-222, doi:10.1016/j.lab.2004.07.006.
33. Ferreira, P.G.; Costa-e-Silva, A.; Oliveira, M.J.; Monteiro, E.; Cunha, E.M.; Aguas, A.P. Severe leukopenia and liver biochemistry changes in adult rabbits after calicivirus infection. *Res Vet Sci* **2006**, *80*, 218-225, doi:10.1016/j.rvsc.2005.05.007.

34. Chen, S.Y.; Shien, J.H.; Ooi, H.K. Hyperlipidemia in rabbit hemorrhagic disease. *Exp Anim* **2008**, *57*, 479-483, doi:10.1538/expanim.57.479.
35. Niedzwiedzka-Rystwej, P.; Deptula, W. Apoptosis of peripheral blood leukocytes from rabbits infected with non-haemagglutinating strains of rabbit haemorrhagic disease virus (RHDV). *Vet Immunol Immunopathol* **2012**, *149*, 54-57, doi:10.1016/j.vetimm.2012.06.012.
36. *Terrestrial manual. Chapter 3.7.2 Rabbit haemorrhagic disease*; World Organisation for Animal Health.
37. Dalton, K.P.; Nicieza, I.; Balseiro, A.; Muguerza, M.A.; Rosell, J.M.; Casais, R.; Alvarez, A.L.; Parra, F. Variant rabbit hemorrhagic disease virus in young rabbits, Spain. *Emerg Infect Dis* **2012**, *18*, 2009-2012, doi:10.3201/eid1812.120341.
38. Rocchi, M.; Dagleish, M. Diagnosis and prevention of RHVD2 infection. *Vet Rec* **2018**, *182*, 604-605, doi:10.1136/vr.k2313.
39. Bębnowska, D.; Niedźwiedzka-Rystwej, P. Characteristics of a new variant of rabbit haemorrhagic disease virus – RHDV2. *Acta Biologica* **2019**, *26*, 83-97, doi:10.18276/ab.2019.26-08.
40. Hall, R.N.; King, T.; O'Connor, T.; Read, A.J.; Arrow, J.; Trought, K.; Duckworth, J.; Piper, M.; Strive, T. Age and Infectious Dose Significantly Affect Disease Progression after RHDV2 Infection in Naive Domestic Rabbits. *Viruses* **2021**, *13*, doi:10.3390/v13061184.
41. Capucci, L.; Cavadini, P.; Schiavitto, M.; Lombardi, G.; Lavazza, A. Increased pathogenicity in rabbit haemorrhagic disease virus type 2 (RHDV2). *Vet Rec* **2017**, *180*, 426, doi:10.1136/vr.104132.
42. Duarte, M.D.; Carvalho, C.L.; Barros, S.C.; Henriques, A.M.; Ramos, F.; Fagulha, T.; Luis, T.; Duarte, E.L.; Fevereiro, M. A real time Taqman RT-PCR for the detection of rabbit hemorrhagic disease virus 2 (RHDV2). *J Virol Methods* **2015**, *219*, 90-95, doi:10.1016/j.jviromet.2015.03.017.
43. Tu, T.; Zhou, Y.; Jiang, D.; Pang, M.; Wu, X.; Yao, X.; Luo, Y.; Yang, Z.; Ren, M.; Wang, Y. The pathogenicity comparison of Lagovirus europaeus GI.1 and GI.2 strains in China by using relative quantitative assay. *Sci Rep* **2022**, *12*, 20518, doi:10.1038/s41598-022-25118-0.
44. O'Toole, A.D.; Mohamed, F.M.; Zhang, J.; Brown, C.C. Early pathogenesis in rabbit hemorrhagic disease virus 2. *Microb Pathog* **2022**, *173*, 105814, doi:10.1016/j.micpath.2022.105814.
45. Abrantes, J.; Lopes, A.M. A Review on the Methods Used for the Detection and Diagnosis of Rabbit Hemorrhagic Disease Virus (RHDV). *Microorganisms* **2021**, *9*, doi:10.3390/microorganisms9050972.
46. Alonso, C.; Oviedo, J.M.; Martin-Alonso, J.M.; Diaz, E.; Boga, J.A.; Parra, F. Programmed cell death in the pathogenesis of rabbit hemorrhagic disease. *Arch Virol* **1998**, *143*, 321-332, doi:10.1007/s007050050289.
47. Park, J.H.; Lee, Y.S.; Itakura, C. Pathogenesis of acute necrotic hepatitis in rabbit hemorrhagic disease. *Lab Anim Sci* **1995**, *45*, 445-449.
48. Ueda, K.; Park, J.H.; Ochiai, K.; Itakura, C. Disseminated intravascular coagulation (DIC) in rabbit haemorrhagic disease. *Jpn J Vet Res* **1992**, *40*, 133-141.
49. Harcourt-Brown, N.; Silkstone, M.; Whitbread, T.J.; Harcourt-Brown, F.M. RHDV2 epidemic in UK pet rabbits. Part 1: clinical features, gross post mortem and histopathological findings. *J Small Anim Pract* **2020**, *61*, 419-427, doi:10.1111/jsap.13141.
50. Bebnowska, D.; Hrynkiewicz, R.; Rzeszotek, S.; Freus, M.; Poniewierska-Baran, A.; Niedzwiedzka-Rystwej, P. Apoptotic Cell Death in an Animal Model of Virus-Induced Acute Liver Failure-Observations during Lagovirus europaeus/GI.2 Infection. *Int J Mol Sci* **2024**, *25*, doi:10.3390/ijms25020798.
51. Bebnowska, D.; Hrynkiewicz, R.; Wisniewska, K.; Zabinska, M.; Rintz, E.; Pierzynowska, K.; Niedzwiedzka-Rystwej, P. Apoptosis activation during Lagovirus europaeus/GI.2 infection in rabbits. *Front Microbiol* **2023**, *14*, 1308018, doi:10.3389/fmicb.2023.1308018.
52. Chen, M.; Liu, X.; Hu, B.; Fan, Z.; Song, Y.; Wei, H.; Qiu, R.; Xu, W.; Zhu, W.; Wang, F. Rabbit Hemorrhagic Disease Virus Non-structural Protein 6 Induces Apoptosis in Rabbit Kidney Cells. *Front Microbiol* **2018**, *9*, 3308, doi:10.3389/fmicb.2018.03308.

53. Jung, J.Y.; Lee, B.J.; Tai, J.H.; Park, J.H.; Lee, Y.S. Apoptosis in rabbit haemorrhagic disease. *J Comp Pathol* **2000**, *123*, 135-140, doi:10.1053/jcpa.2000.0403.
54. Ni, Z.; Wei, W.; Liu, G.Q.; Chen, L.; Yu, B.; Yun, T.; Hua, J.G.; Li, S.M. [Studies on the apoptosis of RK13 cells induced by rabbit hemorrhagic disease virus]. *Bing Du Xue Bao* **2009**, *25*, 316-317.
55. Vallejo, D.; Crespo, I.; San-Miguel, B.; Alvarez, M.; Prieto, J.; Tunon, M.J.; Gonzalez-Gallego, J. Autophagic response in the Rabbit Hemorrhagic Disease, an animal model of virally-induced fulminant hepatic failure. *Vet Res* **2014**, *45*, 15, doi:10.1186/1297-9716-45-15.
56. Trzeciak-Ryczek, A.; Tokarz-Deptula, B.; Deptula, W. The importance of liver lesions and changes to biochemical and coagulation factors in the pathogenesis of RHD. *Acta Biochim Pol* **2015**, *62*, 169-171, doi:10.18388/abp.2014_943.
57. Tunon, M.J.; San Miguel, B.; Crespo, I.; Riezu-Boj, J.I.; Larrea, E.; Alvarez, M.; Gonzalez, I.; Bustos, M.; Gonzalez-Gallego, J.; Prieto, J. Cardiotrophin-1 promotes a high survival rate in rabbits with lethal fulminant hepatitis of viral origin. *J Virol* **2011**, *85*, 13124-13132, doi:10.1128/JVI.05725-11.
58. Ghavami, S.; Hashemi, M.; Kadkhoda, K.; Alavian, S.M.; Bay, G.H.; Los, M. Apoptosis in liver diseases--detection and therapeutic applications. *Med Sci Monit* **2005**, *11*, RA337-345.
59. Porter, A.G.; Janicke, R.U. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* **1999**, *6*, 99-104, doi:10.1038/sj.cdd.4400476.
60. Hu, B.; Wei, H.; Song, Y.; Chen, M.; Fan, Z.; Qiu, R.; Zhu, W.; Xu, W.; Wang, F. NF-kappaB and Keap1 Interaction Represses Nrf2-Mediated Antioxidant Response in Rabbit Hemorrhagic Disease Virus Infection. *J Virol* **2020**, *94*, doi:10.1128/JVI.00016-20.
61. San-Miguel, B.; Crespo, I.; Vallejo, D.; Alvarez, M.; Prieto, J.; Gonzalez-Gallego, J.; Tunon, M.J. Melatonin modulates the autophagic response in acute liver failure induced by the rabbit hemorrhagic disease virus. *J Pineal Res* **2014**, *56*, 313-321, doi:10.1111/jpi.12124.
62. Ramezani, A.; Nahad, M.P.; Faghihloo, E. The role of Nrf2 transcription factor in viral infection. *J Cell Biochem* **2018**, *119*, 6366-6382, doi:10.1002/jcb.26897.
63. Hukowska-Szematowicz, B.; Ostrycharz, E.; Dudzinska, W.; Roszkowska, P.; Siennicka, A.; Wojciechowska-Koszko, I. Digital PCR (dPCR) Quantification of miR-155-5p as a Potential Candidate for a Tissue Biomarker of Inflammation in Rabbits Infected with Lagovirus europaeus/Rabbit Hemorrhagic Disease Virus (RHDV). *Viruses* **2023**, *15*, doi:10.3390/v15071578.
64. Marcato, P.S.; Benazzi, C.; Vecchi, G.; Galeotti, M.; Della Salda, L.; Sarli, G.; Lucidi, P. Clinical and pathological features of viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev Sci Tech* **1991**, *10*, 371-392, doi:10.20506/rst.10.2.560.
65. Ferreira, P.G.; Costa, E.S.A.; Oliveira, M.J.; Monteiro, E.; Aguas, A.P. Leukocyte-hepatocyte interaction in calicivirus infection: differences between rabbits that are resistant or susceptible to rabbit haemorrhagic disease (RHD). *Vet Immunol Immunopathol* **2005**, *103*, 217-221, doi:10.1016/j.vetimm.2004.09.028.
66. Hrynkiewicz, R.; Bebnowska, D.; Niedzwiedzka-Rystwej, P. Myeloperoxidase and Lysozymes as a Pivotal Hallmark of Immunity Status in Rabbits. *Animals (Basel)* **2020**, *10*, doi:10.3390/ani10091581.
67. Hukowska-Szematowicz, B.; Deptuła, W. Non-specific cellular in rabbits experimentlaly infected with four Czech strains of the Rabbit Haemorrhagic Disease Virus with Different patho-genicity. *Pol. J. Environ. Stud.* **2012**, *21*, 879-892.
68. Hukowska-Szematowicz, B. Changes in innate and adaptive immune response to Lagovirus europaeus GI.1 (Rabbit Haemorrhagic Disease Virus-RHDV) infection. *Acta Biol.* **2020**, *27*, 77-91.
69. Ostrycharz, E.; Blatkiewicz, M.; Hukowska-Szematowicz, B. Expression of the Master Anti-Inflammatory Regulator IL-10 during Lagovirus Europaeus GI.1/RHDV (Rabbit Hemorrhagic Disease Virus) Infection. In *Młodzi naukowcy 2.0. Tom 1.* , Korpysa, J., Niedźwiedzka-Rystwej, P., Eds.; Fundacja Centrum Badań Socjologicznych (Centre of Sociological Research): Szczecin, 2021; pp. 59-72.
70. Trzeciak-Ryczek, A.; Tokarz-Deptula, B.; Deptula, W. Expression of IL-1Ra, IL-6, IL-8, IL-18, TNF-alpha and IFN-gamma genes in peripheral blood leukocytes of rabbits infected with

RHDV (Rabbit Haemorrhagic Disease Virus). *Dev Comp Immunol* **2017**, *76*, 310-315, doi:10.1016/j.dci.2017.07.005.
71. Trzeciak-Ryczek, A.; Tokarz-Deptula, B.; Deptula, W. Expression of IL-1beta, IL-2, IL-10, TNF-beta and GM-CSF in peripheral blood leukocytes of rabbits experimentally infected with rabbit haemorrhagic disease virus. *Vet Microbiol* **2016**, *186*, 71-81, doi:10.1016/j.vetmic.2016.02.021.
72. Semerjyan, A.B.; Sargsyan, M.A.; Arzumanyan, H.H.; Hakobyan, L.H.; Abroyan, L.O.; Semerjyan, Z.B.; Avetisyan, A.S.; Karalova, E.M.; Manukyan, D.M.; Matevosyan, H.S.; et al. Immune cell pathology in rabbit hemorrhagic disease. *Vet World* **2019**, *12*, 1332-1340, doi:10.14202/vetworld.2019.1332-1340.
73. Teixeira, L.; Marques, R.M.; Aguas, A.P.; Ferreira, P.G. Regulatory T cells are decreased in acute RHDV lethal infection of adult rabbits. *Vet Immunol Immunopathol* **2012**, *148*, 343-347, doi:10.1016/j.vetimm.2012.05.006.
74. Yu, J.; Li, Y.; Xiao, L.; Xie, J.; Guo, Z.; Ye, Y.; Lin, Y.; Cao, Y.; Wu, X.; Mao, C.; et al. Neglected Spleen Transcriptional Profile Reveals Inflammatory Disorder Conferred by Rabbit Hemorrhagic Disease Virus 2 Infection. *Viruses* **2024**, *16*, doi:10.3390/v16040495.
75. Hukowska-Szematowicz, B.; Deptuła, W. Peripheral blood lymphocytes in rabbits infected with Czech strains, CAMPV-562 and CAMPV-558 of RHD virus. *Cent. Eur. J. Immunol.* **2008**, *33*, 8-13.
76. Niedźwiedzka-Rystwej, P.; Tokarz-Deptuła, B.; Hukowska-Szematowicz, B.; Działo, J.; Trzeciak-Ryczek, A. Peripheral blood lymphocytes B and T and their subpopulation in rabbits infected with rabbit haemorrhagic diseaase virus (RHDV) *Med. Weter.* **2013**, *59*, 484-487.
77. Hefler, J.; Marfil-Garza, B.A.; Pawlick, R.L.; Freed, D.H.; Karvellas, C.J.; Bigam, D.L.; Shapiro, A.M.J. Preclinical models of acute liver failure: a comprehensive review. *PeerJ* **2021**, *9*, e12579, doi:10.7717/peerj.12579.
78. Lai, W.K.; Murphy, W. Management of acute liver failure. *Continuing Education in Anaesthesia Critical Care & Pain* **2004**, *4*, 40-43, doi:10.1093/bjaceaccp/mkh013.
79. Tunon, M.J.; Sanchez-Campos, S.; Garcia-Ferreras, J.; Alvarez, M.; Jorquera, F.; Gonzalez-Gallego, J. Rabbit hemorrhagic viral disease: characterization of a new animal model of fulminant liver failure. *J Lab Clin Med* **2003**, *141*, 272-278, doi:10.1067/mlc.2003.30.
80. Tunon, M.J.; Alvarez, M.; Culebras, J.M.; Gonzalez-Gallego, J. An overview of animal models for investigating the pathogenesis and therapeutic strategies in acute hepatic failure. *World J Gastroenterol* **2009**, *15*, 3086-3098, doi:10.3748/wjg.15.3086.
81. Bonvehi, C.; Ardiaca, M.; Montesinos, A.; Juan-Salles, C.; Gomez, A.; Teso, B.; Barbero, S.; Ferrera, S.E. Clinicopathologic findings of naturally occurring Rabbit Hemorrhagic Disease Virus 2 infection in pet rabbits. *Vet Clin Pathol* **2019**, *48*, 89-95, doi:10.1111/vcp.12701.
82. Esteves, P.J.; Abrantes, J.; Baldauf, H.M.; BenMohamed, L.; Chen, Y.; Christensen, N.; Gonzalez-Gallego, J.; Giacani, L.; Hu, J.; Kaplan, G.; et al. Author Correction: The wide utility of rabbits as models of human diseases. *Exp Mol Med* **2019**, *51*, 1, doi:10.1038/s12276-019-0252-0.
83. Bartel, D.P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **2004**, *116*, 281-297, doi:10.1016/s0092-8674(04)00045-5.
84. Lee, R.; Feinbaum, R.; Ambros, V. A short history of a short RNA. *Cell* **2004**, *116*, S89-92, 81 p following S96, doi:10.1016/s0092-8674(04)00035-2.
85. Dexheimer, P.J.; Cochella, L. MicroRNAs: From Mechanism to Organism. *Front Cell Dev Biol* **2020**, *8*, 409, doi:10.3389/fcell.2020.00409.
86. Saha, S. Role of microRNA in Oxidative Stress. *Stresses* **2024**, *4*, 269-281, doi:10.3390/stresses4020016.
87. Nejad, C.; Stunden, H.J.; Gantier, M.P. A guide to miRNAs in inflammation and innate immune responses. *FEBS J* **2018**, *285*, 3695-3716, doi:10.1111/febs.14482.
88. O'Brien, J.; Hayder, H.; Zayed, Y.; Peng, C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front Endocrinol (Lausanne)* **2018**, *9*, 402, doi:10.3389/fendo.2018.00402.
89. Rajewsky, N. microRNA target predictions in animals. *Nat Genet* **2006**, *38 Suppl*, S8-13, doi:10.1038/ng1798.

90. Berezikov, E.; Guryev, V.; van de Belt, J.; Wienholds, E.; Plasterk, R.H.; Cuppen, E. Phylogenetic shadowing and computational identification of human microRNA genes. *Cell* **2005**, *120*, 21-24, doi:10.1016/j.cell.2004.12.031.
91. Wang, J.; Chen, J.; Sen, S. MicroRNA as Biomarkers and Diagnostics. *J Cell Physiol* **2016**, *231*, 25-30, doi:10.1002/jcp.25056.
92. Chakraborty, C.; Sharma, A.R.; Sharma, G.; Lee, S.S. Therapeutic advances of miRNAs: A preclinical and clinical update. *J Adv Res* **2021**, *28*, 127-138, doi:10.1016/j.jare.2020.08.012.
93. Lee, Y.; Jeon, K.; Lee, J.T.; Kim, S.; Kim, V.N. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J* **2002**, *21*, 4663-4670, doi:10.1093/emboj/cdf476.
94. Ha, M.; Kim, V.N. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* **2014**, *15*, 509-524, doi:10.1038/nrm3838.
95. Ostrycharz, E.; Hukowska-Szematowicz, B. Micro-Players of Great Significance-Host microRNA Signature in Viral Infections in Humans and Animals. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms231810536.
96. Bauer, A.N.; Majumdar, N.; Williams, F.; Rajput, S.; Pokhrel, L.R.; Cook, P.P.; Akula, S.M. MicroRNAs: Small but Key Players in Viral Infections and Immune Responses to Viral Pathogens. *Biology (Basel)* **2023**, *12*, doi:10.3390/biology12101334.
97. Sadri Nahand, J.; Shojaie, L.; Akhlagh, S.A.; Ebrahimi, M.S.; Mirzaei, H.R.; Bannazadeh Baghi, H.; Mahjoubin-Tehran, M.; Rezaei, N.; Hamblin, M.R.; Tajiknia, V.; et al. Cell death pathways and viruses: Role of microRNAs. *Mol Ther Nucleic Acids* **2021**, *24*, 487-511, doi:10.1016/j.omtn.2021.03.011.
98. Verburg, S.G.; Lelievre, R.M.; Westerveld, M.J.; Inkol, J.M.; Sun, Y.L.; Workenhe, S.T. Viral-mediated activation and inhibition of programmed cell death. *PLoS Pathog* **2022**, *18*, e1010718, doi:10.1371/journal.ppat.1010718.
99. Zhou, X.; Jiang, W.; Liu, Z.; Liu, S.; Liang, X. Virus Infection and Death Receptor-Mediated Apoptosis. *Viruses* **2017**, *9*, doi:10.3390/v9110316.
100. Imre, G. Cell death signalling in virus infection. *Cell Signal* **2020**, *76*, 109772, doi:10.1016/j.cellsig.2020.109772.
101. Hacker, G. Apoptosis in infection. *Microbes Infect* **2018**, *20*, 552-559, doi:10.1016/j.micinf.2017.10.006.
102. Chipuk, J.E.; Kuwana, T.; Bouchier-Hayes, L.; Droin, N.M.; Newmeyer, D.D.; Schuler, M.; Green, D.R. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* **2004**, *303*, 1010-1014, doi:10.1126/science.1092734.
103. Galluzzi, L.; Brenner, C.; Morselli, E.; Touat, Z.; Kroemer, G. Viral control of mitochondrial apoptosis. *PLoS Pathog* **2008**, *4*, e1000018, doi:10.1371/journal.ppat.1000018.
104. Liu, N.; Zhang, J.; Jiao, T.; Li, Z.; Peng, J.; Cui, Z.; Ye, X. Hepatitis B virus inhibits apoptosis of hepatoma cells by sponging the MicroRNA 15a/16 cluster. *J Virol* **2013**, *87*, 13370-13378, doi:10.1128/JVI.02130-13.
105. Wu, H.C.; Huang, C.L.; Wang, H.W.; Hsu, W.F.; Tsai, T.Y.; Chen, S.H.; Peng, C.Y. Serum miR-21 correlates with the histological stage of chronic hepatitis B-associated liver fibrosis. *Int J Clin Exp Pathol* **2019**, *12*, 3819-3829.
106. Qiu, X.; Dong, S.; Qiao, F.; Lu, S.; Song, Y.; Lao, Y.; Li, Y.; Zeng, T.; Hu, J.; Zhang, L.; et al. HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene* **2013**, *32*, 3296-3305, doi:10.1038/onc.2013.150.
107. Damania, P.; Sen, B.; Dar, S.B.; Kumar, S.; Kumari, A.; Gupta, E.; Sarin, S.K.; Venugopal, S.K. Hepatitis B virus induces cell proliferation via HBx-induced microRNA-21 in hepatocellular carcinoma by targeting programmed cell death protein4 (PDCD4) and phosphatase and tensin homologue (PTEN). *PLoS One* **2014**, *9*, e91745, doi:10.1371/journal.pone.0091745.
108. Zhang, Y.; Wei, W.; Cheng, N.; Wang, K.; Li, B.; Jiang, X.; Sun, S. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology* **2012**, *56*, 1631-1640, doi:10.1002/hep.25849.
109. Clement, S.; Sobolewski, C.; Gomes, D.; Rojas, A.; Goossens, N.; Conzelmann, S.; Calo, N.; Negro, F.; Foti, M. Activation of the oncogenic miR-21-5p promotes HCV replication and

steatosis induced by the viral core 3a protein. *Liver Int* **2019**, *39*, 1226-1236, doi:10.1111/liv.14112.
110. Sharma, V.K.; Raimondi, V.; Ruggero, K.; Pise-Masison, C.A.; Cavallari, I.; Silic-Benussi, M.; Ciminale, V.; D'Agostino, D.M. Expression of miR-34a in T-Cells Infected by Human T-Lymphotropic Virus 1. *Front Microbiol* **2018**, *9*, 832, doi:10.3389/fmicb.2018.00832.
111. Amini, M.A.; Karimi, J.; Talebi, S.S.; Piri, H. The Association of COVID-19 and Reactive Oxygen Species Modulator 1 (ROMO1) with Oxidative Stress. *Chonnam Med J* **2022**, *58*, 1-5, doi:10.4068/cmj.2022.58.1.1.
112. Gain, C.; Song, S.; Angtuaco, T.; Satta, S.; Kelesidis, T. The role of oxidative stress in the pathogenesis of infections with coronaviruses. *Front Microbiol* **2022**, *13*, 1111930, doi:10.3389/fmicb.2022.1111930.
113. Ivanov, A.V.; Bartosch, B.; Isaguliants, M.G. Oxidative Stress in Infection and Consequent Disease. *Oxid Med Cell Longev* **2017**, *2017*, 3496043, doi:10.1155/2017/3496043.
114. Peterhans, E. Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation. *J Nutr* **1997**, *127*, 962S-965S, doi:10.1093/jn/127.5.962S.
115. Banerjee, J.; Khanna, S.; Bhattacharya, A. MicroRNA Regulation of Oxidative Stress. *Oxid Med Cell Longev* **2017**, *2017*, 2872156, doi:10.1155/2017/2872156.
116. Su, Y.C.; Huang, Y.F.; Wu, Y.W.; Chen, H.F.; Wu, Y.H.; Hsu, C.C.; Hsu, Y.C.; Lee, J.C. MicroRNA-155 inhibits dengue virus replication by inducing heme oxygenase-1-mediated antiviral interferon responses. *FASEB J* **2020**, *34*, 7283-7294, doi:10.1096/fj.201902878R.
117. Yachie, A. Heme Oxygenase-1 Deficiency and Oxidative Stress: A Review of 9 Independent Human Cases and Animal Models. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22041514.
118. Yusoff, F.M.; Maruhashi, T.; Kawano, K.I.; Nakashima, A.; Chayama, K.; Tashiro, S.; Igarashi, K.; Higashi, Y. Bach1 plays an important role in angiogenesis through regulation of oxidative stress. *Microvasc Res* **2021**, *134*, 104126, doi:10.1016/j.mvr.2020.104126.
119. Escalera-Cueto, M.; Medina-Martinez, I.; del Angel, R.M.; Berumen-Campos, J.; Gutierrez-Escolano, A.L.; Yocupicio-Monroy, M. Let-7c overexpression inhibits dengue virus replication in human hepatoma Huh-7 cells. *Virus Res* **2015**, *196*, 105-112, doi:10.1016/j.virusres.2014.11.010.
120. Chen, S.; Ni, M.; Yu, B.; Lv, T.; Lu, M.; Gong, F. Construction and identification of a human liver specific microRNA eukaryotic expression vector. *Cell Mol Immunol* **2007**, *4*, 473-477.
121. Chen, Y.; Shen, A.; Rider, P.J.; Yu, Y.; Wu, K.; Mu, Y.; Hao, Q.; Liu, Y.; Gong, H.; Zhu, Y.; et al. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. *FASEB J* **2011**, *25*, 4511-4521, doi:10.1096/fj.11-187781.
122. Fan, C.G.; Wang, C.M.; Tian, C.; Wang, Y.; Li, L.; Sun, W.S.; Li, R.F.; Liu, Y.G. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. *Oncol Rep* **2011**, *26*, 1281-1286, doi:10.3892/or.2011.1375.
123. Shan, Y.; Zheng, J.; Lambrecht, R.W.; Bonkovsky, H.L. Reciprocal effects of micro-RNA-122 on expression of heme oxygenase-1 and hepatitis C virus genes in human hepatocytes. *Gastroenterology* **2007**, *133*, 1166-1174, doi:10.1053/j.gastro.2007.08.002.
124. Herengt, A.; Thyrsted, J.; Holm, C.K. NRF2 in Viral Infection. *Antioxidants (Basel)* **2021**, *10*, doi:10.3390/antiox10091491.
125. Daskou, M.; Fotooh Abadi, L.; Gain, C.; Wong, M.; Sharma, E.; Kombe Kombe, A.J.; Nanduri, R.; Kelesidis, T. The Role of the NRF2 Pathway in the Pathogenesis of Viral Respiratory Infections. *Pathogens* **2023**, *13*, doi:10.3390/pathogens13010039.
126. Wasik, U.; Milkiewicz, M.; Kempinska-Podhorodecka, A.; Milkiewicz, P. Protection against oxidative stress mediated by the Nrf2/Keap1 axis is impaired in Primary Biliary Cholangitis. *Sci Rep* **2017**, *7*, 44769, doi:10.1038/srep44769.
127. Zhou, Y.; Li, K.S.; Liu, L.; Li, S.L. MicroRNA-132 promotes oxidative stress-induced pyroptosis by targeting sirtuin 1 in myocardial ischaemia-reperfusion injury. *Int J Mol Med* **2020**, *45*, 1942-1950, doi:10.3892/ijmm.2020.4557.
128. Mao, M.; Yang, L.; Hu, J.; Liu, B.; Zhang, X.; Liu, Y.; Wang, P.; Li, H. Oncogenic E3 ubiquitin ligase NEDD4 binds to KLF8 and regulates the microRNA-132/NRF2 axis in bladder cancer. *Exp Mol Med* **2022**, *54*, 47-60, doi:10.1038/s12276-021-00663-2.

129. Koyama, S.; Ishii, K.J.; Coban, C.; Akira, S. Innate immune response to viral infection. *Cytokine* **2008**, *43*, 336-341, doi:10.1016/j.cyto.2008.07.009.
130. Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X.; Zhao, L. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* **2018**, *9*, 7204-7218, doi:10.18632/oncotarget.23208.
131. Lawrence, T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol* **2009**, *1*, a001651, doi:10.1101/cshperspect.a001651.
132. Iacobazzi, D.; Convertini, P.; Todisco, S.; Santarsiero, A.; Iacobazzi, V.; Infantino, V. New Insights into NF-kappaB Signaling in Innate Immunity: Focus on Immunometabolic Crosstalks. *Biology (Basel)* **2023**, *12*, doi:10.3390/biology12060776.
133. Zhao, J.; He, S.; Minassian, A.; Li, J.; Feng, P. Recent advances on viral manipulation of NF-kappaB signaling pathway. *Curr Opin Virol* **2015**, *15*, 103-111, doi:10.1016/j.coviro.2015.08.013.
134. Liu, T.; Zhang, L.; Joo, D.; Sun, S.C. NF-kappaB signaling in inflammation. *Signal Transduct Target Ther* **2017**, *2*, 17023-, doi:10.1038/sigtrans.2017.23.
135. Mahesh, G.; Biswas, R. MicroRNA-155: A Master Regulator of Inflammation. *J Interferon Cytokine Res* **2019**, *39*, 321-330, doi:10.1089/jir.2018.0155.
136. Pareek, S.; Roy, S.; Kumari, B.; Jain, P.; Banerjee, A.; Vrati, S. MiR-155 induction in microglial cells suppresses Japanese encephalitis virus replication and negatively modulates innate immune responses. *J Neuroinflammation* **2014**, *11*, 97, doi:10.1186/1742-2094-11-97.
137. Hu, J.; Huang, S.; Liu, X.; Zhang, Y.; Wei, S.; Hu, X. miR-155: An Important Role in Inflammation Response. *J Immunol Res* **2022**, *2022*, 7437281, doi:10.1155/2022/7437281.
138. Peng, C.; Wang, H.; Zhang, W.J.; Jie, S.H.; Tong, Q.X.; Lu, M.J.; Yang, D.L. Inhibitory effect of miR-125b on hepatitis C virus core protein-induced TLR2/MyD88 signaling in THP-1 cells. *World J Gastroenterol* **2016**, *22*, 4354-4361, doi:10.3748/wjg.v22.i17.4354.
139. Zhang, X.; Guo, Y.; Xu, X.; Tang, T.; Sun, L.; Wang, H.; Zhou, W.; Fang, L.; Li, Q.; Xie, P. miR-146a promotes Borna disease virus 1 replication through IRAK1/TRAF6/NF-kappaB signaling pathway. *Virus Res* **2019**, *271*, 197671, doi:10.1016/j.virusres.2019.197671.
140. Sharma, N.; Verma, R.; Kumawat, K.L.; Basu, A.; Singh, S.K. miR-146a suppresses cellular immune response during Japanese encephalitis virus JaOArS982 strain infection in human microglial cells. *J Neuroinflammation* **2015**, *12*, 30, doi:10.1186/s12974-015-0249-0.
141. Wu, S.; He, L.; Li, Y.; Wang, T.; Feng, L.; Jiang, L.; Zhang, P.; Huang, X. miR-146a facilitates replication of dengue virus by dampening interferon induction by targeting TRAF6. *J Infect* **2013**, *67*, 329-341, doi:10.1016/j.jinf.2013.05.003.
142. Fei, Y.; Chaulagain, A.; Wang, T.; Chen, Y.; Liu, J.; Yi, M.; Wang, Y.; Huang, Y.; Lin, L.; Chen, S.; et al. MiR-146a down-regulates inflammatory response by targeting TLR3 and TRAF6 in Coxsackievirus B infection. *RNA* **2020**, *26*, 91-100, doi:10.1261/rna.071985.119.
143. Shi, M.; Lu, Q.; Zhao, Y.; Ding, Z.; Yu, S.; Li, J.; Ji, M.; Fan, H.; Hou, S. miR-223: a key regulator of pulmonary inflammation. *Front Med (Lausanne)* **2023**, *10*, 1187557, doi:10.3389/fmed.2023.1187557.
144. Olejnik, J.; Hume, A.J.; Muhlberger, E. Toll-like receptor 4 in acute viral infection: Too much of a good thing. *PLoS Pathog* **2018**, *14*, e1007390, doi:10.1371/journal.ppat.1007390.
145. Gao, Z.; Dou, Y.; Chen, Y.; Zheng, Y. MicroRNA roles in the NF- kappaB signaling pathway during viral infections. *Biomed Res Int* **2014**, *2014*, 436097, doi:10.1155/2014/436097.
146. Guo, H.; Callaway, J.B.; Ting, J.P. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med* **2015**, *21*, 677-687, doi:10.1038/nm.3893.
147. Houshmandfar, S.; Saeedi-Boroujeni, A.; Rashno, M.; Khodadadi, A.; Mahmoudian-Sani, M.R. miRNA-223 as a regulator of inflammation and NLRP3 inflammasome, the main fragments in the puzzle of immunopathogenesis of different inflammatory diseases and COVID-19. *Naunyn Schmiedebergs Arch Pharmacol* **2021**, *394*, 2187-2195, doi:10.1007/s00210-021-02163-6.
148. Zamani, P.; Oskuee, R.K.; Atkin, S.L.; Navashenaq, J.G.; Sahebkar, A. MicroRNAs as important regulators of the NLRP3 inflammasome. *Prog Biophys Mol Biol* **2020**, *150*, 50-61, doi:10.1016/j.pbiomolbio.2019.05.004.
149. Chen, S.; Smith, B.A.; Iype, J.; Prestipino, A.; Pfeifer, D.; Grundmann, S.; Schmitt-Graeff, A.; Idzko, M.; Beck, Y.; Prinz, G.; et al. MicroRNA-155-deficient dendritic cells cause less severe

GVHD through reduced migration and defective inflammasome activation. *Blood* **2015**, *126*, 103-112, doi:10.1182/blood-2014-12-617258.
150. Haneklaus, M.; Gerlic, M.; Kurowska-Stolarska, M.; Rainey, A.A.; Pich, D.; McInnes, I.B.; Hammerschmidt, W.; O'Neill, L.A.; Masters, S.L. Cutting edge: miR-223 and EBV miR-BART15 regulate the NLRP3 inflammasome and IL-1beta production. *J Immunol* **2012**, *189*, 3795-3799, doi:10.4049/jimmunol.1200312.
151. Ahmad, A.; Imran, M.; Ahsan, H. Biomarkers as Biomedical Bioindicators: Approaches and Techniques for the Detection, Analysis, and Validation of Novel Biomarkers of Diseases. *Pharmaceutics* **2023**, *15*, doi:10.3390/pharmaceutics15061630.
152. Sunde, R.A. mRNA transcripts as molecular biomarkers in medicine and nutrition. *J Nutr Biochem* **2010**, *21*, 665-670, doi:10.1016/j.jnutbio.2009.11.012.
153. He, J.; Wu, F.; Han, Z.; Hu, M.; Lin, W.; Li, Y.; Cao, M. Biomarkers (mRNAs and Non-Coding RNAs) for the Diagnosis and Prognosis of Colorectal Cancer - From the Body Fluid to Tissue Level. *Front Oncol* **2021**, *11*, 632834, doi:10.3389/fonc.2021.632834.
154. Koziy, R.V.; Bracamonte, J.L.; Katselis, G.S.; Udenze, D.; Hayat, S.; Hammond, S.A.; Simko, E. Putative mRNA Biomarkers for the Eradication of Infection in an Equine Experimental Model of Septic Arthritis. *Vet Sci* **2024**, *11*, doi:10.3390/vetsci11070299.
155. Finucane, D.M.; Bossy-Wetzel, E.; Waterhouse, N.J.; Cotter, T.G.; Green, D.R. Bax-induced caspase activation and apoptosis via cytochrome c release from mitochondria is inhibitable by Bcl-xL. *J Biol Chem* **1999**, *274*, 2225-2233, doi:10.1074/jbc.274.4.2225.
156. Pepper, C.; Hoy, T.; Bentley, D.P. Bcl-2/Bax ratios in chronic lymphocytic leukaemia and their correlation with in vitro apoptosis and clinical resistance. *Br J Cancer* **1997**, *76*, 935-938, doi:10.1038/bjc.1997.487.
157. Li, K.; Wu, D.; Chen, X.; Zhang, T.; Zhang, L.; Yi, Y.; Miao, Z.; Jin, N.; Bi, X.; Wang, H.; et al. Current and emerging biomarkers of cell death in human disease. *Biomed Res Int* **2014**, *2014*, 690103, doi:10.1155/2014/690103.
158. Saddam, M.; Paul, S.K.; Habib, M.A.; Abrar Fahim, M.A.; Mimi, A.; Islam, S.; Paul, B.; Helal, M.U. Emerging biomarkers and potential therapeutics of the BCL-2 protein family: the apoptotic and anti-apoptotic context. *Egypt J Med Hum Genet* **2024**, *25*, doi:10.1186/s43042-024-00485-7.
159. Choi, A.M.; Alam, J. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* **1996**, *15*, 9-19, doi:10.1165/ajrcmb.15.1.8679227.
160. Tanaka, T.; Narazaki, M.; Kishimoto, T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* **2014**, *6*, a016295, doi:10.1101/cshperspect.a016295.
161. Lai, W.Y.; Wang, J.W.; Huang, B.T.; Lin, E.P.; Yang, P.C. A Novel TNF-alpha-Targeting Aptamer for TNF-alpha-Mediated Acute Lung Injury and Acute Liver Failure. *Theranostics* **2019**, *9*, 1741-1751, doi:10.7150/thno.30972.
162. Slaats, J.; Ten Oever, J.; van de Veerdonk, F.L.; Netea, M.G. IL-1beta/IL-6/CRP and IL-18/ferritin: Distinct Inflammatory Programs in Infections. *PLoS Pathog* **2016**, *12*, e1005973, doi:10.1371/journal.ppat.1005973.
163. Pirhonen, J. Regulation of IL-18 expression in virus infection. *Scand J Immunol* **2001**, *53*, 533-539, doi:10.1046/j.1365-3083.2001.00939.x.
164. Kaplanski, G. Interleukin-18: Biological properties and role in disease pathogenesis. *Immunol Rev* **2018**, *281*, 138-153, doi:10.1111/imr.12616.
165. Dinarello, C.A. Interleukin-18, a proinflammatory cytokine. *Eur Cytokine Netw* **2000**, *11*, 483-486.
166. Jain, S.; Gautam, V.; Naseem, S. Acute-phase proteins: As diagnostic tool. *J Pharm Bioallied Sci* **2011**, *3*, 118-127, doi:10.4103/0975-7406.76489.
167. Hukowska-Szematowicz, B.; Maciejak-Jastrzebska, A.; Blatkiewicz, M.; Maciak, K.; Gora, M.; Janiszewska, J.; Burzynska, B. Changes in MicroRNA Expression during Rabbit Hemorrhagic Disease Virus (RHDV) Infection. *Viruses* **2020**, *12*, doi:10.3390/v12090965.
168. He, G.; Ding, J.; Zhang, Y.; Cai, M.; Yang, J.; Cho, W.C.; Zheng, Y. microRNA-21: a key modulator in oncogenic viral infections. *RNA Biol* **2021**, *18*, 809-817, doi:10.1080/15476286.2021.1880756.

169. Gao, X.; Zhao, S. miRNA-16-5p inhibits the apoptosis of high glucose-induced pancreatic beta cells via targeting of CXCL10: potential biomarkers in type 1 diabetes mellitus. *Endokrynol Pol* **2020**, *71*, 404-410, doi:10.5603/EP.a2020.0046.
170. Jiang, D.; Li, M.; Yu, Y.; Shi, H.; Chen, R. microRNA-34a aggravates coxsackievirus B3-induced apoptosis of cardiomyocytes through the SIRT1-p53 pathway. *J Med Virol* **2019**, *91*, 1643-1651, doi:10.1002/jmv.25482.
171. Buscaglia, L.E.; Li, Y. Apoptosis and the target genes of microRNA-21. *Chin J Cancer* **2011**, *30*, 371-380, doi:10.5732/cjc.011.10132.
172. Wei, J.; Feng, L.; Li, Z.; Xu, G.; Fan, X. MicroRNA-21 activates hepatic stellate cells via PTEN/Akt signaling. *Biomed Pharmacother* **2013**, *67*, 387-392, doi:10.1016/j.biopha.2013.03.014.
173. Ghafouri-Fard, S.; Abak, A.; Shoorei, H.; Mohaqiq, M.; Majidpoor, J.; Sayad, A.; Taheri, M. Regulatory role of microRNAs on PTEN signaling. *Biomed Pharmacother* **2021**, *133*, 110986, doi:10.1016/j.biopha.2020.110986.
174. Cimmino, A.; Calin, G.A.; Fabbri, M.; Iorio, M.V.; Ferracin, M.; Shimizu, M.; Wojcik, S.E.; Aqeilan, R.I.; Zupo, S.; Dono, M.; et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A* **2005**, *102*, 13944-13949, doi:10.1073/pnas.0506654102.
175. Wu, G.; Yu, F.; Xiao, Z.; Xu, K.; Xu, J.; Tang, W.; Wang, J.; Song, E. Hepatitis B virus X protein downregulates expression of the miR-16 family in malignant hepatocytes in vitro. *Br J Cancer* **2011**, *105*, 146-153, doi:10.1038/bjc.2011.190.
176. Lai, X.; Dreyer, F.S.; Cantone, M.; Eberhardt, M.; Gerer, K.F.; Jaitly, T.; Uebe, S.; Lischer, C.; Ekici, A.; Wittmann, J.; et al. Network- and systems-based re-engineering of dendritic cells with non-coding RNAs for cancer immunotherapy. *Theranostics* **2021**, *11*, 1412-1428, doi:10.7150/thno.53092.
177. Yamakuchi, M.; Lowenstein, C.J. MiR-34, SIRT1 and p53: the feedback loop. *Cell Cycle* **2009**, *8*, 712-715, doi:10.4161/cc.8.5.7753.
178. Hermeking, H. The miR-34 family in cancer and apoptosis. *Cell Death Differ* **2010**, *17*, 193-199, doi:10.1038/cdd.2009.56.
179. Klieser, E.; Mayr, C.; Kiesslich, T.; Wissniowski, T.; Fazio, P.D.; Neureiter, D.; Ocker, M. The Crosstalk of miRNA and Oxidative Stress in the Liver: From Physiology to Pathology and Clinical Implications. *Int J Mol Sci* **2019**, *20*, doi:10.3390/ijms20215266.
180. Xu, K.; Chen, C.; Wu, Y.; Wu, M.; Lin, L. Advances in miR-132-Based Biomarker and Therapeutic Potential in the Cardiovascular System. *Front Pharmacol* **2021**, *12*, 751487, doi:10.3389/fphar.2021.751487.
181. Tang, B.; Xiao, B.; Liu, Z.; Li, N.; Zhu, E.D.; Li, B.S.; Xie, Q.H.; Zhuang, Y.; Zou, Q.M.; Mao, X.H. Identification of MyD88 as a novel target of miR-155, involved in negative regulation of Helicobacter pylori-induced inflammation. *FEBS Lett* **2010**, *584*, 1481-1486, doi:10.1016/j.febslet.2010.02.063.
182. Bandyopadhyay, S.; Long, M.E.; Allen, L.A. Differential expression of microRNAs in Francisella tularensis-infected human macrophages: miR-155-dependent downregulation of MyD88 inhibits the inflammatory response. *PLoS One* **2014**, *9*, e109525, doi:10.1371/journal.pone.0109525.
183. Imaizumi, T.; Tanaka, H.; Tajima, A.; Yokono, Y.; Matsumiya, T.; Yoshida, H.; Tsuruga, K.; Aizawa-Yashiro, T.; Hayakari, R.; Inoue, I.; et al. IFN-gamma and TNF-alpha synergistically induce microRNA-155 which regulates TAB2/IP-10 expression in human mesangial cells. *Am J Nephrol* **2010**, *32*, 462-468, doi:10.1159/000321365.
184. Zhou, H.; Huang, X.; Cui, H.; Luo, X.; Tang, Y.; Chen, S.; Wu, L.; Shen, N. miR-155 and its star-form partner miR-155* cooperatively regulate type I interferon production by human plasmacytoid dendritic cells. *Blood* **2010**, *116*, 5885-5894, doi:10.1182/blood-2010-04-280156.
185. Wei, T.T.; Cheng, Z.; Hu, Z.D.; Zhou, L.; Zhong, R.Q. Upregulated miR-155 inhibits inflammatory response induced by C. albicans in human monocytes derived dendritic cells via targeting p65 and BCL-10. *Ann Transl Med* **2019**, *7*, 758, doi:10.21037/atm.2019.11.71.
186. Shen, S.M.; Jiang, H.; Zhao, J.N.; Shi, Y. Down-regulation of miR-155 inhibits inflammatory response in human pulmonary microvascular endothelial cells infected with influenza A virus

by targeting sphingosine-1-phosphate receptor 1. *Chin Med J (Engl)* **2020**, *133*, 2429-2436, doi:10.1097/CM9.0000000000001036.
187. Yuan, S.; Wu, Q.; Wang, Z.; Che, Y.; Zheng, S.; Chen, Y.; Zhong, X.; Shi, F. miR-223: An Immune Regulator in Infectious Disorders. *Front Immunol* **2021**, *12*, 781815, doi:10.3389/fimmu.2021.781815.
188. Ma, X.; Tian, D.; Lv, W.; Gao, B.; Ma, Z.; Zheng, X. Anti-inflammatory effects of microRNA-223 on sepsis-induced lung injury in rats by targeting the Toll-like receptor signaling pathway. *Exp Ther Med* **2021**, *22*, 964, doi:10.3892/etm.2021.10396.
189. Li, T.; Morgan, M.J.; Choksi, S.; Zhang, Y.; Kim, Y.S.; Liu, Z.G. MicroRNAs modulate the noncanonical transcription factor NF-kappaB pathway by regulating expression of the kinase IKKalpha during macrophage differentiation. *Nat Immunol* **2010**, *11*, 799-805, doi:10.1038/ni.1918.
190. Bauernfeind, F.; Rieger, A.; Schildberg, F.A.; Knolle, P.A.; Schmid-Burgk, J.L.; Hornung, V. NLRP3 inflammasome activity is negatively controlled by miR-223. *J Immunol* **2012**, *189*, 4175-4181, doi:10.4049/jimmunol.1201516.
191. Lee, H.M.; Kim, T.S.; Jo, E.K. MiR-146 and miR-125 in the regulation of innate immunity and inflammation. *BMB Rep* **2016**, *49*, 311-318, doi:10.5483/bmbrep.2016.49.6.056.
192. Barbu, M.G.; Condrat, C.E.; Thompson, D.C.; Bugnar, O.L.; Cretoiu, D.; Toader, O.D.; Suciu, N.; Voinea, S.C. MicroRNA Involvement in Signaling Pathways During Viral Infection. *Front Cell Dev Biol* **2020**, *8*, 143, doi:10.3389/fcell.2020.00143.
193. Rehermann, B.; Nascimbeni, M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* **2005**, *5*, 215-229, doi:10.1038/nri1573.
194. Su, C.; Hou, Z.; Zhang, C.; Tian, Z.; Zhang, J. Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells. *Virol J* **2011**, *8*, 354, doi:10.1186/1743-422X-8-354.
195. Yu, S.L.; Deng, H.; Li, X.H.; Huang, Y.X.; Xie, D.Y.; Gao, Z.L. Expression of MicroRNA-155 is Downregulated in Peripheral Blood Mononuclear Cells of Chronic Hepatitis B Patients. *Hepat Mon* **2016**, *16*, e34483, doi:10.5812/hepatmon.34483.
196. Sarkar, N.; Panigrahi, R.; Pal, A.; Biswas, A.; Singh, S.P.; Kar, S.K.; Bandopadhyay, M.; Das, D.; Saha, D.; Kanda, T.; et al. Expression of microRNA-155 correlates positively with the expression of Toll-like receptor 7 and modulates hepatitis B virus via C/EBP-beta in hepatocytes. *J Viral Hepat* **2015**, *22*, 817-827, doi:10.1111/jvh.12390.
197. Wang, W.; Bian, H.; Li, F.; Li, X.; Zhang, D.; Sun, S.; Song, S.; Zhu, Q.; Ren, W.; Qin, C.; et al. HBeAg induces the expression of macrophage miR-155 to accelerate liver injury via promoting production of inflammatory cytokines. *Cell Mol Life Sci* **2018**, *75*, 2627-2641, doi:10.1007/s00018-018-2753-8.
198. Bala, S.; Tilahun, Y.; Taha, O.; Alao, H.; Kodys, K.; Catalano, D.; Szabo, G. Increased microRNA-155 expression in the serum and peripheral monocytes in chronic HCV infection. *J Transl Med* **2012**, *10*, 151, doi:10.1186/1479-5876-10-151.
199. Cheng, Y.Q.; Ren, J.P.; Zhao, J.; Wang, J.M.; Zhou, Y.; Li, G.Y.; Moorman, J.P.; Yao, Z.Q. MicroRNA-155 regulates interferon-gamma production in natural killer cells via Tim-3 signalling in chronic hepatitis C virus infection. *Immunology* **2015**, *145*, 485-497, doi:10.1111/imm.12463.
200. Jiang, M.; Broering, R.; Trippler, M.; Wu, J.; Zhang, E.; Zhang, X.; Gerken, G.; Lu, M.; Schlaak, J.F. MicroRNA-155 controls Toll-like receptor 3- and hepatitis C virus-induced immune responses in the liver. *J Viral Hepat* **2014**, *21*, 99-110, doi:10.1111/jvh.12126.
201. Arboleda, J.F.; Fernandez, G.J.; Urcuqui-Inchima, S. Vitamin D-mediated attenuation of miR-155 in human macrophages infected with dengue virus: Implications for the cytokine response. *Infect Genet Evol* **2019**, *69*, 12-21, doi:10.1016/j.meegid.2018.12.033.
202. Natekar, J.P.; Rothan, H.A.; Arora, K.; Strate, P.G.; Kumar, M. Cellular microRNA-155 Regulates Virus-Induced Inflammatory Response and Protects against Lethal West Nile Virus Infection. *Viruses* **2019**, *12*, doi:10.3390/v12010009.
203. Arroyo, M.; Salka, K.; Chorvinsky, E.; Xuchen, X.; Abutaleb, K.; Perez, G.F.; Weinstock, J.; Gaviria, S.; Gutierrez, M.J.; Nino, G. Airway mir-155 responses are associated with TH1

cytokine polarization in young children with viral respiratory infections. *PLoS One* **2020**, *15*, e0233352, doi:10.1371/journal.pone.0233352.
204. Inchley, C.S.; Sonerud, T.; Fjaerli, H.O.; Nakstad, B. Nasal mucosal microRNA expression in children with respiratory syncytial virus infection. *BMC Infect Dis* **2015**, *15*, 150, doi:10.1186/s12879-015-0878-z.
205. Wang, S.; Ling, Y.; Yao, Y.; Zheng, G.; Chen, W. Luteolin inhibits respiratory syncytial virus replication by regulating the MiR-155/SOCS1/STAT1 signaling pathway. *Virol J* **2020**, *17*, 187, doi:10.1186/s12985-020-01451-6.
206. Xu, J.; Wu, C.; Che, X.; Wang, L.; Yu, D.; Zhang, T.; Huang, L.; Li, H.; Tan, W.; Wang, C.; et al. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Mol Carcinog* **2011**, *50*, 136-142, doi:10.1002/mc.20712.
207. Yu, G.; Chen, X.; Chen, S.; Ye, W.; Hou, K.; Liang, M. MiR-19a, miR-122 and miR-223 are differentially regulated by hepatitis B virus X protein and involve in cell proliferation in hepatoma cells. *J Transl Med* **2016**, *14*, 122, doi:10.1186/s12967-016-0888-7.
208. El-Guendy, N.M.; Helwa, R.; El-Halawany, M.S.; Abdel Rahman Ali, S.; Tantawy Aly, M.; Hasan Alieldin, N.; Fouad, S.A.; Saeid, H.; Abdel-Wahab, A.H. The Liver MicroRNA Expression Profiles Associated With Chronic Hepatitis C Virus (HCV) Genotype-4 Infection: A Preliminary Study. *Hepat Mon* **2016**, *16*, e33881, doi:10.5812/hepatmon.33881.
209. Wu, N.; Gao, N.; Fan, D.; Wei, J.; Zhang, J.; An, J. miR-223 inhibits dengue virus replication by negatively regulating the microtubule-destabilizing protein STMN1 in EAhy926 cells. *Microbes Infect* **2014**, *16*, 911-922, doi:10.1016/j.micinf.2014.08.011.
210. Li, Y.; Chan, E.Y.; Li, J.; Ni, C.; Peng, X.; Rosenzweig, E.; Tumpey, T.M.; Katze, M.G. MicroRNA expression and virulence in pandemic influenza virus-infected mice. *J Virol* **2010**, *84*, 3023-3032, doi:10.1128/JVI.02203-09.
211. Liu, C.C.; Miao, Y.; Chen, R.L.; Zhang, Y.Q.; Wu, H.; Yang, S.M.; Shang, L.Q. STIM1 mediates IAV-induced inflammation of lung epithelial cells by regulating NLRP3 and inflammasome activation via targeting miR-223. *Life Sci* **2021**, *266*, 118845, doi:10.1016/j.lfs.2020.118845.
212. Sun, G.; Li, H.; Wu, X.; Covarrubias, M.; Scherer, L.; Meinking, K.; Luk, B.; Chomchan, P.; Alluin, J.; Gombart, A.F.; et al. Interplay between HIV-1 infection and host microRNAs. *Nucleic Acids Res* **2012**, *40*, 2181-2196, doi:10.1093/nar/gkr961.
213. Taganov, K.D.; Boldin, M.P.; Chang, K.J.; Baltimore, D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A* **2006**, *103*, 12481-12486, doi:10.1073/pnas.0605298103.
214. Wang, S.; Zhang, X.; Ju, Y.; Zhao, B.; Yan, X.; Hu, J.; Shi, L.; Yang, L.; Ma, Z.; Chen, L.; et al. MicroRNA-146a feedback suppresses T cell immune function by targeting Stat1 in patients with chronic hepatitis B. *J Immunol* **2013**, *191*, 293-301, doi:10.4049/jimmunol.1202100.
215. Li, J.F.; Dai, X.P.; Zhang, W.; Sun, S.H.; Zeng, Y.; Zhao, G.Y.; Kou, Z.H.; Guo, Y.; Yu, H.; Du, L.Y.; et al. Upregulation of microRNA-146a by hepatitis B virus X protein contributes to hepatitis development by downregulating complement factor H. *mBio* **2015**, *6*, doi:10.1128/mBio.02459-14.
216. Zhang, W.J.; Wang, H.; Tong, Q.X.; Jie, S.H.; Yang, D.L.; Peng, C. Involvement of TLR2-MyD88 in abnormal expression of miR-146a in peripheral blood monocytes of patients with chronic hepatitis C. *J Huazhong Univ Sci Technolog Med Sci* **2015**, *35*, 219-224, doi:10.1007/s11596-015-1414-5.
217. Ren, J.P.; Ying, R.S.; Cheng, Y.Q.; Wang, L.; El Gazzar, M.; Li, G.Y.; Ning, S.B.; Moorman, J.P.; Yao, Z.Q. HCV-induced miR146a controls SOCS1/STAT3 and cytokine expression in monocytes to promote regulatory T-cell development. *J Viral Hepat* **2016**, *23*, 755-766, doi:10.1111/jvh.12537.
218. Pu, J.; Wu, S.; Xie, H.; Li, Y.; Yang, Z.; Wu, X.; Huang, X. miR-146a Inhibits dengue-virus-induced autophagy by targeting TRAF6. *Arch Virol* **2017**, *162*, 3645-3659, doi:10.1007/s00705-017-3516-9.
219. Zhang, F.; Sun, X.; Zhu, Y.; Qin, W. Downregulation of miR-146a inhibits influenza A virus replication by enhancing the type I interferon response in vitro and in vivo. *Biomed Pharmacother* **2019**, *111*, 740-750, doi:10.1016/j.biopha.2018.12.103.

220. Deng, Y.; Yan, Y.; Tan, K.S.; Liu, J.; Chow, V.T.; Tao, Z.Z.; Wang, D.Y. MicroRNA-146a induction during influenza H3N2 virus infection targets and regulates TRAF6 levels in human nasal epithelial cells (hNECs). *Exp Cell Res* **2017**, *352*, 184-192, doi:10.1016/j.yexcr.2017.01.011.
221. Tang, H.; Gao, Y.; Li, Z.; Miao, Y.; Huang, Z.; Liu, X.; Xie, L.; Li, H.; Wen, W.; Zheng, Y.; et al. The noncoding and coding transcriptional landscape of the peripheral immune response in patients with COVID-19. *Clin Transl Med* **2020**, *10*, e200, doi:10.1002/ctm2.200.
222. Bandiera, S.; Pfeffer, S.; Baumert, T.F.; Zeisel, M.B. miR-122--a key factor and therapeutic target in liver disease. *J Hepatol* **2015**, *62*, 448-457, doi:10.1016/j.jhep.2014.10.004.
223. Wang, S.; Qiu, L.; Yan, X.; Jin, W.; Wang, Y.; Chen, L.; Wu, E.; Ye, X.; Gao, G.F.; Wang, F.; et al. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1) -modulated P53 activity. *Hepatology* **2012**, *55*, 730-741, doi:10.1002/hep.24809.
224. Qiu, L.; Fan, H.; Jin, W.; Zhao, B.; Wang, Y.; Ju, Y.; Chen, L.; Chen, Y.; Duan, Z.; Meng, S. miR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. *Biochem Biophys Res Commun* **2010**, *398*, 771-777, doi:10.1016/j.bbrc.2010.07.021.
225. Tili, E.; Michaille, J.J.; Cimino, A.; Costinean, S.; Dumitru, C.D.; Adair, B.; Fabbri, M.; Alder, H.; Liu, C.G.; Calin, G.A.; et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. *J Immunol* **2007**, *179*, 5082-5089, doi:10.4049/jimmunol.179.8.5082.
226. Deng, W.; Zhang, X.; Ma, Z.; Lin, Y.; Lu, M. MicroRNA-125b-5p mediates post-transcriptional regulation of hepatitis B virus replication via the LIN28B/let-7 axis. *RNA Biol* **2017**, *14*, 1389-1398, doi:10.1080/15476286.2017.1293770.
227. Dai, C.Y.; Tsai, Y.S.; Chou, W.W.; Liu, T.; Huang, C.F.; Wang, S.C.; Tsai, P.C.; Yeh, M.L.; Hsieh, M.Y.; Huang, C.I.; et al. The IL-6/STAT3 pathway upregulates microRNA-125b expression in hepatitis C virus infection. *Oncotarget* **2018**, *9*, 11291-11302, doi:10.18632/oncotarget.24129.
228. Zhang, X.; Huang, F.; Yang, D.; Peng, T.; Lu, G. Identification of miRNA-mRNA Crosstalk in Respiratory Syncytial Virus- (RSV-) Associated Pediatric Pneumonia through Integrated miRNAome and Transcriptome Analysis. *Mediators Inflamm* **2020**, *2020*, 8919534, doi:10.1155/2020/8919534.
229. Makkoch, J.; Poomipak, W.; Saengchoowong, S.; Khongnomnan, K.; Praianantathavorn, K.; Jinato, T.; Poovorawan, Y.; Payungporn, S. Human microRNAs profiling in response to influenza A viruses (subtypes pH1N1, H3N2, and H5N1). *Exp Biol Med (Maywood)* **2016**, *241*, 409-420, doi:10.1177/1535370215611764.
230. Mantri, C.K.; Pandhare Dash, J.; Mantri, J.V.; Dash, C.C. Cocaine enhances HIV-1 replication in CD4+ T cells by down-regulating MiR-125b. *PLoS One* **2012**, *7*, e51387, doi:10.1371/journal.pone.0051387.
231. Huang, C.W.; Tsai, K.N.; Chen, Y.S.; Chang, R.Y. Differential miRNA Expression Profiling Reveals Correlation of miR125b-5p with Persistent Infection of Japanese Encephalitis Virus. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22084218.
232. Wei, X.; Tan, C.; Tang, C.; Ren, G.; Xiang, T.; Qiu, Z.; Liu, R.; Wu, Z. Epigenetic repression of miR-132 expression by the hepatitis B virus x protein in hepatitis B virus-related hepatocellular carcinoma. *Cell Signal* **2013**, *25*, 1037-1043, doi:10.1016/j.cellsig.2013.01.019.
233. Zhang, F.; Lin, X.; Yang, X.; Lu, G.; Zhang, Q.; Zhang, C. MicroRNA-132-3p suppresses type I IFN response through targeting IRF1 to facilitate H1N1 influenza A virus infection. *Biosci Rep* **2019**, *39*, doi:10.1042/BSR20192769.
234. Buggele, W.A.; Johnson, K.E.; Horvath, C.M. Influenza A virus infection of human respiratory cells induces primary microRNA expression. *J Biol Chem* **2012**, *287*, 31027-31040, doi:10.1074/jbc.M112.387670.
235. Mulik, S.; Xu, J.; Reddy, P.B.; Rajasagi, N.K.; Gimenez, F.; Sharma, S.; Lu, P.Y.; Rouse, B.T. Role of miR-132 in angiogenesis after ocular infection with herpes simplex virus. *Am J Pathol* **2012**, *181*, 525-534, doi:10.1016/j.ajpath.2012.04.014.

236. Feili, X.; Wu, S.; Ye, W.; Tu, J.; Lou, L. MicroRNA-34a-5p inhibits liver fibrosis by regulating TGF-beta1/Smad3 pathway in hepatic stellate cells. *Cell Biol Int* **2018**, *42*, 1370-1376, doi:10.1002/cbin.11022.
237. Smith, J.L.; Jeng, S.; McWeeney, S.K.; Hirsch, A.J. A MicroRNA Screen Identifies the Wnt Signaling Pathway as a Regulator of the Interferon Response during Flavivirus Infection. *J Virol* **2017**, *91*, doi:10.1128/JVI.02388-16.
238. Fan, N.; Wang, J. MicroRNA 34a contributes to virus-mediated apoptosis through binding to its target gene Bax in influenza A virus infection. *Biomed Pharmacother* **2016**, *83*, 1464-1470, doi:10.1016/j.biopha.2016.08.049.
239. Centa, A.; Fonseca, A.S.; da Silva Ferreira, S.G.; Azevedo, M.L.V.; de Paula, C.B.V.; Nagashima, S.; Machado-Souza, C.; Dos Santos Miggiolaro, A.F.R.; Pellegrino Baena, C.; de Noronha, L.; et al. Deregulated miRNA expression is associated with endothelial dysfunction in post-mortem lung biopsies of COVID-19 patients. *Am J Physiol Lung Cell Mol Physiol* **2021**, *320*, L405-L412, doi:10.1152/ajplung.00457.2020.
240. Othumpangat, S.; Beezhold, D.H.; Umbright, C.M.; Noti, J.D. Influenza Virus-Induced Novel miRNAs Regulate the STAT Pathway. *Viruses* **2021**, *13*, doi:10.3390/v13060967.
241. Kumarswamy, R.; Volkmann, I.; Thum, T. Regulation and function of miRNA-21 in health and disease. *RNA Biol* **2011**, *8*, 706-713, doi:10.4161/rna.8.5.16154.
242. Jenike, A.E.; Halushka, M.K. miR-21: a non-specific biomarker of all maladies. *Biomark Res* **2021**, *9*, 18, doi:10.1186/s40364-021-00272-1.
243. Fu, R.Q.; Hu, D.P.; Hu, Y.B.; Hong, L.; Sun, Q.F.; Ding, J.G. miR-21 promotes alpha-SMA and collagen I expression in hepatic stellate cells via the Smad7 signaling pathway. *Mol Med Rep* **2017**, *16*, 4327-4333, doi:10.3892/mmr.2017.7054.
244. Chen, Y.; Chen, J.; Wang, H.; Shi, J.; Wu, K.; Liu, S.; Liu, Y.; Wu, J. HCV-induced miR-21 contributes to evasion of host immune system by targeting MyD88 and IRAK1. *PLoS Pathog* **2013**, *9*, e1003248, doi:10.1371/journal.ppat.1003248.
245. Ouyang, X.; Jiang, X.; Gu, D.; Zhang, Y.; Kong, S.K.; Jiang, C.; Xie, W. Dysregulated Serum MiRNA Profile and Promising Biomarkers in Dengue-infected Patients. *Int J Med Sci* **2016**, *13*, 195-205, doi:10.7150/ijms.13996.
246. Shi, J.; Feng, P.; Gu, T. MicroRNA-21-3p modulates FGF2 to facilitate influenza A virus H5N1 replication by refraining type I interferon response. *Biosci Rep* **2020**, *40*, doi:10.1042/BSR20200158.
247. Zhao, H.; Chen, M.; Tellgren-Roth, C.; Pettersson, U. Fluctuating expression of microRNAs in adenovirus infected cells. *Virology* **2015**, *478*, 99-111, doi:10.1016/j.virol.2015.01.033.
248. Garg, A.; Seeliger, B.; Derda, A.A.; Xiao, K.; Gietz, A.; Scherf, K.; Sonnenschein, K.; Pink, I.; Hoeper, M.M.; Welte, T.; et al. Circulating cardiovascular microRNAs in critically ill COVID-19 patients. *Eur J Heart Fail* **2021**, *23*, 468-475, doi:10.1002/ejhf.2096.
249. Sanchez-Del Cojo, M.; Lopez-Huertas, M.R.; Diez-Fuertes, F.; Rodriguez-Mora, S.; Bermejo, M.; Lopez-Campos, G.; Mateos, E.; Jimenez-Tormo, L.; Gomez-Esquer, F.; Diaz-Gil, G.; et al. Changes in the cellular microRNA profile by the intracellular expression of HIV-1 Tat regulator: A potential mechanism for resistance to apoptosis and impaired proliferation in HIV-1 infected CD4+ T cells. *PLoS One* **2017**, *12*, e0185677, doi:10.1371/journal.pone.0185677.
250. He, F.; Xiao, Z.; Yao, H.; Li, S.; Feng, M.; Wang, W.; Liu, Z.; Liu, Z.; Wu, J. The protective role of microRNA-21 against coxsackievirus B3 infection through targeting the MAP2K3/P38 MAPK signaling pathway. *J Transl Med* **2019**, *17*, 335, doi:10.1186/s12967-019-2077-y.
251. Pandey, N.; Rastogi, M.; Singh, S.K. Chandipura virus dysregulates the expression of hsa-miR-21-5p to activate NF-kappaB in human microglial cells. *J Biomed Sci* **2021**, *28*, 52, doi:10.1186/s12929-021-00748-0.
252. Aqeilan, R.I.; Calin, G.A.; Croce, C.M. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. *Cell Death Differ* **2010**, *17*, 215-220, doi:10.1038/cdd.2009.69.
253. Yan, X.; Liang, H.; Deng, T.; Zhu, K.; Zhang, S.; Wang, N.; Jiang, X.; Wang, X.; Liu, R.; Zen, K.; et al. The identification of novel targets of miR-16 and characterization of their biological functions in cancer cells. *Mol Cancer* **2013**, *12*, 92, doi:10.1186/1476-4598-12-92.
254. Zhu, B.; Wei, X.X.; Wang, T.B.; Zhou, Y.C.; Liu, A.M.; Zhang, G.W. Increased miR-16 expression induced by hepatitis C virus infection promotes liver fibrosis through

downregulation of hepatocyte growth factor and Smad7. *Arch Virol* **2015**, *160*, 2043-2050, doi:10.1007/s00705-015-2474-3.
255. Zou, C.; Li, Y.; Cao, Y.; Zhang, J.; Jiang, J.; Sheng, Y.; Wang, S.; Huang, A.; Tang, H. Up-regulated MicroRNA-181a induces carcinogenesis in hepatitis B virus-related hepatocellular carcinoma by targeting E2F5. *BMC Cancer* **2014**, *14*, 97, doi:10.1186/1471-2407-14-97.
256. Zou, C.; Chen, J.; Chen, K.; Wang, S.; Cao, Y.; Zhang, J.; Sheng, Y.; Huang, A.; Tang, H. Functional analysis of miR-181a and Fas involved in hepatitis B virus-related hepatocellular carcinoma pathogenesis. *Exp Cell Res* **2015**, *331*, 352-361, doi:10.1016/j.yexcr.2014.11.007.
257. Elhelw, D.S.; Mekky, R.Y.; El-Ekiaby, N.; Ahmed, R.; Eldin, M.A.; El-Sayed, M.; Abouelkhair, M.M.; Salah, A.; Zekri, A.R.; Esmat, G.; et al. Predictive prognostic role of miR-181a with discrepancy in the liver and serum of genotype 4 hepatitis C virus patients. *Biomed Rep* **2014**, *2*, 843-848, doi:10.3892/br.2014.343.
258. Li, G.Y.; Zhou, Y.; Ying, R.S.; Shi, L.; Cheng, Y.Q.; Ren, J.P.; Griffin, J.W.; Jia, Z.S.; Li, C.F.; Moorman, J.P.; et al. Hepatitis C virus-induced reduction in miR-181a impairs CD4(+) T-cell responses through overexpression of DUSP6. *Hepatology* **2015**, *61*, 1163-1173, doi:10.1002/hep.27634.
259. Patra, T.; Meyer, K.; Ray, R.B.; Ray, R. Hepatitis C Virus Mediated Inhibition of miR-181c Activates ATM Signaling and Promotes Hepatocyte Growth. *Hepatology* **2020**, *71*, 780-793, doi:10.1002/hep.30893.
260. Peng, F.; He, J.; Loo, J.F.; Yao, J.; Shi, L.; Liu, C.; Zhao, C.; Xie, W.; Shao, Y.; Kong, S.K.; et al. Identification of microRNAs in Throat Swab as the Biomarkers for Diagnosis of Influenza. *Int J Med Sci* **2016**, *13*, 77-84, doi:10.7150/ijms.13301.
261. Atherton, L.J.; Jorquera, P.A.; Bakre, A.A.; Tripp, R.A. Determining Immune and miRNA Biomarkers Related to Respiratory Syncytial Virus (RSV) Vaccine Types. *Front Immunol* **2019**, *10*, 2323, doi:10.3389/fimmu.2019.02323.
262. Su, J.L.; Chen, P.S.; Johansson, G.; Kuo, M.L. Function and regulation of let-7 family microRNAs. *Microrna* **2012**, *1*, 34-39, doi:10.2174/2211536611201010034.
263. Letafati, A.; Najafi, S.; Mottahedi, M.; Karimzadeh, M.; Shahini, A.; Garousi, S.; Abbasi-Kolli, M.; Sadri Nahand, J.; Tamehri Zadeh, S.S.; Hamblin, M.R.; et al. MicroRNA let-7 and viral infections: focus on mechanisms of action. *Cell Mol Biol Lett* **2022**, *27*, 14, doi:10.1186/s11658-022-00317-9.
264. Wang, Y.; Lu, Y.; Toh, S.T.; Sung, W.K.; Tan, P.; Chow, P.; Chung, A.Y.; Jooi, L.L.; Lee, C.G. Lethal-7 is down-regulated by the hepatitis B virus x protein and targets signal transducer and activator of transcription 3. *J Hepatol* **2010**, *53*, 57-66, doi:10.1016/j.jhep.2009.12.043.
265. Qiu, D.; Chen, J.; Liu, J.; Luo, Z.; Jiang, W.; Huang, J.; Qiu, Z.; Yue, W.; Wu, L. Expression of microRNA let-7a positively correlates with hepatitis B virus replication in hepatocellular carcinoma tissues. *Exp Biol Med (Maywood)* **2017**, *242*, 939-944, doi:10.1177/1535370217697382.
266. Li, Q.; Lowey, B.; Sodroski, C.; Krishnamurthy, S.; Alao, H.; Cha, H.; Chiu, S.; El-Diwany, R.; Ghany, M.G.; Liang, T.J. Cellular microRNA networks regulate host dependency of hepatitis C virus infection. *Nat Commun* **2017**, *8*, 1789, doi:10.1038/s41467-017-01954-x.
267. Zhang, S.; Gu, D.; Ouyang, X.; Xie, W. Proinflammatory effects of the hemagglutinin protein of the avian influenza A (H7N9) virus and microRNA-mediated homeostasis response in THP-1 cells. *Mol Med Rep* **2015**, *12*, 6241-6246, doi:10.3892/mmr.2015.4142.
268. Bakre, A.; Mitchell, P.; Coleman, J.K.; Jones, L.P.; Saavedra, G.; Teng, M.; Tompkins, S.M.; Tripp, R.A. Respiratory syncytial virus modifies microRNAs regulating host genes that affect virus replication. *J Gen Virol* **2012**, *93*, 2346-2356, doi:10.1099/vir.0.044255-0.
269. Chen, B.; Han, J.; Chen, S.; Xie, R.; Yang, J.; Zhou, T.; Zhang, Q.; Xia, R. MicroLet-7b Regulates Neutrophil Function and Dampens Neutrophilic Inflammation by Suppressing the Canonical TLR4/NF-kappaB Pathway. *Front Immunol* **2021**, *12*, 653344, doi:10.3389/fimmu.2021.653344.
270. Tahamtan, A.; Teymoori-Rad, M.; Nakstad, B.; Salimi, V. Anti-Inflammatory MicroRNAs and Their Potential for Inflammatory Diseases Treatment. *Front Immunol* **2018**, *9*, 1377, doi:10.3389/fimmu.2018.01377.

271. Tan, Y.; Ge, G.; Pan, T.; Wen, D.; Gan, J. Serum MiRNA panel as potential biomarkers for chronic hepatitis B with persistently normal alanine aminotransferase. *Clin Chim Acta* **2015**, *451*, 232-239, doi:10.1016/j.cca.2015.10.002.
272. Horii, R.; Honda, M.; Shirasaki, T.; Shimakami, T.; Shimizu, R.; Yamanaka, S.; Murai, K.; Kawaguchi, K.; Arai, K.; Yamashita, T.; et al. MicroRNA-10a Impairs Liver Metabolism in Hepatitis C Virus-Related Cirrhosis Through Deregulation of the Circadian Clock Gene Brain and Muscle Aryl Hydrocarbon Receptor Nuclear Translocator-Like 1. *Hepatol Commun* **2019**, *3*, 1687-1703, doi:10.1002/hep4.1431.
273. Wang, Y.; Brahmakshatriya, V.; Lupiani, B.; Reddy, S.M.; Soibam, B.; Benham, A.L.; Gunaratne, P.; Liu, H.C.; Trakooljul, N.; Ing, N.; et al. Integrated analysis of microRNA expression and mRNA transcriptome in lungs of avian influenza virus infected broilers. *BMC Genomics* **2012**, *13*, 278, doi:10.1186/1471-2164-13-278.
274. Yao, Y.; Zhao, Y.; Smith, L.P.; Lawrie, C.H.; Saunders, N.J.; Watson, M.; Nair, V. Differential expression of microRNAs in Marek's disease virus-transformed T-lymphoma cell lines. *J Gen Virol* **2009**, *90*, 1551-1559, doi:10.1099/vir.0.009902-0.
275. Skovgaard, K.; Cirera, S.; Vasby, D.; Podolska, A.; Breum, S.O.; Durrwald, R.; Schlegel, M.; Heegaard, P.M. Expression of innate immune genes, proteins and microRNAs in lung tissue of pigs infected experimentally with influenza virus (H1N2). *Innate Immun* **2013**, *19*, 531-544, doi:10.1177/1753425912473668.
276. Chen, L.; Song, Y.; He, L.; Wan, X.; Lai, L.; Dai, F.; Liu, Y.; Wang, Q. MicroRNA-223 Promotes Type I Interferon Production in Antiviral Innate Immunity by Targeting Forkhead Box Protein O3 (FOXO3). *J Biol Chem* **2016**, *291*, 14706-14716, doi:10.1074/jbc.M115.700252.
277. Stewart, C.R.; Marsh, G.A.; Jenkins, K.A.; Gantier, M.P.; Tizard, M.L.; Middleton, D.; Lowenthal, J.W.; Haining, J.; Izzard, L.; Gough, T.J.; et al. Promotion of Hendra virus replication by microRNA 146a. *J Virol* **2013**, *87*, 3782-3791, doi:10.1128/JVI.01342-12.
278. Hou, J.; Wang, P.; Lin, L.; Liu, X.; Ma, F.; An, H.; Wang, Z.; Cao, X. MicroRNA-146a feedback inhibits RIG-I-dependent Type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. *J Immunol* **2009**, *183*, 2150-2158, doi:10.4049/jimmunol.0900707.
279. Stenfeldt, C.; Arzt, J.; Smoliga, G.; LaRocco, M.; Gutkoska, J.; Lawrence, P. Proof-of-concept study: profile of circulating microRNAs in Bovine serum harvested during acute and persistent FMDV infection. *Virol J* **2017**, *14*, 71, doi:10.1186/s12985-017-0743-3.
280. J, A.H.; Yoo, D.; Liu, H.C. Characterization of the microRNAome in porcine reproductive and respiratory syndrome virus infected macrophages. *PLoS One* **2013**, *8*, e82054, doi:10.1371/journal.pone.0082054.
281. He, T.; Feng, G.; Chen, H.; Wang, L.; Wang, Y. Identification of host encoded microRNAs interacting with novel swine-origin influenza A (H1N1) virus and swine influenza virus. *Bioinformation* **2009**, *4*, 112-118, doi:10.6026/97320630004112.
282. Zhao, P.; Zhao, L.; Zhang, K.; Feng, H.; Wang, H.; Wang, T.; Xu, T.; Feng, N.; Wang, C.; Gao, Y.; et al. Infection with street strain rabies virus induces modulation of the microRNA profile of the mouse brain. *Virol J* **2012**, *9*, 159, doi:10.1186/1743-422X-9-159.
283. Shi, N.; Zhang, X.Y.; Dong, C.Y.; Hou, J.L.; Zhang, M.L.; Guan, Z.H.; Li, Z.Y.; Duan, M. Alterations in microRNA expression profile in rabies virus-infected mouse neurons. *Acta Virol* **2014**, *58*, 120-127, doi:10.4149/av_2014_02_120.
284. Peng, X.; Gao, Q.S.; Zhou, L.; Chen, Z.H.; Lu, S.; Huang, H.J.; Zhan, C.Y.; Xiang, M. MicroRNAs in avian influenza virus H9N2-infected and non-infected chicken embryo fibroblasts. *Genet Mol Res* **2015**, *14*, 9081-9091, doi:10.4238/2015.August.7.17.
285. Burnside, J.; Morgan, R. Emerging roles of chicken and viral microRNAs in avian disease. *BMC Proc* **2011**, *5 Suppl 4*, S2, doi:10.1186/1753-6561-5-S4-S2.
286. Tian, F.; Luo, J.; Zhang, H.; Chang, S.; Song, J. MiRNA expression signatures induced by Marek's disease virus infection in chickens. *Genomics* **2012**, *99*, 152-159, doi:10.1016/j.ygeno.2011.11.004.
287. Ji, J.; Shang, H.; Zhang, H.; Li, H.; Ma, J.; Bi, Y.; Xie, Q. Temporal changes of microRNA gga-let-7b and gga-let-7i expression in chickens challenged with subgroup J avian leukosis virus. *Vet Res Commun* **2017**, *41*, 219-226, doi:10.1007/s11259-017-9681-1.

288. Thomson, B.J. Viruses and apoptosis. *Int J Exp Pathol* **2001**, *82*, 65-76, doi:10.1111/j.1365-2613.2001.iep0082-0065-x.
289. Santus, P.; Danzo, F.; Zuffi, A.; Pini, S.; Saad, M.; Visconti, A.; Radovanovic, D. Oxidative stress and viral Infections: rationale, experiences, and perspectives on N-acetylcysteine. *Eur Rev Med Pharmacol Sci* **2022**, *26*, 8582-8590, doi:10.26355/eurrev_202211_30395.
290. Sun, W.; Liu, S.; Yan, Y.; Wang, Q.; Fan, Y.; Okyere, S.K. Pseudorabies virus causes splenic injury via inducing oxidative stress and apoptosis related factors in mice. *Sci Rep* **2023**, *13*, 23011, doi:10.1038/s41598-023-50431-7.
291. Verma, A.K.; Ghosh, S.; Basu, A. Chandipura Virus Induced Neuronal Apoptosis via Calcium Signaling Mediated Oxidative Stress. *Front Microbiol* **2018**, *9*, 1489, doi:10.3389/fmicb.2018.01489.
292. Yang, X.; Liu, X.; Nie, Y.; Zhan, F.; Zhu, B. Oxidative stress and ROS-mediated cellular events in RSV infection: potential protective roles of antioxidants. *Virol J* **2023**, *20*, 224, doi:10.1186/s12985-023-02194-w.
293. Su, Z.; Yang, Z.; Xu, Y.; Chen, Y.; Yu, Q. MicroRNAs in apoptosis, autophagy and necroptosis. *Oncotarget* **2015**, *6*, 8474-8490, doi:10.18632/oncotarget.3523.
294. Hao, R.; Song, X.; Sun-Waterhouse, D.; Tan, X.; Li, F.; Li, D. MiR-34a/Sirt1/p53 signaling pathway contributes to cadmium-induced nephrotoxicity: A preclinical study in mice. *Environ Pollut* **2021**, *282*, 117029, doi:10.1016/j.envpol.2021.117029.
295. Miyashita, T.; Reed, J.C. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* **1995**, *80*, 293-299, doi:10.1016/0092-8674(95)90412-3.
296. Duprez, L.; Wirawan, E.; Vanden Berghe, T.; Vandenabeele, P. Major cell death pathways at a glance. *Microbes Infect* **2009**, *11*, 1050-1062, doi:10.1016/j.micinf.2009.08.013.
297. Chaitanya, G.V.; Steven, A.J.; Babu, P.P. PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. *Cell Commun Signal* **2010**, *8*, 31, doi:10.1186/1478-811X-8-31.
298. Jafarzadeh, A.; Naseri, A.; Shojaie, L.; Nemati, M.; Jafarzadeh, S.; Bannazadeh Baghi, H.; Hamblin, M.R.; Akhlagh, S.A.; Mirzaei, H. MicroRNA-155 and antiviral immune responses. *Int Immunopharmacol* **2021**, *101*, 108188, doi:10.1016/j.intimp.2021.108188.
299. Marshall, J.S.; Warrington, R.; Watson, W.; Kim, H.L. An introduction to immunology and immunopathology. *Allergy Asthma Clin Immunol* **2018**, *14*, 49, doi:10.1186/s13223-018-0278-1.
300. Fajgenbaum, D.C.; June, C.H. Cytokine Storm. *N Engl J Med* **2020**, *383*, 2255-2273, doi:10.1056/NEJMra2026131.
301. Zheng, Q.; Hua, C.; Liang, Q.; Cheng, H. The NLRP3 inflammasome in viral infection (Review). *Mol Med Rep* **2023**, *28*, doi:10.3892/mmr.2023.13047.
302. Peng, Q.; Yin, R.; Zhu, X.; Jin, L.; Wang, J.; Pan, X.; Ma, A. miR-155 activates the NLRP3 inflammasome by regulating the MEK/ERK/NF-kappaB pathway in carotid atherosclerotic plaques in ApoE(-/-) mice. *J Physiol Biochem* **2022**, *78*, 365-375, doi:10.1007/s13105-022-00871-y.
303. Escoubet-Lozach, L.; Benner, C.; Kaikkonen, M.U.; Lozach, J.; Heinz, S.; Spann, N.J.; Crotti, A.; Stender, J.; Ghisletti, S.; Reichart, D.; et al. Mechanisms establishing TLR4-responsive activation states of inflammatory response genes. *PLoS Genet* **2011**, *7*, e1002401, doi:10.1371/journal.pgen.1002401.

# Załącznik 1

# Kopie publikacji naukowych wchodzących w skład rozprawy doktorskiej

**Ewa Ostrycharz-Jasek**

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# Micro-Players of Great Significance—Host microRNA Signature in Viral Infections in Humans and Animals

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**Abstract:** Over time, more and more is becoming known about micro-players of great significance. This is particularly the case for microRNAs (miRNAs; miR), which have been found to participate in the regulation of many physiological and pathological processes in both humans and animals. One such process is viral infection in humans and animals, in which the host miRNAs—alone or in conjunction with the virus—interact on two levels: viruses may regulate the host's miRNAs to evade its immune system, while the host miRNAs can play anti- or pro-viral roles. The purpose of this comprehensive review is to present the key miRNAs involved in viral infections in humans and animals. We summarize the data in the available literature, indicating that the signature miRNAs in human viral infections mainly include 12 miRNAs (i.e., miR-155, miR-223, miR-146a, miR-122, miR-125b, miR-132, miR-34a, miR -21, miR-16, miR-181 family, let-7 family, and miR-10a), while 10 miRNAs are commonly found in animals (i.e., miR-155, miR-223, miR-146a, miR-145, miR-21, miR-15a/miR-16 cluster, miR-181 family, let-7 family, and miR-122) in this context. Knowledge of which miRNAs are involved in different viral infections and the biological functions that they play can help in understanding the pathogenesis of viral diseases, facilitating the future development of therapeutic agents for both humans and animals.

**Keywords:** microRNA; viral infections; hepatitis viruses; hemorrhagic viruses; respiratory viruses; *Lagovirus europaeus*/RHDV; Marek's disease virus; foot-and-mouth disease virus; porcine reproductive and respiratory syndrome virus; rabies virus

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## 1. Introduction

MicroRNAs (miRNAs, miR) are small (i.e., consisting of 17–25 nucleotides) non-coding RNA molecules involved in the regulation of gene expression [1,2]. MiRNAs are found in most eukaryotes, including humans and animals (referred to as host miRNAs in the native organism) [2]. MiRNAs are critical for correct development in all organisms. They also play a crucial role in regulating gene expression, as well as controlling cell differentiation, apoptosis, and metabolic pathways, including homeostasis [3,4]. In the human genome, miRNAs account for 1–5% and regulate at least 30% of protein-coding genes, where the aberrant expression of miRNAs has been found to be associated with many human diseases [5,6]. Furthermore, miRNAs can also be secreted into extracellular biological fluids, where they may serve as potential biomarkers [7]. MiRNAs are also involved in the regulation of the immune system and response, including the response to viral infections in humans [8,9] and animals [10,11].

MiRNA precursors are commonly located within the genome. As such, miRNA biogenesis initiates in the nucleus, and involves a two-step process [2] (Figure 1). The first step of miRNA formation is gene transcription by Pol II or Pol III in order to generate primary miRNA (pri-miRNA). The pri-miRNA maturation process begins with nuclear cleavage into stem-loop structures (70 nucleotides on average), thus forming precursor

miRNA (pre-miRNA) [12]. This process is accomplished by a protein complex consisting of the RNase III endonucleases Drosha and DiGeorge syndrome critical region gene 8 (DGCR8), which control miRNA biogenesis through stabilization by Drosha. Afterwards, the pre-miRNA is exported to the cytoplasm in an Exportin5/RangGTP-dependent manner, and cleaved near the loop by Dicer and an RNase III to form an miRNA duplex containing the mature miRNA [13]. The duplex unwinds to produce single-stranded mature miRNA, which assembles into a ribonucleoprotein complex (RNA-induced silencing complex; RISC) which contains the Argonaute (Ago) protein. The resulting complex mediates the recognition of target mRNA and participates in gene silencing via translation repression or mRNA cleavage [14]. Most miRNAs in animals are thought to function through the inhibition of the effective mRNA translation of target genes via imperfect base pairing with the 3′-untranslated region (3′UTR) of target mRNAs [2].

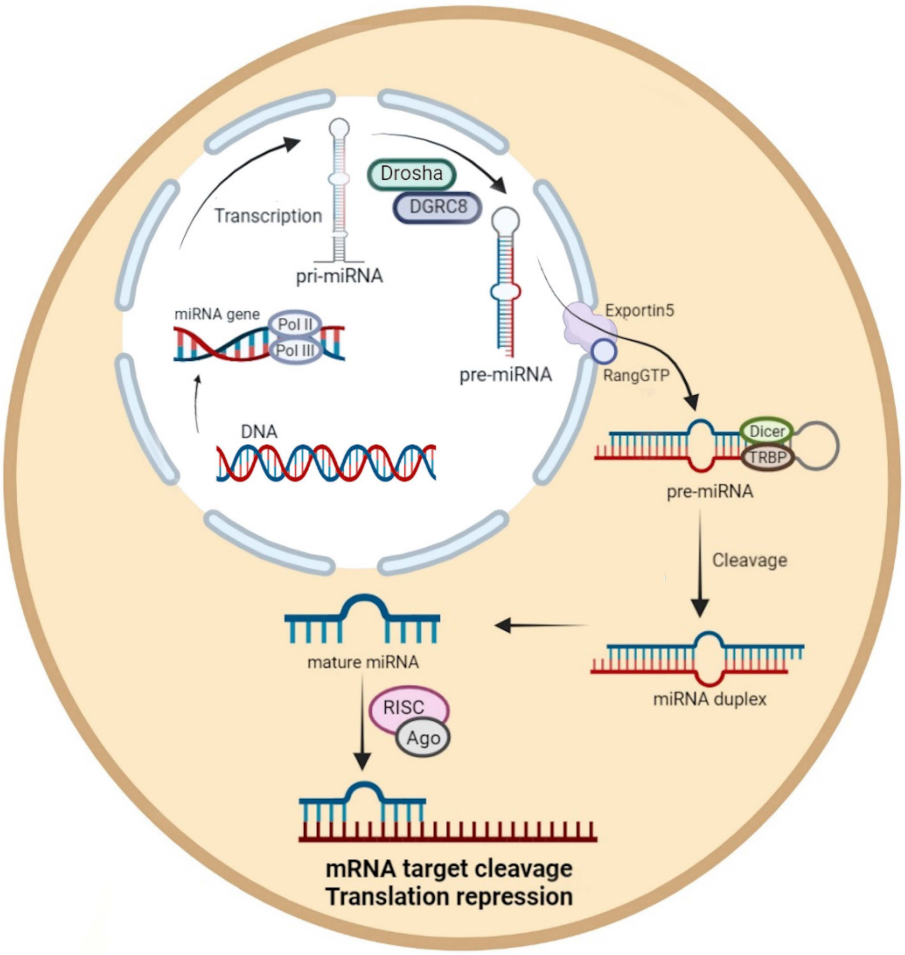


**Figure 1.** Biogenesis and functions of microRNA in humans and animals. MiRNA genes are transcribed in the cell nucleus to pri-miRNA, then, transformed to pre-miRNA by Drosha. Then, the pre-miRNA is transported to the cytoplasm by Exportin5 and cleaved to create an miRNA duplex containing the mature miRNA. After strand separation, the single-stranded mature miRNAs are incorporated into the miRNA-induced silencing complex. The resulting complex mediates the recognition of target mRNA and participates in gene silencing via translation repression or mRNA cleavage.

*MicroRNAs and Viral Infections in Humans and Animals*

MiRNAs have been also shown to play an important role in viral infections in humans and animals, and can therefore be considered micro-players of great significance. MiRNAs in humans and animals are referred to as host miRNAs [2]. In recent years, it has been shown that some viruses can also synthesize their own miRNAs [15]. According to the

above, Barbu et al. [11] has classified miRNAs based on their source and role. With respect to their source, they were classified into host miRNAs and viral miRNAs. However, due to their various roles, the host miRNAs were further divided into (i) pro-viral miRNAs and (ii) anti-viral miRNAs. Considering the former, the interaction between viruses and host miRNAs may facilitate viral replication and infection, thus exerting a pro-viral function. Furthermore, pro-viral miRNAs can promote viral infection by suppressing anti-viral factors, such as interferon (IFN), thus allowing the virus to escape the immune response of the host. In contrast, various host miRNAs can exert anti-viral functions by influencing the production of viral RNA, blocking viral replication, suppressing pro-viral proteins, or inducing the virus to enter a latent phase [11,16].

The purpose of this comprehensive review is to present the key miRNAs involved in the course of viral infections in humans and animals. Infections caused by viruses constantly threaten the lives of humans and animals, constituting a global health problem. In the case of humans, it is safe to say that they pose a significant challenge for modern medicine; meanwhile, in the case of animals, they may lead to huge economic losses, requiring effective and rapid diagnostic and therapeutic pathways. Knowledge of which miRNAs are involved in different viral infections and their biological functions can help to understand the pathogenesis of viral diseases, as well as facilitating the future development of therapeutic agents for both humans and animals.

## 2. MicroRNA Signature in Human Viral Infections

In the scope of this study, we distinguished 12 host miRNAs which are extremely important, from the point of view of their participation in viral infections (Figure 2A).

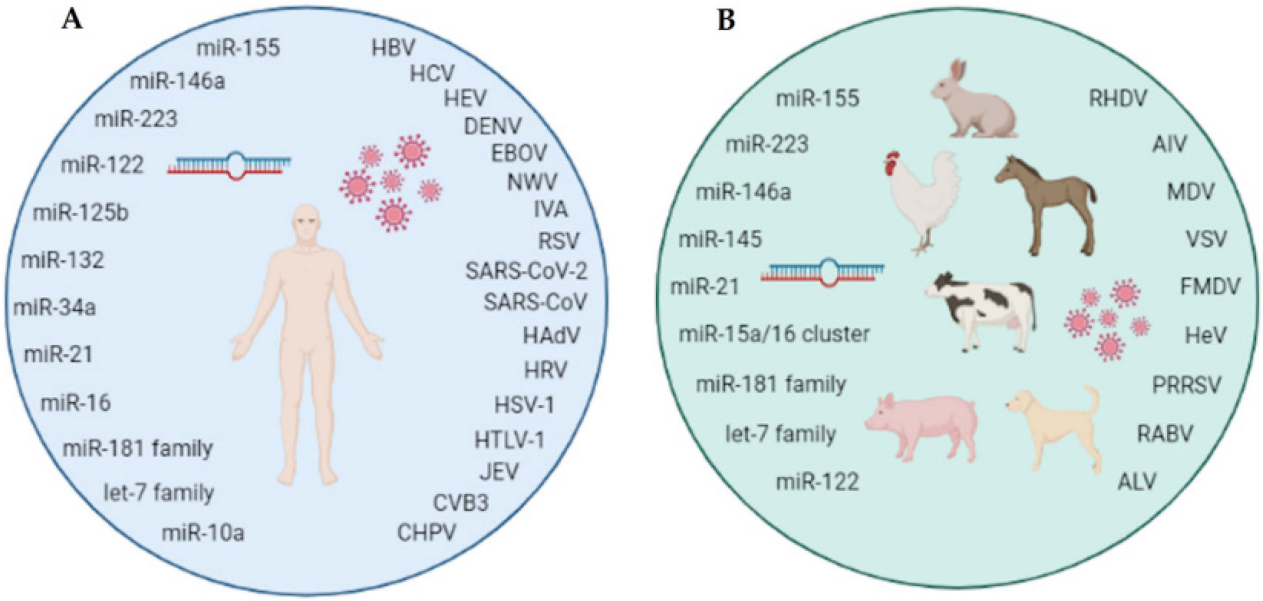


**Figure 2.** Key miRNAs: (**A**) in human viral infections and (**B**) in animal viral infections.

### 2.1. MicroRNA-155 (miR-155)

MiR-155 is a multi-functional miRNA which is highly expressed in several immune cells [17]. The expression of miR-155 was first reported in human spleen and thymus, liver, lung, and kidney [18]. MiR-155 is particularly responsive to many inflammatory stimuli, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IFN, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), alarmins (e.g., IL-1α), and hypoxia, as well as the Toll-like receptor (TLR) ligand in various cell types, particularly in monocytes/macrophages [19]. MiR-155 has an important function in the

modulation of humoral and cellular immune responses during viral infections, and can be inhibited by viruses [20].

#### 2.1.1. Hepatitis Viruses

Hepatitis B virus (HBV) belongs to the family *Hepadnaviridae*, with a DNA genome [21]. HBV, as an etiological agent of viral hepatitis, is a public health concern. Chronic HBV infection leads to persistent liver inflammation and damage, which may ultimately result in hepatocellular carcinoma (HCC) due to the development of oncogenic changes [21]. The expression level of miR-155 is significantly down-regulated in healthy hepatocytes; however, it may be increased in different pathological processes. Due to its role in regulating the immune response, miR-155 plays a significant role in infections with hepatotropic viruses, such as HBV or hepatitis C virus (HCV) [22]. Su et al. [23] performed a study to identify the effects of miR-155 on the immune response during HBV infection in human hepatoma cells. They found that the ectopic expression of miR-155 up-regulated the expression of several IFN-inducible anti-viral genes. This study also showed that enhanced expression of miR-155 suppressed the suppressor of cytokine signaling 1 (SOCS1) and enhanced the phosphorylation of signal transducer and activator of transcription (STAT) 1 and STAT3. In addition, miR-155 may inhibit HBV X gene expression, to some extent, in vitro. They also showed that miR-155 can promote intrinsic anti-viral immunity by targeting SOCS1, thus enhancing the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, which suppresses HBV infection in hepatocytes [23]. In a liver biopsy specimen, serum and peripheral blood mononuclear cells (PBMCs) from HBV-infected patients were observed to significantly reduce miR-155 [24–26]; however, interestingly, chronic hepatitis B (CHB) patients with elevated alanine aminotransferase (ALT) presented higher levels of miR-155 [25,26]. These results suggest that miR-155 levels in PBMCs are correlated with immune state in patients with chronic HBV infection [25]. Furthermore, the down-regulation of miR-155 in the natural killer (NK) cells of immune-active patients impaired IFN-γ production by targeting SOCS1, which may contribute to immune dysfunction [26]. The miR-155 level was also reduced in hepatoma cells (HepG2.2.15) stably replicating HBV. Furthermore, due to the fact that miR-155 has heterogenous roles in the immune responses mediated by TLRs, Sarkar et al. [24] investigated the relationship between TLRs and miR-155, and the consequent effect on the replication status of HBV. They observed a positive correlation between TLR7 and miR-155 expression, which modulated HBV replication. In addition, their results indicated that TLR7 stimulation induces the synthesis of miR-155 through the NF-κB pathway [24]. Meanwhile, the ectopic expression of miR-155 in HepG2.2.15 cells reduced viral DNA via targeted suppression of CCAAT/enhancer-binding protein-β (C/EBP-β), which is a positive regulator of viral transcription [24]. During CHB and HBV infection in carriers, higher miR-155 expression in CD4+ and CD8+ T-cells has been observed, which was positively correlated with T-cell activation. In addition, HBV carriers expressed higher amounts of miR-155 in their CD8+ T-cells compared to healthy individuals [27]. Wang et al. [28] reported that hepatitis B antigen (HBeAg) augmented the expression of miR-155 in macrophages through the phosphatidylinositol 3-kinase (PI3K) and nuclear factor kappa-light-chain-enhancer of activated B-cells' (NF-κB) signal pathway, where the increase in miR-155 promoted HBeAg-induced inflammatory cytokine production by inhibiting the expression of B-cell lymphoma 6 (BCL-6), inositol polyphosphate 5-phosphatase 1 (SHIP-1), and SOCS-1. An interesting observation, made by Chen et al. [29], is that miR-155 was inhibited by HBV infection, while miR-155 transfection remarkably reinforced HBV replication and antigen expression in HepG2.2.15. Moreover, miR-155 impaired the inhibition of the SOCS1/protein kinase B/mammalian target of the rapamycin kinase (SOCS1/Akt/mTOR) axis and reinforced autophagy, resulting in increased replication of HBV [29]. Another interesting fact is that over-expression of miR-155 in serum was correlated with non-responsiveness to the hepatitis B (HB) vaccine. Researchers have hypothesized that increased miR-155 expression may dampen the generation of the T-cell-mediated immune response, resulting in failure

to respond to the HB vaccine [30]. Furthermore, rs767649 T/A polymorphisms in miR-155 may be a risk factor for CHB. It has been shown that AT and AA genotypes are related to the risk of developing chronic hepatitis. Polymorphism of miR-155 can also affect the binding sites of interferon regulatory factor 1 (IRF1), interferon regulatory factor 2 (IRF2), and PR domain zinc finger protein 1 (PRDM1), thus playing a part in the pathogenesis of CHB [31].

Hepatitis C virus (HCV) belongs to the family *Flaviviridae*, with the ssRNA genome. HCV, as opposed to HBV, causes chronic viral hepatitis in around 55–80% of infected individuals. Around 5–20% of chronic hepatitis C (CHC) patients develop cirrhosis or HCC [32]. In patients with CHC infection, the expression of miR-155 has been found to be elevated in serum, monocytes, and liver tissue [33,34]. The stimulation of normal monocytes with TLR4 and TLR8 ligands or the HCV core, non-structural protein 3 (NS3), and non-structural protein 5 (NS5) recombinant proteins induced a robust increase in both miR-155 expression and TNFα production, thus identifying potential mechanisms for the in vivo induction of miR-155 during chronic HCV infection [33]. The up-regulation of miR-155 expression was also observed in PBMCs from HCV-infected patients, and was associated with HCV replication in PBMCs cells [35]. Similarly to HBV infection, miR-155 was down-regulated in NK cells from chronically HCV-infected individuals. Lower levels of miR-155 were correlated with up-regulated T-cell immunoglobulin mucin-3 (TIM-3) and T-box expression in T-cells (T-bet) and NK cells during chronic HCV infection. The transfection of miR-155 to NK cells affected T-bet/Tim-3 expression and increased in IFN-γ production. From the above, it was concluded that miR-155 may regulate Tim-3/T-bet/STAT-5 signaling and cytokine expression in NK cells, potentially balancing immune clearance and immune injury during chronic viral infection [36]. In non-parenchymal liver cells (NPCs), miR-155 expression was induced by the TLR3 ligand, while transforming growth factor beta (TGF-β) and IL-10 inhibited the TLR3-induced anti-viral response through the inhibition of NF-κB and IRF3. However, in liver biopsies from patients infected with HCV, a lower level of miR-155 expression was associated with higher expression of interferon-stimulated gene 15 (ISG15) and TLR3 [34]. In contrast to other genotypes of HCV, investigation of the HCV genotype 4 indicated that the expression of miR-155 was similar in both healthy and infected PBMCs [37,38]. This could be attributed to the attenuation of the IFN pathway by HCV, which was assessed by investigating the expression of an ISG, which showed lower expression in HCV-infected PBMCs. HCV might also interfere with miR-155 expression through the TLR-7 pathway [38]. Moreover, miR-155 also plays an important role in regulating tumorigenesis; in particular, HCV infection may promote the initiation and progression of HCC. Zhang et al. [39] indicated that miR-155 levels were markedly increased in patients infected with HCV. MiR-155 transcription was found to be regulated by NF-κB, where p300 increased NF-κB-dependent miR-155 expression. The over-expression of miR-155 significantly inhibited hepatocyte apoptosis and promoted cell proliferation by repressing the adenomatous polyposis coli (APC) gene and activating wingless-related integration site (Wnt) signaling. The up-regulation of miR-155 and activation of the Wnt pathway resulted in nuclear accumulation of β-catenin and a concomitant increase in cyclin D1, MYC Proto-Oncogene (c-Myc), and survivin, contributing to HCC growth [39].

#### 2.1.2. Hemorrhagic Viruses

Dengue virus (DENV) is a member of the *Flaviviviridae* family, with an ssRNA genome, and includes four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), each of which is capable of causing dengue fever (DF) and dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) [40]. MiR-155 also plays a significant role in DENV infection, and has been determined to have a major function in regulating the TLR/NF-κB-induced signaling pathways in human macrophages in DENV infection [41,42]. Su et al. [43] indicated that miR-155 is down-regulated in DENV-infected Huh-7 cells and infected mice. However, the exogenous over-expression of miR-155 appeared to limit viral replication in vitro, suggesting that low miR-155 levels would be beneficial for DENV replication. In vivo,

the over-expression of miR-155 protected mice from the life-threatening effects of DENV infection and reduced the propagation of the virus. Their investigation revealed that the effect of miR-155 on the inhibition of DENV replication was due to target BTB domain and CNC homolog 1 (Bach1), resulting in the induction of the heme oxygenase-1 (HO-1)-mediated inhibition of DENV NS2B/NS3 protease activity and enhanced anti-viral IFN responses [43]. Interestingly, researchers have also suggested that the TLR4/NF-κB/miR-155-5p/SOCS-1 axis in human macrophages may be regulated by vitamin D during DENV infection [41].

West Nile virus (WNV) is a member of the *Flaviviviridae* family, with an ssRNA genome, which has disseminated globally and is a significant cause of viral encephalitis in humans [40,44]. It was reported that miR-155 was up-regulated in the brains of mice infected with WNV. This up-regulation was correlated with neuroinflammatory molecules and target genes for miR-155, including IL-13, brain-derived neurotrophic factor (BDNF), and C-C motif chemokine receptor 9 (CCR9) [45]. One of the target genes, IL-13, is involved in cell survival, and the reduced levels of IL-13 observed in WNV-infected mice may promote apoptosis [46]. Natekar et al. [47] demonstrated the critical role of miR-155 in WNV infection in mice. MiR-155 knockout (miR-155−/−) mice exhibited significantly higher morbidity and mortality after infection with a lethal strain. Increased mortality in miR-155−/− mice was associated with a significantly high WNV load in sera and brains. In the same mice, higher levels of protein IFN-α were observed, as well as significantly lower levels of anti-viral interleukins (i.e., IL-1β, IL-12, IL-6, and IL-15) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Additionally, the over-expression of miR-155 in human neuronal cells modulated the anti-viral cytokine response, resulting in significantly lower WNV replication [47].

#### 2.1.3. Respiratory Viruses

Influenza virus (IV) belongs to the *Orthomyxoviridae* family, with an ssRNA genome [48]. Influenza is an acute infectious respiratory disease that occurs seasonally in temperate climates while, in tropical regions, it can occur year-round, thus causing epidemics. These viruses circulate in all parts of the world and cause influenza of varying severity, sometimes leading to hospitalization and even death [49]. Patients with severe influenza exhibit bilateral pulmonary infiltration and, often, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) with associated hypoxemic respiratory failure [50]. MiR-155 might serve as a positive pro-inflammatory regulator in the inflammatory response induced by influenza H1N1 infection in human pulmonary microvascular endothelial cells (HPMECs). In virus-infected HPMECs, the miR-155 level was significantly increased at 16 h and 24 h. Sphingosine-1-phosphate receptor 1 (S1PR1) can be targeted by miR-155, where the over-expression of miR-155 may decrease the expression of S1PR1, leading to NF-κB activation, cytokine production, and increased inflammatory response. An in vitro study showed that the over-expression of miR-155 in IV-infected pulmonary vascular endothelial cells promoted the expression of IFN-β, IL-1β, TNF-α, IL-6, IL-8, C-C motif chemokine ligand (CCL) 2, and CCL5; however, the down-regulation of miR-155 by an miR-155 inhibitor in infected cells promoted the expression of S1PR1, thus decreasing the levels of pro-inflammatory cytokines [51]. Another study indicated that the introduction of miR-155 into the non-structural gene segment of influenza A virus (IAV) increased the immunogenicity of the virus after experimental vaccination in mice. Immunization with the recombinant influenza virus promoted the proliferation of influenza-specific CD8 + T-cells and produced higher titers of neutralizing antibodies [52]. An in vivo study carried out in mice showed that miR-155 deficiency profoundly impairs in vivo CD8+ T-cell responses against viral infections. Additionally, miR-155 deficiency resulted in increased type I IFN signaling and reduced CD8+ T-cell proliferation, which may contribute to impaired viral clearance [53]. However, miR-155 may contribute to a more severe course of the influenza. Studies have demonstrated that miR-155 knockout mice recovered faster after influenza

infection, with decreased lung inflammation, endoplasmic reticulum stress (ER stress), and pathological process, despite increased collagen expression [54].

Respiratory syncytial virus (RSV) belongs to the *Pneumoviridae* family, with an RNA genome [55]. RSV is the most important cause of viral lower respiratory tract illness (VLRTI) in infants, children, the elderly, and immunocompromised patients. Clinical manifestations of the disease range from asymptomatic infection to a form with bronchospasm and pneumonia [56]. Similarly to influenza virus infection, increased miR-155 expression has been observed in RSV infection [57–59]. The airway secretion of miR-155 during RSV infections in young children was associated with enhanced anti-viral immunity. These results provide evidence that miR-155 is strongly linked to high IFN-γ production and enhanced airway Th1 cytokine polarization (IFN-γ/IL-4 ratio). Additionally, high airway miR-155 levels have been linked to decreased respiratory disease severity [57]. Furthermore, increased miR-155 expression has been observed in the PBMCs of infants with RSV infection with pneumonia, which was positively related to the expression of TNF-α, IL-1β, IL-6, and IL-8 [59]. NF-κB activation following RSV-antigen binding to the pathogen recognition receptors TLR4 or retinoic acid-inducible gene 1 (RIG-1) is a primary stage in the immunological response to RSV, leading to increased miR-155 expression [58,60]. MiR-155 produced by targeting SHIP1 positively regulates myeloid proliferation, while that produced by the regulation of the $p27^{kip1}$ protein level through the targeting of kinesin-like protein (Kif1) leads to dendritic cell maturation, which is a necessary step for dendritic cell migration to the lymph nodes and antigen presentation [61,62]. Interestingly, miR-155 may be a target for the treatment of RSV infection. Luteolin inhibited RSV replication in both in vitro and in vivo studies through the induction of miR-155, which targets SOCS1, leading to enhanced STAT1 phosphorylation and expression of interferon-stimulated genes (ISGs) [63].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the *Coronaviridae* family, with an RNA genome [64]. SARS-CoV-2 is the etiological agent of the coronavirus disease 2019 (COVID-19), an acute respiratory infectious disease. Most cases of COVID-19 have a benign course, but some can lead to life-threatening pneumonia and multiple organ failure [65]. In SARS-CoV-2 infection, hyper-inflammation, cytokine storm, and an inability to properly produce IFN are among the pathogenic mechanisms of coronavirus disease 2019 (COVID-19) [66–68]. In patients with COVID-19, an elevated expression of miR-155 has been observed in the nasopharynx, plasma, and PBMCs [69–72]. Interestingly, the miR-155 level has been found to be correlated with the severity of COVID-19, with the highest expression observed in non-surviving COVID-19 patients [69,71]. Furthermore, in SARS-CoV-2-infected cells, significant up-regulation of miR-155 has been observed in vitro [73,74]. Additionally, miR-155 has presented significant correlations with the clinicopathological characteristics of COVID-19 patients, such as chest computed tomography (CT) scan findings, C-reactive protein (CRP), ferritin, mortality, D-dimer, white blood cell (WBC) count, and lymphocyte and neutrophil percentages [69]. Donyavi et al. [71] demonstrated the existence of an inverse correlation between miR-155-5p and the SARS-CoV-2 N-gene and SARS-CoV-2 RdRp-gene, which may indicate that the expression of miR-155 increases due to an immune response to fight SARS-CoV-2. Based on bioinformatic analyses, it has been indicated that miR-155 may be associated with some genes involved in the immune response, apoptosis, and COVID-19 progression, such as STAT1, STAT3, transforming growth factor beta 1 (TGFB1), mothers against decapentaplegic homolog 3 (SMAD3), IRF1, AKT1, MYB proto-oncogene (MYB), BCL6, TP6, hypoxia inducible factor 1 subunit alpha (HIF1A), forkhead box P3 (FOXP3), AP-1 transcription factor subunit (JUNB), and nuclear factor kappa B subunit 1 (NFKB1) [71]. Qi et al. [75] suggested that human hsa-miR-155-5p might regulate the host immune response by controlling the expression of SOCS1, IL6, IL1B, colony stimulating factor 1 receptor (CSF1R), programmed death-ligand 1 CD274, TLR6, and TNF. Additionally, they hypothesized that the competing endogenous RNA (ceRNA) network could be utilized to modulate the host immune response to SARS-CoV-2 infection, such as the nuclear paraspeckle assembly transcript 1 (NEAT1)–miR-155-5p–IL-6/TNF/IL-1β axis; however, the specific mechanisms still need to be uncovered [75]. The in vivo

suppression of miR-155 in SARS-CoV-2-infected mice transgenic for human angiotensin I-converting enzyme 2 receptor (tg-mice hACE2) improved survival and mitigated the cytokine storm and lung inflammation. This study reported that treatment with an miR-155 inhibitor improved the survival rate, promoted survival, and attenuated inflammation and the lung cytokine storm induced by the virus through the down-regulation of IL-1α, granulocyte colony-stimulating factor (G-CSF), IL-9, major intrinsic protein of lens fiber (MIP)-2, and interleukin 12 p70 (IL-12-P70), while increasing vascular endothelial growth factor (VEGF), interferon-induced protein 10 (IP-10), IFN-γ, monocyte chemoattractant protein-1 (MCP-1), C-X-C motif chemokine ligand 9 (MIG), MIP-1α, macrophage colony-stimulating factor (M−CSF), TNF-α, and MIP-1β production in lung secretions [72]. There have also been reports that miR-155, due to various correlations, could serve as a diagnostic clinical biomarker for the detection of COVID-19 and the severity of infection and, additionally, might be a predictor of chronic myocardial damage and inflammation in patients after COVID-19 [69,71,76].

Human adenovirus (HAdV) belongs to the *Adenoviridae* family, with a dsDNA genome [77,78]. It has a dramatic impact on its host cell, causing degenerative changes and necrosis of the epithelium of the respiratory tract, gastrointestinal tract, and conjunctiva. In severe cases of HAdV infection, systemic infection may occur [79]. Similarly to the infections described above, HAdV infection leads to the up-regulation of miR-155 [57,80]. Zhao et al. [80] showed that early stages of HAdV type 2 infection in human lung fibroblast cell culture led to increased miR-155 expression [80]. It has been speculated that miR-155 plays a role in the host anti-viral response, as an early gene in primary macrophages responding to different types of inflammation mediators (e.g., cytokine IFN-β) [80,81]. Additionally, miR-155 may inhibit SOCS1, subsequently enhancing type I IFN effector gene expression and the type I IFN-mediated anti-viral response [82].

Human rhinovirus (HRV) belongs to the *Picornaviridae* family, with an ssRNA genome [83]. HRV is the etiological agent of most common colds in healthy subjects, and is a major trigger of chronic obstructive pulmonary disease (COPD) and asthmatic exacerbations, representing a significant problem for disease management [84]. In young children, HRV infection has been linked with the airway secretion of extracellular vesicles containing miR-155, promoting local Th1 cell-mediated anti-viral responses. MiR-155 targets a set of target genes (similar to those in HAdV infection) which control the immune responses to rhinoviruses [85]. Additionally, miR-155 may act as a negative regulator of SHIP1, hence enhancing IFN type I signaling [20]. Bondanese et al. [84] have suggested that the over-expression of miR-155 in human bronchial epithelial cells can affect human rhinovirus 1B (HRV1B) replication by inhibiting its replication, thus participating in the anti-viral response.

In conclusion, miR-155, as the main immune regulator, plays an extremely important role in a range of viral infections. However, the most important role of miR-155 is in the regulation of genes related to the immune response, including SOCS1, SHIP1, and TNF, which influence the regulation of the production of IFN types I and II. Additionally, researchers have noted the ability of miR-155 to inhibit the replication of viruses through the regulation of genes related to the anti-viral response and oxidative stress.

### *2.2. MicroRNA-223 (miR-223)*

MiR-223 plays a key role in the development and homeostasis of the immune system. To date, miR-223 has been demonstrated to be involved in many types of cancers, inflammatory diseases, autoimmune diseases, and other pathological processes [86]. Studies have shown that miR-223 expression is mainly altered during the inflammatory response of various cell types, including granulocytes, macrophages, dendritic cells (DCs), T-cells, endothelial cells, and epithelial cells. Alterations in miR-223 expression regulate the various functions of these cells, attenuating or exacerbating the associated tissue inflammation, thus also playing a role during viral infection [87,88].

#### 2.2.1. Hepatitis Viruses

MiR-223 is abundantly expressed in the liver, and may play a role in the pathology of viral hepatitis. A study revealed elevated serum miR-223 not only in patients with HCC, but also in patients with chronic hepatitis B (CHB) [89]. Meanwhile, in liver tissue with HCC, miR-223 has been found to be down-regulated [90]. This may be due to the fact that hepatocytes contain abundant miR-223, and their damage—for example, caused by inflammation due to viral infection or cancer—can be expected to lead to the release of a significant amount of this miRNA into the circulation [89]. However, another study has shown lower miR-223 levels in the sera of HBV-positive patients than in healthy controls [91,92]. In vitro studies have demonstrated that miR-223 is down-regulated in HBx- or HBV-transfected HepG2 cells, as well as in HepG2.2.15 cells, and the repression of miR-223 has been associated with the up-regulation of c-Myc in infected cells [92].

Furthermore, during HCV infection, the level of miR-223 in serum was decreased [93]. In addition, liver biopsies from CHC patients presented lower miR-223 levels. Notably, low miR-223 levels likely contribute to chronic liver inflammation and subsequent complications through targeting of the NF-κB pathway during viral infection [94]. It has been potentially indicated that this miRNA has an anti-viral function due to high levels of miR-223 being detected in patients who had reached a sustained virological response after treatment [93]. Thus, researchers have suggested that miR-223 might serve as a novel and potential non-invasive biomarker correlated with the therapeutic effect and pathological features of liver disease [92,93].

#### 2.2.2. Hemorrhagic Viruses

Similarly to the viruses discussed earlier, DENV has also been shown to decrease the miR-223 levels in infected cells. Moreover, a study showed that miR-223 over-expression in a human endothelial-like cell line inhibited DENV2 replication. Further analysis showed that miR-223 directly targeted the 3′UTR of the mRNA for microtubule-destabilizing protein stathmin 1 (STMN1), thereby reducing its mRNA and protein levels [95]. The study also indicated that STMN1 is up-regulated during DENV2 infection and, so, the inhibition of STMN1 expression had a negative effect on DENV replication. Moreover, researchers showed that decreased C/EBPα and increased E2F transcription factor 1 (E2F1) played a major role in the DENV2-induced down-regulation of miR-223 through a negative feedback loop. The results suggested that miR-223 downregulation may be associated with the induction of pro-inflammatory stimuli, and that miR-223 itself may act as a novel anti-viral factor, which may open an avenue to limiting DENV infection [95].

#### 2.2.3. Respiratory Viruses

Studies have indicated that miR-223 is differentially expressed in response to influenza viruses, and may contribute to the virulence of the influenza A virus (IAV) [96–98]; in particular, during IAV infection, the up-regulation of miR-223 in lung tissue, whole blood, and infected cells has been observed [96,97,99]. In addition, a higher level of miR-223 was observed with infections causing severe and lethal pulmonary disease, such as those of r1918 (the reconstructed 1918 influenza virus), A/Vietnam/1203/04, or H5N2 ma81, in contrast with non-lethal infections of some other H1N1 influenza virus strains, including A/Texas/36/91 (Tx/91), A/Kawasaki/173/01 (K173), or H5N1 w81 [96,97,100]. A study showed that, along the duration of the infection, the up-regulation of miR-223 occurred [100]. The up-regulation of miR-223 was also observed in 2009 pandemic H1N1 virus and PR8 infection [98]. Interestingly, research showed that the inhibition of miR-223-3p reduced IV replication in the lungs, while over-expression of this miRNA in the lungs augmented the infection. Mice treated with anti-miR-223-3p presented increased survival and started to gain weight 6 days after infection [100]. Moreover, studies have indicated that increased miR-223 is associated with the regulation of inflammation in mouse lungs through down-regulation of the pro-inflammatory cytokine production of TNF-α [97,101], and also promotes granulocytic differentiation [100]. Additionally, the upregulation of miR-223

may repress the activity of cyclic AMP responsive element-binding protein (CREB)—which is a transcription factor involved in critical functions, including T-cell development and cell survival—by targeting PI3K, insulin-like growth factor 1 receptor (IGF1R), G protein coupled receptors (GPCR), protein phosphatase 2 (PP2A), cAMP-dependent protein kinase (PKA), and the $Ca^{2+}$ channel [96]. Other analyses have shown that miR-223 may regulate interleukin 1 receptor antagonist (IL1RN), melanoma differentiation-associated protein 5 (MDA5), and STAT1 genes, and can participate in the regulation of cell death and apoptosis [100]. The research results obtained by Liu et al. [102] indicated that miR-223 was down-regulated in patients during IAV infection and in cell cultures infected with H1N1 (A/Puerto Rico/8/34). Further research showed that miR-223 over-expression by a mimic had inhibitory effects on IAV-induced elevated ROS intensity, inflammatory cytokine (TNF-α, IL-1β, IL-6, and IL-18) contents, and cell apoptotic rate, and also lowered the expression of NOD-like (NLR) receptor family pyrin domain containing 3 (NLRP3) [102], the over-expression of which can lead to a disordered inflammatory state and the immune pathogenesis of cells under stimulus [103].

Severe acute respiratory syndrome coronavirus (SARS-CoV) is a member of the *Coronaviridae* family, with an RNA genome [64]. SARS-CoV is the etiological agent of severe acute respiratory syndrome (SARS). It is characterized by severe symptoms associated with lower respiratory tract infection, causing alveolar damage. Atypical pneumonia with rapid deterioration and failure may occur due to increased levels of activated pro-inflammatory chemokines and cytokines. In severe cases of SARS, ARDS may be observed as a severe life-threatening immune-mediated disease [104]. In the case of SARS-CoV infection, miR-223 is down-regulated in bronchoalveolar stem cells (BASCs), which may contribute to the development of infection. Mallick et al. [105] indicated that the N and S proteins of SARS-CoV down-regulate miR-223, which may help the N protein to enter the host cell. Furthermore, decreased expression of miR-223 may help SARS-CoV to escape miRNA-mediated repression, rescuing the virus for effective transmission at the initial stage of viral infection. In addition, researchers have suggested that, upon successful entry, the N protein may use miR-223 once again to activate C-C motif chemokine receptor 1 (CCR1), the inflammatory chemokine receptor for CCL3 and CCL5, via NF-κB at its replicative stage, in order to enhance lung fibrosis [105].

As with SARS, miR-223 is decreased in SARS-CoV-2 infection [106,107]. Additionally, it has been predicted that miR-223 directly binds to the receptor of the S protein, mediating the membrane fusion and entry of SARS-CoV-2 into cells. An in vitro study indicated that an miR-223-3p mimic significantly inhibited the expression of the S protein, and also significantly inhibited SARS-CoV-2 replication [106]. Due to the fact that important targets for miR-223 involved in infection and inflammation are TNF receptor-associated factor 6 (TRAF6), forkhead box O1 (FOXO1), TLR4, STMN1, PI3K/AKT, C-X-C motif chemokine ligand 2 (CXCL2), CCL3, IL-6, IFN-I, IL-1β, Caspase-1 (and, mainly, NLRP3), inhibitor of nuclear factor kappa-B kinase subunit alpha (IKKα), and NF-κB, it participates in regulating the inflammatory process while also indicating antioxidant and anti-viral roles, suggesting miR-223 as a potential regulatory factor in the process of COVID-19 immunopathogenesis [108].

#### 2.2.4. Human Immunodeficiency Viruses

Human immunodeficiency virus-1 (HIV-1) is a member of the *Retroviridae* family, with an ssRNA genome [109]. Human immunodeficiency virus (HIV) causes acquired immune deficiency syndrome (AIDS), which kills or impairs cells of the immune system and progressively destroys the body's ability to fight infections and certain cancers. RNA viruses, such as HIV-1, are replicated in the cytoplasm. Given that miRNAs primarily regulate mRNA in the cytoplasm, it is possible that miRNAs function as anti-viral factors by directly targeting and controlling the replication of RNA viruses. Huang et al. [110] showed that miR-223 potently inhibits HIV-1 production in resting primary CD4+ T-cells [110]. The reduction of miR-223 or inhibition of its activity accelerated HIV-1 replication in dormant

CD4+ T-cells [110,111]. A higher miR-223 level was also observed in monocytes, whereas HIV-1 infectivity was greater in macrophages than in monocytes, potentially indicating a role of miR-223 in regulating the susceptibility of immune cells [111]. However, Sun et al. [112] pointed out that, due to the low expression levels of miR-223 in T-cells and the poor accessibility of the target site, it is unlikely that miR-223 directly inhibits HIV-1 in these cells. Meanwhile, the expression of miR-223 is significantly increased in myeloid, cells which may indicate that it has an inhibitory function on HIV-1 in NK cells, macrophages, and monocytes. It is more likely, though, that miR-223 influences HIV-1 infection by targeting cellular factors required for replication, such as ras homolog family member B (RhoB), Sp3 transcription factor (Sp3), and leukemia inhibitory factor (LIF) [112]. RhoB functions as an anti-apoptosis gene, and can activate the AKT–NF-κB pathway. It has also been reported that RhoB is down-regulated in HIV-1-infected cells obtained from patients. Sp3 can repress HIV-1 LTR activity directly, and may also activate apolipoprotein B mRNA editing enzyme catalytic subunit 3G (APOBEC3G), which is a host viral restriction factor. LIF can restrict HIV-1 replication, and has been reported to be down-regulated in early HIV-1 infection. Therefore, miR-223 may function as a negative factor in HIV-1 infection by reducing RhoB-mediated activation of the AKT–NF-κB pathway. This miRNA may also function as a positive factor in HIV-1 infection by targeting HIV-1-suppressive Sp3 and LIF [112].

In conclusion, miR-223 plays an important role in the regulation of the immune response. Altered expression of miR-223 is commonly observed during viral infections, which is crucial for maintaining immune homeostasis to avoid excessive inflammation and tissue damage as a result of the infection. In addition, miR-223 over-expression inhibits replication of viruses, which may constitute a good basis for the development of new anti-viral therapies in the future.

### *2.3. MicroRNA-146a (miR-146a)*

MiR-146a, as a modulator of the differentiation and function of cells of innate as well as adaptive immunity, has particular importance. In T-cells, miR-146a might be involved in the determination of the fate of Th1 and Th2 cells due to their differential expression [113]. In addition, miR-146a is induced by T-cell receptor (TCR) stimulation in memory T-cells and has also been shown to be critical for Treg functions [114,115]. Furthermore, miR-146a is quickly induced upon the activation of human monocytes, and is also inducible by inflammation in an NF-κB-dependent manner, targeting the TRAF6 and IL-1 receptor-associated kinase (IRAK) 1 genes [114]. Studies have reported that negative regulation of miR-146a may contribute to promoting viral replication in macrophages through type I IFN down-regulation in an RIG-I/NF-κB-dependent manner, and the down-regulation of TRAF6, IRAK1, and IRAK2 [116]. Considering the above, miR-146a seems to be an important regulator in viral infection. MiR-146a plays a significant role in inflammatory settings and in the T-lymphocyte-mediated adaptive immune response, which has a pivotal role in viral infections [117,118].

#### 2.3.1. Hepatitis Viruses

In CHB and acute chronic liver failure (ACLF) caused by HBV, miR-146a-5p expression levels have been observed to be up-regulated in sera and in a human hepatic cell line [118–120]. HBV infection led to higher levels of miR-146a and pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-8, IL-12, and IL-18), along with a reduction in X-linked inhibitor of apoptosis (XIAP) expression via NF-κB activation [118]. In chronic HBV infection, inflammatory cytokines and viral factors may induce miR-146a expression in T-cells. The up-regulation of miR-146a may subsequently lead to the suppression of the anti-viral function of CD4+ and CD8+ T-cells by targeting STAT1, and may contribute to the persistence of the virus. MiR-146a also decreases the cytotoxicity of T-cells, as well as the production of anti-viral cytokines (e.g., IFN-γ, IL-2, and TNF-α). Additionally, an in vitro study has shown that the blockage of miR-146a in T-cells in CHB greatly enhanced virus-specific T-cell activity [117]. Li et al. [121]

found that HBx promoted the expression of miR-146a through the NF-κB signaling pathway, and that increasingly expressed miR-146a down-regulated its target, complement factor H (CFH)—an important negative regulator of the alternative complement pathway. These results demonstrate that the HBx–miR-146a–CFH–complement activation regulation pathway might play an important role in the immunopathogenesis of chronic HBV infection and the promotion of liver inflammation [121]. Interestingly, a single-nucleotide polymorphism within the miR-146a gene may contribute to acute-on-chronic hepatitis B liver failure. Jiang et al. [122] reported that the GG genotype within the pre-miR-146a in PBMCs was associated with higher expression of miR-146a, lower levels of serum TNF-α concentration, and a relatively higher survival rate [122]. Research has shown that miR-146a may affect HBV replication [118–120]. It has been reported that HBV induces the autophagic response of the miR-146a-5p–XIAP–mouse double minute 2 homolog (MDM2)/cellular tumor antigen p53 (p53) pathway, leading to enhanced HBV replication [118]. Another target of miR-146a affecting HBV replication is zinc finger E-box-binding homeobox 2 (ZEB2) [119], which has been found to suppress HBV transcription and replication by targeting its core promoter [123]. The in vitro study of Wang and Li [119] demonstrated that the over-expression of miR-146a or knockdown of ZEB2 promoted HBV replication and expression, while the down-regulation of miR-146a or over-expression of ZEB2 suppressed viral replication [119]. MiR-146a may also regulate HBV replication indirectly through flap endonuclease 1 (FEN1), which repairs relaxed circular DNA to form covalently closed circular DNA, thus promoting HBV DNA replication [120,124]. The regulation of FEN1 takes place through the down-regulation of TRAF6 and IRAK1 by miR-146a, decreasing the activity of the NF-κB pathway. In addition, Argonaute-2 (Ago2) cooperates with miR-146a to regulate the transcription and expression levels of the FEN1 protein through the downstream target gene IRAK1/TRAF6, promoting HBV replication [120].

MiR-146a has also been found to be up-regulated in monocytes from HCV-infected patients [125,126]. Zhang et al. [126] indicated that the HCV core protein could promote the expression of miR-146a through the TLR2–myeloid differentiation primary response 88 (MyD88) pathway [126]. However, the level of miR-146a in monocytes with HCV infection decreased following TLR stimulation [125]. Another mechanism regulating miR-146a expression in hepatocytes during HCV infection is NF-κB signaling [127]. Another study showed that the inhibition of miR-146a in monocytes from HCV-infected patients led to decreased IL-23, IL-10, and TGF-β expression through the induction of the SOCS1/STAT3 pathway [125]. Ren et al. [125] have suggested that miR-146a increases cytokine production in monocytes and increases regulatory T-cells during HCV infection through SOCS1/STAT3 signaling induction, which may lead to immune injury of the liver during chronic viral infection [125]. As in HBV infection, miR-146a-5p promotes HCV infection by enhancing the production of infectious viral particles and, furthermore, contributes to the development of liver disease and HCC by targeting genes associated with inflammation, fibrosis, and cancer development, including CXC motif chemokine receptor 4 (CXCR4), TLR2, TRAF6, IRAK1, breast cancer gene 1 (BRCA1), NFKB1, epidermal growth factor receptor (EGFR), CD40 ligand (CD40LG), SMAD4, hepatocyte nuclear factor 1 homeobox A (HNF1), SHIP1, and TLR4 [127].

#### 2.3.2. Hemorrhagic Viruses

DENV infection induces massive immune activation and the production of high amounts of pro-inflammatory cytokines, which can be regulated by miR-146a [128]. Wu et al. [129] found that expression of miR-146a was significantly up-regulated after DENV-2 infection of human primary monocytes and THP-1 cells. Moreover, they indicated that the over-expression of miR-146a inhibited the production of IFN-β and IL-28A/B by TRAF6, and also contributed to increased DENV2 replication [129]. MiR-146a, by targeting TRAF6, also decreased the conversion of the microtubule-associated protein light-chain 3-I (LC3-I) to LC3-II, as well as negatively regulating the autophagy process of A549 cells and THP-1 cells during DENV2 infection, thus contributing to efficient viral replication [130]. Similarly, up-regulation of miR-146a has been observed in the hepatic tissue of patients

with dengue hemorrhagic fever, which may promote the inflammatory response and pathological liver damage [131]. Interestingly, Ouyang et al. [132] demonstrated that miR-146a was significantly decreased in the sera of dengue patients. Additionally, they showed that miR-146a-5p was negatively correlated with serum aspartate aminotransferase (AST) and ALT activities in dengue-infected patients, suggesting that miR-146a might mediate the development of liver inflammation. However, the physiological or pathological significance of reduced miR-146a in the sera of dengue-infected patients remains ambiguous and, thus, requires further research [132].

#### 2.3.3. Respiratory Viruses

Research has indicated that miR-146a is induced during IAV infection and, so, may play an important role in viral replication in this context [133–136]. Zhang et al. [133] showed that the over-expression of miR-146a in A549 cells diminished IFN type I (IFN-I) responses by decreasing IFN-β production and ISG expression, thereby promoting IAV replication [133]. In addition, they found that miR-146a directly targets TRAF6, which is involved in the production of IFN-I, where TRAF6 over-expression reversed the replication-promoting effect of miR-146a on IAV. Furthermore, the in vivo inhibition of miR-146a alleviated IAV-induced lung injury in mice and enhanced survival rates by promoting type I IFN anti-viral activities [133]. Another study has indicated that, during IAV infection, miR-146a may negatively regulate IRAK1 and exert an influence on the neurotrophin and Toll-like signaling pathway, IL-7 signaling pathway, VEGF signaling pathway, or JAK-STAT pathway [134]. Functional analysis has additionally revealed that miR-146a is strongly associated with the innate immune response, cytokine production, and apoptosis [135]. In human nasal epithelial cells (hNECs), after infection with influenza H3N2 virus, miR-146a was induced, regulating TRAF6 expression but not IRAK expression in the nasal epithelium; in contrast, it targeted IRAK1 in the lower airway [136]. Interestingly, Terrier et al. [135] indicated that the inhibition of miR-146a significantly increased viral propagation. Therefore, more research is needed to clarify the anti-viral effect of miR-146a in IAV infection and influenza–host interactions.

As in the case of IAV infection, RSV infection strongly increased the expression of miR-146a-5p [137]; however, in vitro studies have shown that miR-146a targets 70 kilodalton heat shock proteins (HSP-70) during RSV infection, and not (as with IAV) TRAF6 and IRAK1, which may have an effect on viral replication [137,138].

In contrast to other respiratory infections, during SARS-CoV-2 infection, miR-146a down-regulation has been observed, where the level of miR-146a was correlated with the severity of COVID-19 [139,140]. Tang et al. [139] have pointed out that miR-146a plays fundamental roles during SARS-CoV-2 infection by targeting TRAF6, IRAK1, and IRAK2 which participate in the NF-κB pro-inflammatory signaling pathway in immune cells. Additionally, analysis has revealed the broad up-regulation of STAT1, targeted by miR-146a-5p, which encodes a key element of the JAK/STAT pathway [139]. The miR-146a deficiency observed in diabetes leads to enhanced inflammation and increased synthesis of MCP-1, followed by further reduction in miR-146a by enhancing the signaling of systemic effects by TGF-β1/Erb-B2 receptor tyrosine kinase 4 (ErbB4)/neurogenic locus notch homolog protein 1 (Notch1) accompanying severe COVID-19; this may explain the more severe COVID-19 cases occurring in these patients [141]. However, in the oral fluids of patients with type 2 diabetes, increased levels of miR-146a have been observed, which may lead to the up-regulation of angiotensin converting enzyme 2 (ACE2) expression; these are essential SARS-CoV-2 receptors, and modulate the host anti-viral response in these patients [142]. Sabbatinelli et al. [140] showed that COVID-19 patients presented increased IL-6 levels and reduced miR-146a-5p levels compared to healthy age-matched subjects, pointing out the imbalance in the IL-6/miR-146a-5p physiological axis in the pathogenesis of SARS-CoV-2 infection [140]. Additionally, patients with multi-focal interstitial pneumonia due to SARS-CoV-2 infection who did not respond to anti-IL-6 therapy had lower serum levels of miR-146a-5p, and experienced a more severe course of the disease [140]. The down-

regulation of miR-146a in COVID-19 may cause hyperactivation of the immune response, a loss in T-cell function, and immune dysregulation in patients with severe COVID-19 and, so, it might be key regulator of COVID-19 pathogenesis [139]. Moreover, the down-regulation of miR-146a may cause excessive cytokine production and the lack of a feed-back mechanism to limit inflammatory damage to tissues [141].

In conclusion, miR-146a regulates genes influencing the activity of the NF-κB transcription factor and, thus, may affect the production of pro-inflammatory cytokines during viral infections. Additionally, miR-146a indirectly influences viral replication by regulating NF-κB activity.

### *2.4. MicroRNA-122 (miR-122)*

MiR-122 is an miRNA that is conserved among vertebrate species. MiR-122 typically has a liver-enriched expression and is one of the most abundant miRNAs in the liver, accounting for about 70% and 52% of the whole hepatic miRNome in adult mice and humans, respectively [143,144]. MiR-122 is a central player in liver biology (including liver development) and differentiation, supports spontaneous regeneration, and takes part in liver homeostasis, lipid metabolism, and cholesterol synthesis [145–147]. Hence, alterations in intrahepatic miR-122 have been associated with liver disease, including hepatitis with viral etiology (HBV, HCV), steatosis, cirrhosis, and hepatocellular carcinoma (HCC) [147].

#### 2.4.1. Hepatitis Viruses

As liver-specific miRNA, miR-122 has been found to be closely related to HBV replication and liver injury [148]. MiR-122 expression in the liver was significantly down-regulated in patients with HBV infection compared with healthy controls, and the miR-122 levels were negatively correlated with intrahepatic viral load and hepatic necroinflammation [149]. Additionally, Wu et al. [150] showed that serum miR-122 levels were significantly lower in patients who developed a virological response (VR) compared with the non-VR group [150]. The decreased miR-122 expression in HBV can lead to increased expression of the cyclin G1 gene. Then, cyclin G1 can attenuate the activity of P53, which increases HBV replication; therefore, a loss in miR-122 expression in HBV patients may activate modulating cyclin G1 and increase HBV replication [149]. On the other hand, it has been reported that serum miR-122 levels were positively associated with serum HBV DNA levels in chronic hepatitis B (CHB) patients [150]. Ebrahimifard et al. [151] also showed that miR-122 levels in sera from CHB patients were higher than those of the control group [151]. Van de Ree et al. [152] shown that plasma miR-122 levels were approximately 60 times higher in CHB patients compared to healthy controls, and approximately 2 times higher in hepatitis B antigen (HBeAg)-positive patients vs. HBeAg-negative patients [152]. Such increases in circulating miR-122 levels in plasma might be caused by the HBV-induced up-regulation of miR-122 expression and increased secretion from the liver [152]. In an in vitro study, researchers reported that the transfection of an miR-122 mimic inhibited HBV expression, whereas the anti-sense inhibition of miR-122 led to increased HBV production in transfected cells. In another study, it was observed that the down-regulation of HO-1 by miR-122 played a negative role in the miR-122-mediated inhibition of viral expression [153–155]. MiR-122 can also inhibit HBV replication by modulating the expression of type I IFN, which can play a significant role in the host anti-viral response, such as protecting against HBV infection [156]. The activity of the JAK/STAT signaling pathway can be negatively regulated by SOCS, and miR-122 may also down-regulate SOCS3 [157]. Although the expression of miR-122 is transcriptionally regulated by liver-enriched transcription factors, including hepatocyte nuclear factor 4 alpha (HNF4) and C/EBPα, it may also be regulated by viral infection [145,158,159]. The transfection of HBV genes into liver cells (Huh7 and HepG2) suppressed the expression of miR-122, which subsequently induced the expression of apolipoprotein B mRNA-editing enzyme catalytic subunit 2 (APOBEC2). These findings further suggest that the effects of HBV on APOBEC2 occur via the down-regulation of cellular miR-122 expression, which may contribute to the tumorigenesis of liver cells [160]. Additionally, research has sug-

gested that hepatitis B virus X protein (HBx) is an important negative regulator of miR-122 expression, highlighted by the fact that HBx binds to peroxisome proliferator activated receptor gamma and inhibits the transcription of miR-122 [92,161]. Another study has shown that the down-regulation of miR-122 may involve HBx through the down-regulation of germline development 2 (Gld2) [162]. More interestingly, other studies have found that serum miR-122 levels may serve as an indicator for viral translation and a potential marker for risk stratification in patients infected with HBV [163,164].

MiR-122 is an abundant, liver-specific miRNA that is an unusual host factor for HCV, an important cause of liver disease in humans [165]. MiR-122 has been shown to be required for the replication of HCV in the hepatoma cell line Huh7. Jopling et al. [166] have shown that sequestration of miR-122 leads to a marked loss in replicating viral RNAs, and simultaneous recognition of the binding site within the 5′-NCR by miR-122 is required for miR-122-induced viral RNA accumulation; this suggests that miR-122 is likely to facilitate viral RNA replication through interaction with the viral 5′-NCR [166]. There have been reports that miR-122 could also enhance HCV replication in non-hepatic human embryonic kidney epithelial cells (HEK-293) [167]. Interestingly, miR-122 expression has been shown to endow the ability of supporting efficient HCV RNA replication, virion production, and virion release in HepG2 cells. These results support the notion that miR-122 is required for HCV RNA replication, but does not greatly enhance viral translation [168]. Henke et al. [169] have shown that miR-122 stimulates HCV RNA translation at an early stage of association with the small ribosomal sub-unit in the viral RNA. The sequestration of miR-122 in liver cell lines strongly reduced HCV translation, whereas the addition of miR-122 stimulated HCV translation in liver cell lines, as well as in non-liver HeLa cells and in rabbit reticulocyte lysate. This stimulation effect was confirmed by the direct interaction of miR-122 with two target sites in the 5′-UTR of the HCV genome [169]. It has also been shown that miR-122 binds to HCV RNA in association with the Ago2 protein complex, slowing the decay of the viral genome in infected cells. Data have demonstrated that an RISC-like complex mediates the stability of HCV RNA, suggesting that Ago2 and miR-122 act in a coordinated manner to protect the viral genome from the 5′ exonuclease activity of the host mRNA decay machinery. Thus, miR-122 acts in an unconventional way to stabilize HCV RNA and slow its decay, expanding the repertoire of mechanisms by which miRNAs modulate gene expression [165]. In another study, the results of an Huh7 cell experiment indicated that the silencing of miR-122 with antagomir decreases HCV RNA abundance, whereas the transfection of miR-122 mimics increases the HCV level. Additionally, the antagomir of miR-122 up-regulates heme oxygenase-1 (HO-1), likely by decreasing transcription repressor Bach1, where HO-1 significantly inhibits HCV replication [170]. In addition, miR-122 has also been shown to be regulated by IFN; therefore, the down-regulation of its expression by IFN could serve as a general anti-viral mechanism. However, due to the cell-type-specific expression of miR-122, this effect may be limited to viruses that infect hepatocytes, such as HCV [171]. Researchers have also shown that the HCV core expression activates an miR122–transforming growth factor β receptor-associated protein 1 (TGFBRAP1) signaling pathway, which might be associated with promoting HCC progression [172]. Additionally, attention should be paid to circulating miR-122 as a diagnostic marker for chronic viral hepatitis detection [164]. In patients with CHC, the serum level of miR-122 has been found to be strongly correlated with serum ALT activity, as well as with necroinflammatory activity in patients with CHC and elevated ALT levels; however, this was not correlated with the fibrosis stage or functional capacity of the liver [173]. However, in the future, well-designed, large-scale, and accurate research is still needed to expand knowledge in this context.

#### 2.4.2. Hemorrhagic Viruses

The liver is one of the most important target tissues in severe cases of dengue due to its intense viral replication and metabolic role. Determining the roles of miRNA during infection is crucial in order to understand the regulatory mechanisms of DENV infection. De

Oliveira et al. [131] studied the expression profile of miR-122 in liver tissue under dengue hemorrhagic fever (DHF). They observed a down-regulation of miR-122 in fatal cases compared to controls. Additionally, they selected target genes for miR-122 in the course of dengue, such as cytochrome P450 family 7 subfamily A member 1 (CYP7A1), IGF1R, serum response factor (SRF), Rac family small GTPase 1 (RAC1), Ras homolog family member A (RHOA), and cyclin G1 (CCNG), which are indirectly involved in immune processes [131]. Meanwhile, another study revealed the up-regulation of miR-122 in sera. The highest level of miR-122 was observed in patients with DHF. Elevated serum levels of miR-122 may be due to its release from damaged hepatocytes as a result of dengue infection [174]. MiR-122 may also modulate the replication of the DENV replicon. In hepatic Huh-7 cells, endogenous miR-122 effectively suppressed the translation levels of replicon D2R2A-30 X/122pmT before entering a more vigorous replication process, whereas stable expression of miR-122 in baby hamster kidney fibroblast (BHK)-21 cells effectively reduced the viral replication of D2R2A-30X/122pmT [175].

In the early stage of Ebola virus (EBOV) infection of Huh-7 cells with the RESTV strain, the expression level of miR-122 was reduced by half. Meanwhile, at 96 h of infection, a two-fold increase in miR-122 was observed in cells infected with the ZEBOV strain [176]. These studies also demonstrated that miR-122 can target the viral genomes of ZEBOV and RESTV; namely, the viral structural protein vp40 gene, which is responsible for regulating viral transcription and coordinating virion assembly [176,177].

#### 2.4.3. Respiratory Viruses

MiR-122 may be also regulated in respiratory viral infections. In the peripheral blood of infants infected with RSV, the down-regulation of miR-122 was observed. Pathway analysis indicated that the dysregulated miRNA was involved in inflammatory and immune responses, including Wnt [178]. Additionally, as a target gene of miR-122-5p, interleukin-1 receptor type I (IL1R1) may be activated by increased interleukin-1 receptor antagonist (IL1RA) after RSV infection, which is one of the RSV-induced genes [179,180]. Another target gene of miR-122-5p is TLR4, which is stimulated by the RSV F protein [180]. TLR4-deficient mice infected with RSV exhibited enhanced disease, and the expression of IL-13, TGF-β, and IL-6 was induced [181,182]. In addition to pulmonary symptoms, RSV can also present with cardiovascular symptoms [183]. Infants with severe acute bronchiolitis-caused RSV and coexisting cardiac dysfunction presented a sharp elevation in serum miR-122. In the case of RSV with cardiovascular disorders, the up-regulation of miR-122 may down-regulate the expression of cationic amino acid transporter-1 (CAT-1) or/and prevent the translation of inducible nitric oxide synthase (iNOS) mRNA, thereby weakening the anti-viral effect [184].

Collison et al. [185] found that HRV infection induced the expression of miR-122 in mouse lungs and human airway epithelial cells. The in vivo inhibition of miR-122 in the lung reduced neutrophilic inflammation and CXCL2 expression, enhanced the innate IFN response, and ameliorated airway hyper-reactivity in the absence and presence of allergic lung inflammation. Additionally, the inhibition of miR-122 in the lung increased the level of suppressor SOCS1, which is an in vitro-validated target of miR-122. Importantly, the gene silencing of SOCS1 in vivo completely reversed the protective effects of miR-122 inhibition on HRV-induced lung disease. These results suggest that miR-122 promotes HRV-induced lung disease through the suppression of the target gene SOCS1 in vivo [185].

In summary, miR-122—considered a liver-specific miRNA—plays a major role in the replication of hepatitis and EBOV viruses. During infection with hepatitis viruses, miR-122 can serve as a biomarker of viral infection. On the other hand, the data highlighted in our review indicate that the role of miR-122 in other viral infections related to the regulation of genes involved in innate immune response is poorly understood at present.

### 2.5. MicroRNA-125b (miR-125b)

MiR-125b is involved in regulating NF-κB, p53, PI3K/Akt/mTOR, v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (ErbB2), Wnt, and other signaling pathways, thereby controlling cell proliferation, differentiation, metabolism, and apoptosis [186]. However, the expression of miR-125b-5p may be also modulated by NF-κB signaling. MiR-125b negatively regulates the inflammatory response by targeting TNF-α [187]. In addition, inducing the inflammation of human macrophages in vitro through lipopolysaccharides (LPS) can suppress the expression of miR-125b [188]. It has been shown that miR-125b may inhibit the expression of a gene encoding 5-lipoxygenase—a key enzyme in the biosynthesis of leukotrienes, which are essential for the innate immune response and inflammatory processes [189]. Researchers have also demonstrated that miR-125b can promote macrophage-mediated inflammation. MiR-125b, by targeting interferon regulatory factor 4 (IRF4), potentiates the functional role of macrophages in inducing immune responses and anti-tumor activities [190]. Other direct target genes of miR-125b are p53 and p38 mitogen-activated protein kinase 1 (MAPK), which can activate the apoptotic pathway and induce mitochondrial apoptosis, respectively [186,191]. Furthermore, the levels of induced myeloid leukemia cell differentiation protein (Mcl-1), B-cell lymphoma-extra-large (Bcl-xL), and Bcl-2-like protein 2 (Bcl-w)—the anti-apoptotic members of the B-cell lymphoma 2 (Bcl-2) family—are increased by miR-125b [192,193]. MiR-125b also regulates T-cell proliferation and activation, is highly expressed in naive CD4 T-cells, and can inhibit the T-cell immune response; however, it may also promote T-cell apoptosis [186].

#### 2.5.1. Hepatitis Viruses

MiR-125b plays an important role in HBV and HCV infection, as well as in HCC development. Research has indicated that miR-125b-5p over-expression increased HBV replication in HepG2.2.15 cells [194,195]. However, Deng et al. [194] found that miR-125b increased HBV replication without altering HBV transcription. Their data demonstrated that miR-125b-5p targeted the LIN28B/let-7 axis in order to stimulate HBV replication in a post-transcriptional step [194]. In addition, miR-125b in serum has been positively correlated with the serum HBV DNA level and with grades of liver necroinflammation [195]. Meanwhile, plasma levels of miR-125b were remarkably decreased in HBV-HCC patients compared to healthy controls and HBV subjects without HCC. Moreover, the low plasma miR-125b levels in HBV-HCC patients were associated with a higher prevalence of metastasis [196]. In addition, it has been reported that miR-125b-5p inhibits the phosphorylation of retinoblastoma protein and blocks cell cycle progression at the G1/S phase in hepatoma cell lines [194]. An in vitro study by Zhang et al. [197] has shown that miR-125b regulates HBV expression by targeting the sodium channel, non-voltage-gated 1 alpha (SCNN1A) gene, and inhibits HBV core protein expression, as well as HBsAg and HBeAg secretion. Additionally, it has been shown that HBV infection of Hep.G2 cells reduces the level of miR-125b [197]. Due to the importance of the data presented, further research is needed to elucidate the role of miR-125b in HBV infection and its potential role as an anti-HBV therapeutic target.

Peng et al. [198] indicated that, in response to HCV core protein stimulation, miR-125b expression was down-regulated in THP-1 cells in contrast to up-regulated cytokine production (e.g., TNF-α, IL-10). Nevertheless, in vitro forced miR-125b expression abolished the HCV core protein-induced enhancement of cytokine expression by targeting TLR2/MyD88 signaling in monocytes, including the phosphorylation of NF-κBp65, extracellular signal-regulated kinases (ERKs), and P38 mitogen-activated protein kinase [198]. Other studies have found that the serum level of miR-125b is increased in HCV infection, in a manner correlated with HCV infection [199–201]. These results were confirmed through in vitro studies, showing that the promoter activity and expression of miR-125b were increased in HCV replicon cells, whereas the miR-125b inhibitor reduced HCV expression levels. The results also indicated that the IL-6/STAT3 pathway plays an inducible role in miR-125b expression and may contribute indirectly—through increased expression of miR-125b—to

enhanced HCV replication [199]. MiR-125b is involved in translational regulation and may regulate virus replication through human antigen R (HuR), a positive regulator of HCV replication. MiR-125b may also serve as a liver fibrosis biomarker in the context of the viral etiology of HCV [200]. Up-regulated expression of miR-125b has been observed in plasma samples from patients with advanced liver fibrosis in CHC, suggesting that miR-125b has potential as a novel prognostic biomarker, independent of viral replication [201]. Studies have indicated that miR-125b-5p is up-regulated in HCV-infected liver carcinoma cells and down-regulated in exosomes from serum [200,202]. In addition, exosomal miR-125b levels have been used to predict recurrence and survival in HCC patients [202].

Hepatitis E virus (HEV) belongs to the family *Hepeviridae*, with an ssRNA genome [203]. It is probably the most common cause of acute viral hepatitis—about 20 million cases are diagnosed annually, and the number of deaths annually has been estimated at 70,000 [204]. Down-regulated miR-125b has been observed in sera from patients with HEV. Interestingly, during acute hepatitis E infection, lower expression of miR-125b was observed compared to samples from patients with chronic hepatis E. Based on these results, researchers have suggested that miR-125b may be a useful biomarker to differentiate acute from chronic viral hepatitis [204].

#### 2.5.2. Respiratory Viruses

Inchley et al. [58] indicated that miR-125b was down-regulated in RVS-infected infants compared to healthy controls [58]; however, based on the results of samples from children with severe RSV-associated pneumonia and samples from mild-RSV-infected children, Zhang et al. [205] found that hsa-miR-125b-5p was significantly increased in samples from children with severe RSV-associated pneumonia. Through gene ontology (GO) enrichment analysis of the target genes of the miRNA, the researchers showed that most target genes were involved in the NF-κB and mitogen-activated protein kinase 1 (MAPK) signaling pathways, which are crucial components of the immune response in humans. Thus, the activation of NF-κB signaling may result in serious complications during severe RSV infection [205].

A reduction in miR-125b expression has also been observed in patients infected with H5N1 and H1N1 influenza virus [99,206]. The down-regulation of miR-125b can trigger the MAPK signaling pathway, which regulates various cellular responses, including cell proliferation and apoptosis [206].

Interesting observations have been made by Chen et al. [207], who noted that the miR-125b-5p–ACE2–IL-6 axis could alter the risk of SARS-CoV-2 infection in lung adenocarcinoma patients. Reduced miR-125b-5p might be the primary inhibitor of ACE2 in lung adenocarcinoma. Whereas ACE2 was dysregulated, IL-6 in the TLR pathway might activate the immune system as a downstream effector [207].

#### 2.5.3. Human Immunodeficiency Viruses

Another target of miR-125b is cleavage and polyadenylation specificity factor 6 (CPSF6), which plays a key role in HIV-1 infection; specifically, during nuclear import and integration targeting. Researchers reported that HIV-1 infection down-regulated miR-125b expression concurrently with the up-regulation of cleavage and CPSF6, which can contribute to promoting HIV-1 nuclear entry and replication [208]. The inhibitory effect of miR-125b on HIV replication was also demonstrated by Mantri et al. [209], who showed that miR-125b knockdown enhanced HIV-1 replication, whereas the over-expression of miR-125b decreased HIV-1 replication in CD4+ T-cells. The replication control mechanism of HIV may be mediated by targeting of the 3′ UTR regions of HIV-1 transcripts by miR-125b, inhibiting viral protein translation [209]. Additionally, an interesting observation is that miR-125b expression levels are especially high in resting CD4 + T-cells, which may explain the resistance of these cells to HIV-1 infection in comparison to activated CD4 + T-cells, which present down-regulation of miR-125b [110].

#### 2.5.4. Neurotropic Viruses

Japanese encephalitis virus (JEV) belongs to the *Flaviviridae* family, with an RNA genome [210]. Japanese encephalitis virus (JEV) is a neurotropic virus that mainly infects children between 1 and 5 years of age, leading to permanent neuronal damage, motor deficits, and memory loss. The hallmark of JEV is neuroinflammation [211]. MiR-125b, as with other viral infections, plays a significant role in JEV infection. During the infection of cells with JEV, high expression of miR-125b-5p has been observed, which may have an effect on viral replication. These assumptions were confirmed by an in vitro study. The transfection of miR-125b-5p into acutely infected cells reduced the genome replication and virus titers of JEV by targeting STAT3, mitogen-activated protein kinase kinase 7 (Map2k7), and TP53-regulated inhibitor of apoptosis 1 (Triap1). In addition, miR-125b targets the viral genome, suggesting that it could act as a key regulator, providing a balance between viral replication and the host anti-viral response [212].

In summary, the most important action of miR-125b is its participation in viral replication and the immune response.

### *2.6. MicroRNA-132 (miR-132)*

MiR-132 is known to control many cellular processes in various tissues, including the modulation of inflammatory processes [213]. It regulates a large number of immune response- and cell cycle-related genes, and may play a role in inhibiting fibrosis through the TGF-β1 signaling pathway [214,215]. MiR-132 also has anti-oxidative stress and anti-apoptotic effects through targeting of the PTEN/Akt pathway [213]. The over-expression of miR-132 induces NF-κB and p65 acetylation [216]. Moreover, miR-132 decreases the production of chemokines (e.g., TNFα, IL-1β) and the capability to attract leukocytes by suppressing the NF-κB pathway [214].

#### 2.6.1. Hepatitis Viruses

Research has shown that miR-132 has a relationship with HBV infection. Interestingly, increased miR-132 expression is influenced by HBx-induced hypermethylation of the DNA promoter [217]. An increase in the expression level of miR-132 can inhibit the expression of some viral proteins, thereby decreasing the expression of anti-viral proteins. However, in liver cancer related to HBV infection, the expression level of miR-132 is down-regulated and its level in serum is significantly correlated with its levels in tumor tissue [217]. Further analysis has revealed that miR-132 exerts tumor-suppressing effects through inactivation of the Akt-signaling pathway, specifically by reducing Akt phosphorylation and the concentration of cyclin D1; therefore, decreased expression of miR-132 may contribute to the development of HBV-related HCC [217]. Liu et al. [218] obtained similar results. They reported significant differences in HBV levels among three groups—CHB, liver cirrhosis, and HCC—in which the HBV-DNA level was highest in the liver cancer group and lowest in the CHB group [218]. Moreover, the level of miR-132 differed remarkably among the three groups, but presented a negative correlation with the HBV-DNA level. In addition, significant differences were detected in the expression levels of genes and protein which regulate cell apoptosis, such as PI3K and p-Akt, in the liver tissues of patients with CHB, liver cirrhosis, and HCC; these, like the level of HBV-DNA, were also negatively correlated with the level of miR-132 [218]. However, further data and more in-depth studies are needed to investigate the role of miR-132 in liver function and the progression of viral liver diseases.

#### 2.6.2. Respiratory Viruses

Zhang et al. [219] showed that miR-132 was up-regulated in the blood of patients with IAV, and also that IAV infection up-regulated its expression in a dose- and time-dependent manner. Further, an in vitro study in A549 cells indicated that the up-regulation of miR-132-3p promoted IAV replication, whereas the knockdown of miR-132-3p repressed viral replication [219]. Additionally, it was shown that the over-expression of miR-132-3p

could inhibit IAV-triggered IFN-α and IFN-β production and ISG expression; however, the suppression of the type I IFN response and the promotion of IAV replication occurred through direct targeting of the miR-132 target gene interferon regulatory factor 1 (IRF1) [219]. Buggele et al. [134] reported that infection with the IAV A/Udorn/72 H3N2 and A/WSN/33 H1N1 strains increased miR-132 expression in the human lung epithelial cell lines A549 and BEAS-2B. Furthermore, miR-132 may regulate innate immune signaling pathways by targeting MAPK3 [134]. Interestingly, miR-132 has been also shown to be regulated transcriptionally through an MAPK3 (ERK1) pathway, and a study on IAV virus has suggested that MAPK3 may use miR-132 to regulate its activity in a negative feedback loop [134]. The expression of miR-132 was also increased in primary bronchial epithelial cells from COPD patients. Enhanced levels of miR-132 decreased the transcriptional coactivator p300, which is essential for the activation of IRF3 and the induction of IFN-β. The up-regulation of miR-132 has been shown to modulate IFN-β induction and impair its function during IAV infection [220].

#### 2.6.3. Human Herpesviruses

Herpes simplex viruses (HSVs) type 1 (HSV-1, or human herpesvirus 1—HHV-1) and type 2 (HSV-2, or human herpesvirus 2—HHV-2) are members of the *Herpesviridae* family, with a dsDNA genome [221,222]. Both are closely related, but differ in epidemiology. HSV-1 is associated with orofacial disease and may cause a chronic immuno-inflammatory response in the eye, which is a significant cause of human blindness, whereas HSV-2 is associated with genital disease [222,223]. Mulik et al. [224] showed that miR-132 expression was up-regulated (10-to 20-fold) after ocular infection with HSV-1. This increased miR-132 expression may be caused by VEGF-A and/or IL-17A during infection. The over-expression of miR-132 may lead to the activation of angiogenic Ras, as well as contributing to the immunopathology of stromal keratitis [224].

In summary, it can be concluded that the most important role of miR-132, with respect to the considered viral infections, is the regulation of the immune and inflammatory responses.

### *2.7. MicroRNA-34a (miR-34a)*

MiR-34a is widely expressed in immune cells (e.g., dendritic cells, macrophages, mast cells, B-cells, and T-cells) and regulates their development, function, and survival. This miRNA, by targeting over 30 genes across different cellular pathways, controls the immune response. MiR-34a expression is controlled through the transcription level of p53 [225]. In addition, miR-34a, through the regulation of Bcl-2, sirtuin 1 (SITR1), cyclin-dependent kinase (CDK) 4, and cyclin D1, among others, induces apoptosis, cell cycle arrest, and/or senescence [226]. MiR-34a plays a significant role in viral infection.

#### 2.7.1. Hepatitis Viruses

HBV infection has a crucial role in cirrhosis and primary liver cancer development. Researchers have shown that miR-34a is down-regulated during HBV infection, and is further lowered in expression in liver cancer [227,228]. HBV, by reducing the level of miR-34a, may modulate the up-regulation of the Wnt/β-catenin pathway through the indirect up-regulation of Wnt1 [229]. In HBV infection, the HBx protein exerts various biological functions related to liver cancer progression, contributing to proliferation, invasion, and venous metastasis [227]. Evidence that HMGB1 (high-mobility group box 1) has enhanced expression in HBV-related liver cancer has been presented, accounting for the epithelial–mesenchymal transition (EMT) and angiogenesis in cancer. A study has proven that HBx-mediated HMGB1 expression is dependent on miR-34a reduction by IL-6/STAT3 [227]. Moreover, it was shown that inhibited miR-34a led to the down-regulation of NF-κB, promoting the expression of HMGB1 and potentially contributing to portal vein tumor thrombus and cancer metastasis [227]. During HBV infection, elevated TGF-β activity in the liver tissue has been observed, which suppresses the expression of miR-34a and leads to the enhanced production of chemokine CCL22 [228,230]. Meanwhile, miR-34a/CCL2

regulation contributes to recruiting regulatory T-cells to facilitate immune escape, favoring the colonization of disseminated HCC cells in the portal venous system [230]. Elevated TGF-β/Smad3 pathway activity and reduced expression of miR-34a also contribute to liver fibrosis in HBV infection, whereas the over-expression of miR-34a in human hepatic stellate cells significantly attenuated fibrosis and TGF-β1/Smad3 activation by targeting Smad4 [228].

Interestingly, during HCV infection, the level of miR-34a is up-regulated. Increased expression of miR-34a has been observed in Huh7.5 HCV-infected cells, as well as in sera from chronic HCV patients [231]. Additionally, miR-34a levels in sera were positively correlated with disease severity, as well as with liver enzyme levels, fibrosis stage, and inflammation activity. Therefore, researchers have postulated that miR-34a may represent a novel, non-invasive biomarker for diagnosis and histological disease-severity determination in patients with CHC [231].

#### 2.7.2. Hemorrhagic Viruses

Rossi et al. [232] have found hsa-miR-34a-5p to be down-regulated in response to DENV-2 infection. Interestingly, miR-34a displays anti-viral activity against flaviviruses, including DENV and WNV [233]. MiR-34a dampens Wnt signaling, allowing the TBK1-mediated phosphorylation of IRF3 in response to pathogen-associated molecular-pattern detection. Moreover, miR-34a may act as a potent activator of the type I IFN response due to the down-regulation of the Wnt/β-catenin signaling members Wnt2 and Wnt3, thereby promoting an anti-viral state [233].

#### 2.7.3. Respiratory Viruses

IAV is a cytolytic virus that induces apoptosis in numerous cell types, and miR-34a is involved in virus-induced apoptosis. A study showed that miR-34a was significantly down-regulated in the sera of infected patients, as well as in IAV-infected A549 cells. Furthermore, an in vitro study indicated that the over-expression of miR-34a could inhibit IV-induced apoptosis by targeting the pro-apoptotic gene Bax [234]. MiR-34 may play a role in IAV infection through regulation of the STAT pathway. Othumpangat et al. [235] demonstrated that, during H9N1 sub-type IAV infection, the level of miR-34 was significantly reduced. Moreover, it was shown that cells transfected with a mimic of miR-34 presented modulated phosphorylation and decreased expression of STAT3, which plays a critical role in anti-inflammatory function and inhibits NF-κB gene reporters. In addition, STAT3, by inhibiting NF-κB, may inhibit IAV replication [235].

MiR-34a has also been found to be down-regulated in the lung tissues of COVID-19 patients. In silico analysis indicated that miR-34a may have an effect on apoptosis through Bcl-2, BCL2 associated X (Bax), and Kruppel-Like Factor 4 (KLF4) genes, as well as on inflammation through the interleukin 6 receptor (IL-6R) [236]. However, further research showed that in lung tissues, only CASP-1 (caspase 1)—which is involved in the signaling pathways of apoptosis, necrosis, and inflammation—underwent increased expression, and was negatively correlated with miR-34a levels [236]. Additionally, a reduction in host miR-34a-3p in COVID-19 could increase X-box-binding protein 1 (XBP1s) expression by URP, increasing the ER folding capacity, inhibiting lung fibrosis, and protecting against over-activation of the immune system, thus promoting survival [237]. There have also been reports that miR-34a, along with other miRNAs, may target the membrane (M) protein gene of SARS-CoV-2, defining the shape of the viral envelope and the central organizer of CoV assembly [238].

#### 2.7.4. Human T-Lymphotropic Virus Type 1

Human T-lymphotropic virus type 1 (HTLV-1) is a member of the *Retroviridae* family, with an ssRNA genome [109]. The virus can cause a type of cancer called adult T-cell leukemia/lymphoma [239]. Sharma et al. [240] showed that infected cell lines expressed higher levels of miR-34a compared to normal PBMC or purified CD4+ T-cells. Further

analysis indicated that the primary miR-34a transcript contained binding motifs for NF-κB and p53. Moreover, the treatment of infected cell lines with the p53 activator resulted in a further increase in miR-34a levels [240]. This increase in miR-34a contributed to the down-regulation of the deacetylase SIRT1, as well as the pro-apoptotic factor Bax. These findings suggest a functional role for miR-34a in fine-tuning the expression of target genes which influence the turnover of HTLV-1-infected cells [240].

Our review indicates that the most important role of miR-34a in the considered viral infections is modulating the immune response and the course of apoptosis.

### *2.8. MicroRNA-21 (miR-21)*

MiR-21 plays a crucial role in many biological functions and diseases, including development, cancer, cardiovascular diseases, and inflammation [241]. The down-regulation of miR-21 increases the rate of cell death, most probably by targeting HIF-1α, phosphatase and tensin homolog (PTEN), and programmed cell death 4 (PDCD4). Meanwhile, the upregulation of miR-21 by cytokines during most viral infections indicates it role in inflammation, and may lead to host immune system dysfunction and viral replication [242]. MiR-21 has also been accepted as an activator of regeneration processes in tissue damage repair [243].

#### 2.8.1. Hepatitis Viruses

Liver fibrosis has been considered as a healing response to various chronic liver injuries, including viral hepatitis [244]. Chronic HBV infection is a major risk factor for HCC, which is one of the most common cancers worldwide [245]. HBx protein is known to be involved in the initiation and progression of HCC through the modulation of the host gene response [246,247]. Wu et al. [248] demonstrated that miR-21 was expressed in the sera of patients with CHB. In addition, the expression level of miR-21 was significantly correlated with the histological stage of liver fibrosis and cirrhosis [248,249]. Studies have shown that miR-21 activates hematopoietic stem cells (HSCs) in the liver through PTEN/Akt signaling, and may also promote α-SMA and collagen I expression in HSCs through the Smad 7 signaling pathway [250,251]. Another study has shown that miR-21 may play a role in liver fibrosis with HBV etiology, mediated via transforming growth factor beta 1 (TGF-β1) signaling [249]. HBV infection up-regulated TGF-β1/miR-21-5p mRNA expression in NTCP-Huh7.5.1 cells. Cells incubated with TGF-β1 presented significantly increased miR-21-5p levels, as well as the mRNA and protein expression of α-smooth muscle actin (α-SMA), collagen type 1 α1 (CoL1A1), and tissue inhibitor of metalloproteinase 1 (TIMP-1), along with reduced Smad7 expression in human hepatic stellate (LX2) cells. Interestingly, the over-expression of miR-21 in LX2 cells can cause a positive feedback loop and up-regulate TGF-β1 activation in cells, contributing to fibrosis in HBV infection [249]. Researchers have noted the role of miR-21 in carcinogenesis caused by HBV infection [252–257]. Qiu et al. [252] indicated that HBx down-regulated PDCD4 through the up-regulation of miR-21 expression. The over-expression of HBx in Huh.7 and HepG2 cells enhanced miR-21 expression. The up-regulation of miR-21 can increase proliferation and decrease target proapoptotic protein (PDCD4 and PTEN) expression, as well as activating Akt [253]. PDCD4 and PTEN have strong tumor-suppressive effects both in vitro and in vivo, and may induce cell apoptosis to suppress the development of HCC; however, their suppression by miR-21 may lead to the progression of HCC [252,253]. Another mechanism of miR-21 regulation by HBx and subsequent HCC development is mediated by the HBx-induced interleukin-6 pathway followed by activation of the STAT3 transcriptional factor. The induction of miR-21 by the IL-6/STAT3 pathway is essential for transforming non-tumor hepatocytes, implying a critical role in early HCC development during chronic HBV infection [254,255]. Yin et al. [257] have additionally shown that IL-12 is a direct target of miR-21 in HBV infection and HCC growth. IL-12 promotes the effective destruction of cancer cells by inducing the proliferation of NK and T-cells, and enhances the generation and activity of cytotoxic T-lymphocytes [258,259]. In an in vitro study, the

inhibition of miR-21 resulted in a significant increase in apoptosis and increased IL-12 expression. Therefore, the results suggest that HCC cell apoptosis was suppressed—at least partially—through HBx-induced miR-21 targeting IL-12 [257].

In hepatic tissues from HCV-infected patients, the expression of miR-21 has also been found to be increased [260–262]. Additionally, in Huh.7 cells, the transduction of the HCV-3a core up-regulated miR-21 expression and/or its activity. Enhanced miR-21-5p bioavailability and binding to specific targets, such as PTEN, and its down-regulation affect lipid accumulation in Huh-7 cells. Additionally, research showed that the genetic deletion of miR-21-5p in mice reduced HCV-3a-induced steatosis in vivo, suggesting that miR-21-5p activation/up-regulation may represent a key event in the pathogenesis of steatosis-associated oncogenesis [260]. Interestingly, miR-21-5p may also promote HCV replication and increased virion production [260,261]. The up-regulation of miR-21 by HCV suppresses HCV-triggered type I IFN production, thus promoting HCV replication [261]. Furthermore, Chen et al. [261] have identified a virus–host interaction pathway, in which HCV infection results in the stimulation of two signaling pathways: The NS5A/protein kinase C (PKC) ε/c-Jun N-terminal (JNK)/c-Jun pathway and the NS3/4A/PKCα/ERK/c-Fos pathway. After infection, c-Jun and c-Fos bind to the AP-1 binding sites in the miR-21 promoter and mediate the induction of miR-21. The induced miR-21 targets two important factors in the TLR signaling pathway—myeloid MyD88 and IRAK1—which are involved in HCV-induced type I IFN production. The silencing of MyD88 and IRAK1 by miR-21 and negative regulation of type I IFN may be potential therapeutic targets for anti-viral intervention [261]. Similarly to HBV, in HCV infection, miR-21 has been found to be correlated with fibrosis stage and, by targeting SMAD7, could increase TGF-β signaling, leading to increased fibrogenesis. Moreover, in HCV infection, miR-21 was also correlated with viral load and serum liver transaminase levels, making it a good candidate as a biomarker [262].

#### 2.8.2. Hemorrhagic Viruses

MiR-21 may also serve as a biomarker in DENV infection. Ouyang et al. [132] have indicated that miR-21 is up-regulated in the course of dengue, and could be used to distinguish dengue-infected patients with preferable sensitivity and specificity. In addition, they showed that miR-21 had a positive correlation with serum AST and ALT activities, and negative correlations with WBC, platelet (PLT), neutrophil, and lymphocyte numbers [132]. Other researchers have noted the role of miR-21 in dengue virus replication. The results showed a significant reduction in DENV 2 production in HepG2 cells after treatment with an anti-miR-21 (AMO-21), clearly suggesting that miR-21 plays a key role in DENV 2 replication [263]. Furthermore, in hemorrhagic fever caused by Ebola, upregulation of miR-21 was also observed [264].

#### 2.8.3. Respiratory Viruses

In response to influenza A virus (IAV) infection, a wide range of innate immune factors and miRNAs, such as miR-21, may be involved in controlling acute influenza. Down-regulation of miR-21 has been observed in the sera of H5N1-infected patients and in A549-infected cells [265,266]. In infected cells, the level of miR-21-3p was conspicuously down-regulated with prolonged infection. H5N1-infected A549 cells transfected with mimic-21-3p contributed to the augmentation of infectious progeny virions, whereas infectious virions were effectively decreased in the inhibitor-21-3p group. Additionally, researchers reported that mimic-21-3p dramatically down-regulated the levels of IFN-β and IFN-α in infected cells, while the silencing of miR-21-3p produced the opposite results [265]. Moreover, this miRNA has been shown to be involved in the NF-κB signaling pathway, and NF-κB affects the production of a type I IFN response [267]. Thus, miR-21 may affect IAV replication. Shi et al. [265] have demonstrated that miR-21, by targeting FGF2, can accelerate H5N1 replication in H5N1-infected A549 cells by inhibiting the type I IFN response [265]. Another study has shown that promoting IAV replication by miR-21 may take place also through

histone deacetylase-8 (HDAC8) [266]. Lam et al. [268] showed that influenza H5N1 and H1N1 infection down-regulated miR-21 expression. Interestingly, the researchers noted that, in H1N1 infection, miR-21 was increased 24 h after infection. Research using the TargetScan software also indicated that miR-21 may regulate CCL1 (small inducible cytokine A1 precursor), CCL17, CCL19, IL22, apoptosis-related protein 3 precursor (C2orf28), and tumor necrosis factor ligand superfamily member 12 (TNFSF13) in the context of influenza infection [268]. Meanwhile, the elevated miR-21 level on day 15 of IAV infection was identified to be important in the late repair phase through the targeting of proliferation-suppressing factors [269,270]. In addition, its elevated expression during repair coincides with increased proliferation in repairing lungs. Notably, increased miR-21 levels may lead to detrimental effects, such as fibrosis, through targets in the TGF-β pathway [271].

MiR-21 has also been shown to be down-regulated in adenovirus type 2 infection of human lung fibroblasts. The researchers showed that its expression was also the most strongly down-regulated (more than 9-fold) 12 h after infection, and that it may target an important cellular network of tumor-suppressor genes, such as p53, TGF-β, and mitochondrial apoptosis genes [80].

In contrast to the main trend of decreased miR-21 expression in IAV infection, in SARS-CoV-2-infected patients, significantly up-regulated miR-21 levels have been observed, which may be fibrosis-associated [76,272]. The up-regulation of miR-21 may also serve as a predictor of chronic myocardial damage and inflammation. Interestingly, the level of miR-21 was decreased in patients who died due to COVID-19, and was also correlated with a higher rate of extracorporeal membrane oxygenation (ECMO) and renal replacement therapy [76]. Researchers have shown that miR-21-3p can bind sites on the open reading frame (ORF) 1ab, ORF3a, and spike of SARS-CoV-2, which encodes the spike protein necessary for viral entry and is a promising target for anti-viral therapy. In connection with the impact of miR-21-3p, host and virus genome experimental validation of miR-21's involvement in both binding to the SARS-CoV-2 genome and modulating the host transcriptome have been suggested [273]. During COVID-19, miR-21-5p may directly target CCL20, which is up-regulated in the inflamed airway epithelium, as well as MYC, the over-expression of which fosters the inflammatory response and T-cell metabolic reprogramming, respectively [139]. Other targets for miR-21 are IRAK1, which participates in the NF-κB pro-inflammatory signaling pathway, as well as CXCL-10 [139,273], a biomarker for viral infection. Increased levels of this chemokine have been observed in the lungs of COVID-19-infected patients compared to healthy ones [273].

In RSV-infected cells, significant up-regulation in the composition of exosome miR-21 has been observed [274,275]. Exosomes released from virus-infected A549 cells can alter innate immune responses through the induction of pro-inflammatory mediators. Antagonistic miR-21 treatment inhibited eosinophil inflammation and AHR in an RSV-induced steroid-insensitive mouse airway allergic-disease model [276]. Therefore, miR-21 may be a key signal regulating the balance and transition between pro-inflammatory and immune activation [277].

#### 2.8.4. Human Immunodeficiency Viruses

In HIV patients, a low level of exosomal miR-21 in the plasma, along with a decrease in CD4+ T-cells, was observed [278]. In Jurkat cells, the stable intracellular expression of Tat (HIV gene) induced an increase in miR-21 expression, which led to apoptosis- and cell cycle-related proteins being down-regulated, including PTEN, PDCD4, and cyclin-dependent kinase inhibitor 1B (CDKN1B) [279]. It has been further demonstrated that the over-expression of miR-21 significantly inhibits IP-10, a key inflammatory cytokine that causes immune dysfunction and facilitates HIV infection [280].

#### 2.8.5. Cardiotropic Viruses

Coxsackievirus B3 (CVB3) belongs to the *Picornaviridae* family, with an RNA genome [83]. CVB3 is a common and important pathogen of viral myocarditis, pancreatitis, and aseptic

meningitis in young children and infants. CVB3 infection may lead to acute heart failure and sudden death due to direct cytopathic effects induced by viral replication in the early phase of infection [281,282]. Similar to DENV, miR-21 plays an important role in viral replication during CVB3 infection. In CVB3-infected HeLa cells, significant up-regulation of miR-21 expression, followed by the suppression of mitogen-activated protein kinase kinase 3 (MAP2K3)/P38 MAPK signaling, has been observed. Furthermore, miR-21 over-expression significantly inhibited the release of virions from CVB3-infected cells. This decreased viral release was accompanied by a significantly alleviated myocyte cell apoptosis rate, reduced viral titers, lower necrosis in the heart, and remarkably prolonged survival time in an in vivo study [283]. Ye et al. [284] have indicated that over-expressed miR-21 during CVB3 infection may target deubiquitinating enzyme (YOD1) to enhance the lysine 48-linked ubiquitination and degradation of desmin; this results in the disruption of desmosomes, which are substantial connections maintaining cardiac structures and mediating signal communications among cardiomyocytes. In addition, miR-21 directly targets vinculin, leading to disturbed fascia adherens, as evidenced by the suppression and disorientation of pan-cadherin and α-E-catenin proteins, two fascia adherens components. Thus, miR-21 may contribute to the pathogenesis of viral myocarditis [284].

#### 2.8.6. Vector-Borne Viruses

Chandipura virus (CHPV) belongs to the *Rhabdoviridae* family, with an RNA genome [285]. It causes acute encephalitis with a case fatality rate of 70%. An in vitro study has indicated over-expression of miR-21 in human microglial cells infected with CHPV. The higher level of miR-21 leads to the down-regulation of PTEN, which promotes the phosphorylation of AKT and NF-kB (p65). Moreover, the activation of NF-kB increases the transcription of pro-inflammatory cytokines (e.g., IL-6 and TNF-α) [286].

In conclusion, invalid miR-21 expression is closely associated with viral infections, where increased levels of miR-21 may enhance viral replication. In addition, research has shown that miR-21 may be involved in organ damage associated with active viral infection. As such, miR-21 has increasingly been described as a potential biomarker of infection.

### *2.9. MicroRNA-16 (miR-16)*

MiR-16 can modulate the cell cycle, inhibit cell proliferation, and promote cell apoptosis [287]. These effects can be explained with respect to the targeting of miR-16 in the anti-apoptotic gene Bcl-2 (B-cell lymphoma 2); numerous genes involved in the G1-S transition, such as cyclin D1, cyclin D3, cyclin E1, and CDK6 (cyclin-dependent kinase 6); and genes involved in the Wnt signaling pathway, such as WNT3A (wingless-type MMTV integration site family member 3A) [288]. Additionally, miR-16 directly targets PDCD4 to suppress the activation of inflammatory macrophages through the mitogen-activated protein kinase (MAPK) and NF-κB pathways. In these ways, miR-16 contributes to decreasing the levels of inflammatory cytokines (e.g., IL-6, TNF-α, and IFN-β), while simultaneously enhancing the secretion and mRNA expression of the anti-inflammatory factor IL-10 [289].

#### 2.9.1. Hepatitis Viruses

MiR-16 may a play a significant role in infection with hepatotropic viruses and fibrosis. Wu et al. [290] indicated that the HBx protein decreased the expression of miR-16 in host malignant hepatocytes (i.e., human HepG2, SK-HEP-1, and Huh7 HCC cell lines) in vitro. They also examined the expression of target genes for miR-16, indicating that cyclin D1 (CCND1)—which functions in the G1/S transition of the cell cycle—was significantly up-regulated [290]. Furthermore, the HBx-induced down-regulation of HepG2 cells was c-Myc mediated. Meanwhile, ectopically expressed miR-16 repressed the proliferation, clonogenicity, and growth of HepG2-hbx cells by inducing cell cycle arrest (CCND1), and apoptosis by targeting Bcl-2 [290]. However, further studies are needed in order to clarify the roles played by miR-16 in HBx-induced hepatocyte apoptosis (or the inhibition of apoptosis) and acute/chronic HBV infection.

HCV is involved in the initiation and progression of liver fibrosis by miRNA and regulating genes encoding host proteins. It was observed that miR-16 levels were increased in the PBMCs and sera of patients with chronic HCV and in liver cell lines infected with HCV. Additionally, the miR-16 level was negatively correlated with HGF and Smad7 expression [291]. This suggests that miR-16 may contribute to the development of liver fibrosis. Interestingly, a study showed that the treatment of HCV- cells infected with IFN-α led to the down-regulation of miR-16 and up-regulation of hepatocyte growth factor (HGF) and Smad7 [291]. MiR-16 has also been shown to be correlated with ALT and AST levels, but not with the stage of fibrosis [231,291].

#### 2.9.2. Respiratory Viruses

The cellular response to viral infection is initiated by recognition of the invading pathogen and subsequent changes in gene expression mediated by both transcriptional and translational mechanisms. Buggele et al. [134] showed that the expression of miR-16 during IAV infection in primary airway epithelial cells increased 8 h after infection, while it was significantly decreased at 24 h [134]. In addition, the miR-16 level was negatively correlated with that of IFN-β. Interestingly, in A549 and BEAS-2B cells infected with A/Udorn/72 and A/WSN/33 strains of influenza virus, miR-16 presented constant expression levels throughout the infection [134]. Research has also demonstrated that NS1 in a specific strain of IAV may contribute to the translocation of miR-16 and AGO2 from the cytoplasm to the nucleus, thus enhancing the in vivo virulence of IAV [292].

In silico analysis has indicated that miR-16 may bind, with high probability, to the RNAs of the human coronaviruses MERS-CoV, SARS-CoV, and SARS-CoV-2. Interestingly, most binding sites were found within NS proteins located in the polyprotein 1ab coding region [272]. During SARS-CoV-2 infection, miR-16 was predicted to target the largest number of differentially expressed host genes. Given that the majority of miR-16 targets, including mitogen-activated protein kinase-activated protein kinase 2 (MK2) and CCND1, were down-regulated in response to SARS-CoV-2, the expression of miR-16 may be increased in the lungs of COVID-19 patients [273].

In children with RSV infection, miR-16 has been shown to be significantly up-regulated [58]. It may have an effect on the NF-κB pathway, which is activated following RSV-antigen binding to the pathogen recognition receptors TLR 4 or RIG-1, as a primary stage in the immunological response to RSV. However, excessive NF-κB activation may also have deleterious effects and, so, negative regulation is also important. Therefore, miR-16 may have a significant role related to the consistent fine-tuning of the immune response to RSV [58].

Our review demonstrates that the most important function of miR-16 is the regulation of hepatocyte apoptosis in infection with hepatotropic viruses, as well as the fine-tuning of the immune response in the course of infection with respiratory viruses.

### *2.10. MicroRNA-181 Family (miR-181)*

The miRNA family miR-181 has diverse roles, in terms of regulating key aspects of cellular growth, development, and activation. By regulating critical signaling pathways, such as NF-κB, the miR-181 family plays a key role in inflammation [293]. Moreover, it targets importin-α3, a protein critical for NF-κB nuclear translocation, and negatively regulates pro-inflammatory cytokines [294]. The miR-181 family also regulates apoptosis by directly targeting relevant genes [295,296]. Therefore, increasing attention has been paid to the implications of the miR-181 family in the field of immunology and viral disease.

#### 2.10.1. Hepatitis Viruses

The miR-181 family plays a significant role in hepatitis virus, and may also be a potential marker in hepatitis diagnostic and liver failure. Yu et al. [297] demonstrated that miR-181 levels were up-regulated and correlated with liver and serum HBV DNA levels and disease progression. Additionally, the serum miR-181b level was associated with the fibrosis score, suggesting that miR181b acts as a pro-fibrosis miRNA in the liver and, so, has

potential as a marker for disease progression in CHB patients [297]. Studies have shown that miR-181b promotes hepatic stellate cell proliferation by targeting the p27 and PTEN/Akt pathways, and is elevated in the sera of cirrhosis patients, further indicating its role as a pro-fibrotic factor [298,299]. Another miR from the miR-181 family which targets PTEN is miR-181a. In vitro research indicated that HBV (HBx protein) increased the expression of miR-181a [300–302], which then reduced PTEN protein expression [300]. The miR-181a/PTEN pathway during HBV infection contributes to cell proliferation and suppresses apoptosis, leading to the development of hepatocellular carcinoma [300]. Another mechanism of miR-181a affecting HCC formation is that the inhibition of the expression of transcription promotes tumor cell growth in vivo through the suppression of TNF receptor superfamily member 6 (Fas) expression in hepatoma cells [302]. These data indicate that miR-181a plays an essential role in the regulation of HCC cell proliferation, and may function as an onco-miRNA in HBV-related HCC [300–302].

In HCV infection, researchers have noted the up-regulation of miR-181a in infected patients [303], as well as its down-regulation in liver tissues [304]. Additionally, serum miR-181a expression in HCV patients is inversely correlated with the level of viremia, as well as liver enzymes (ALT and AST). They also observed significant up-regulation of miR-181a serum levels in interferon-treated patients who had developed a sustained virological response. However, Elhelw et al. [303] did not observe a difference in miR-181a expression in the liver tissues of patients, compared to controls [303]. MiR-181a was also down-regulated in liver fibrosis stages 2 and 3 caused by HCV infection. In addition, miR-181a was negatively correlated with the grade of necroinflammatory activity during HCV genotype 2 infection. An analysis of different types of necroinflammatory activity demonstrated the expression of miR-181a to be involved in periportal/periseptal inflammation [304]. HCV infection also decreased the expression of miR-181a in CD4+ T-cells, where the decline in miR-181a expression impaired CD4+ T-cell responses via the over-expression of dual specific phosphatase 6 (DUSP6) [305]. In particular, a significant decline in miR-181a expression, along with the over-expression of DUSP6, was observed in CD4+ T-cells from chronically HCV-infected individuals. However, treatment with miR-181a precursors in CD4+ T-cells led to improved T-cell responses and increased expression of IL-2 [305] and factor E2F5, which is a key regulator of cell growth [301]. During HBV infection, miR-181a inhibits apoptosis in vitro.

These data suggest that miR-181a may be considered to be a possible prognostic marker in HCV infection [303–305]. Another member of the miR-181 family that is decreased in HCV is miR-181c [305,306]. Mukherjee et al. [306] observed that HCV infection of hepatocytes transcriptionally down-regulated miR-181c expression by modulating C/EBP-β. The reduction in miR-181c levels led to enhanced expression of HOXA1 (homeobox A1), a cell growth regulator that can enhance oncogenic transformation through STAT3 and STAT5 [306]. Cell transfection with an miR-181c mimic contributed to the downregulation of HOXA1, potentially reducing the risk of developing HCC. Interestingly, the over-expression of miR-181c also inhibited HCV replication through direct binding with the E1 and NS5A sequences [306]. Another direct target of miR-181c is ATM (ataxia-telangiectasia mutated). A study demonstrated that ATM expression was higher in HCV-infected hepatocytes and chronic HCV-infected liver biopsy specimens [307]. The exogenous expression of miR-181c inhibited ATM expression and the activation of its downstream molecules, Chk2 (checkpoint kinase 2) and Akt. Furthermore, the over-expression of miR-181c significantly inhibited phospho-CDK2 and Cyclin-A expression, arresting cell cycle progression while simultaneously promoting the apoptosis of HCV-infected hepatocytes [307]. Considering the presented data, miR-181c seems to be an important factor in HCV–hepatocyte interactions, and may serve as a target for therapeutic intervention [306,307].

#### 2.10.2. Respiratory Viruses

Researchers reported that a member of the miR-181 family, miR-181c, was up-regulated in A549 cells infected with H5N1, H3N2, and H1N1 influenza A viruses [206]. In addition,

the increase in the level of this miRNA leads to the targeting of many genes associated with cellular immune defense, such as BCL2, IL-2, and TNF-α [206]. Researchers are increasingly focusing on miRNAs as potential biomarkers of infectious diseases. Lim et al. [308] have identified miR-181c-5p as a potential biomarker for the detection of pandemic influenza A H1N1 virus infection [308]. Moreover, miR-181a-5p has been selected as a biomarker to differentiate IAV or influenza B virus (IBV) patients from healthy controls. Furthermore, it is useful for the diagnosis of H1N1 and H3N2 infection. However, in contrast to miR-181c, influenza A virus down-regulates the level of miR-181a in infected patients [309].

Interestingly, during RSV1 and RSV3 infection, the expression of miR-181a is up-regulated [178]. Furthermore, the vaccination of mice with a live attenuated candidate for RSV resulted in increased miR-181a levels [310]. Therefore, these results suggest that miR-181a may have a significant role in the response to RSV infection [178,310].

In viral infections, members of the miR-181 family regulate many genes related to the cellular immune response. The most important aspect of this family is the use of these molecules as potential biomarkers for the diagnosis of viral infections due to their good correlations with viral load levels and various markers (e.g., liver enzymes).

### *2.11. Let-7 Family (Let-7)*

Let-7 miRNA was first discovered in *Caenorhabditis elegans*, and is highly conserved in human tissues. The human let-7 family of miRNA contains 12 members [311]. The let-7 family is involved in many biological processes. Studies have revealed the role of let-7 members in the regulation of the cell cycle, proliferation, and apoptosis and, above all, in the processes of oncogenesis [311,312]. The let-7 family has also been implicated in the post-transcriptional control of innate immune responses to various pathogenic agents, including viruses [313,314].

#### 2.11.1. Hepatitis Viruses

The Let-7 family is frequently down-regulated in multiple human tumors, including hepatocellular carcinoma (HCC), caused (inter alia) by chronic infection with HBV and HCV. Studies have shown that the HBx protein down-regulates the entire let-7 family in HepG2 line cells [315,316]. In addition, it has been indicated that let-7a negatively regulates cellular proliferation, partly through the targeting of STAT3, which is involved in many cellular processes (including cell growth, survival, metastasis, angiogenesis, and immune suppression), all of which favor tumor formation and progression [315,317]. Therefore, the downregulation of let-7 by HBx supports cell proliferation and may contribute to HCC [315]. The lowered let-7 levels in HBV infection may be caused by the over-expression of Lin-28 Homolog B (Lin28B). Lin28B is over-expressed in HBx-transfected cells and HBV-infected liver tissues, and the HBx–c-Myc–Lin-28 homolog B (Lin28B) axis was found to mediate the repression of let-7 in HepG2 cells [316]. Interestingly, Deng et al. [194] have discussed the implications of Lin28B/let-7 and miR-125b in HBV infection. They demonstrated that miR-125b-5p targets the Lin28B/let-7 axis and contributes to the down-regulation of lin28B and up-regulation of let-7 in order to stimulate HBV replication in a post-transcriptional step [194]. Qiu et al. [318] have shown that the level of let-7a was lower in malignant tissues than in adjacent normal tissues; however, patients with highly active HBV replication demonstrated a significantly higher level of let-7a in hepatocarcinoma tissue than patients with less-active HBV replication [318]. In addition, an in vitro study demonstrated that the down-regulation of let-7a by anti-sense oligonucleotides led to a reduction in HBV DNA copy numbers. This indicated a correlation between the let-7a level and HBV replication, suggesting that the down-regulation of let-7a reduces HBV replication and could prevent the development of HCC [318]. Furthermore, let-7g may affect HBV replication. In an in vitro study, Takata et al. [319] showed that cells over-expressing let-7g presented suppressive effects on replication and HBV protein levels. However, HBV mRNA in the surface protein preS2 region can sequester let-7g which, in turn, impairs the intrinsic let-7g function and is crucial in the pathogenesis of chronic viral infection [319].

Similarly as in HBV infection, in HCV, the down-regulation of let-7a and let-7b was also observed in tissue, particularly, during HCV infection and in HCV-infected cell culturing. However, an in vitro study has shown the function of let-7 in terms of restricting multiple steps of the HCV life cycle; namely, entry, translation, and RNA replication. The over-expression of let-7a in Huh.7.5.1 cells significantly reduced HCV core production expression, HCV RNA production, and viral infectivity, confirming an anti-viral role of let-7a in hepatocytes. Let-7a targets component of inhibitor of nuclear factor kappa B kinase complex (CHUK), inhibitor of nuclear factor kappa b kinase subunit epsilon (IKBKE), and X-prolyl aminopeptidase 1 (XPNPEP1), preferentially acting on HCV assembly or secretion; this implies that let-7a also acts in the late stage of the HCV life-cycle. In addition, let-7a also targets CLDN1 and represses its translation to block HCV entry [320]. Another observation, made by Cheng et al. [321], indicated that let-7b was induced during the early stage of HCV infection and, similarly to let-7a, suppressed HCV replication. Their data suggest that let-7b suppressed HCV replicon activity and down-regulated HCV accumulation, leading to reduced infectivity by binding to the coding sequences of NS5B and the 5′-UTR of the HCV genome. However, the mechanism for the let-7b-mediated suppression of HCV RNA accumulation was not dependent on the inhibition of HCV translation [321]. Another study has shown that let-7b directly targets negative regulators of type I IFN signaling, thereby limiting HCV replication in the early stage of HCV infection [322]. Additionally, let-7b targets SOCS1, leading to the increased expression of downstream ISGs. On the other hand, let-7b directly targets the ATG12 and IκB kinase alpha (IKKα) transcripts and reduces the interaction of the ATG5–ATG12 conjugate with RIG-I, leading to the increased expression of IFN, which may then stimulate JAK/STAT signaling [322]. Studies have also shown that let-7b can affect HCV replication by binding and, thereby, reducing the expression of the factor insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), which is required for HCV replication [323]. In addition to changes in the expression of miRNA in tissue samples or in cell cultures, miRNAs in the let-7 family also present changes in sera. Studies have shown that, along with the severity of the disease, decreased levels of let-7a/7c/7d-5p have been observed in the sera of HCV patients. In addition, levels of let-7 family members have been correlated with the advanced histological hepatic fibrosis stage and other fibrotic markers, including Mac-2-binding protein glycan isomer (M2BPGi), fibrosis-4 (FIB-4) index, and the aspartate aminotransferase-to-platelet ratio index (APRI) [324,325]. Furthermore, pathway analysis has suggested that low levels of let-7 may influence hepatic fibrogenesis through the activation of TGF-β signaling in hepatic stellate cells [325].

#### 2.11.2. Hemorrhagic Viruses

In an in vitro study, a role of let-7c in DENV infection has been also demonstrated. During dengue infection (DENV2 and DENV4) of the liver culture cell Huh.7, as well as in the macrophage–monocytic cell line U937-DC-SIGN, the over-expression of let-7c was observed [326]. As with other infections, let-7 family miRNA—more specifically, let-7c—inhibited DENV infection. Escalera-Cueto et al. [326] have demonstrated that let-7c inhibits infection with DENV2 and DENV4 through the down-regulation of Bach1 and over-expression of HO-1, which is caused by Bach1 depression. The indirect up-regulation of HO-1 by let-7c induces anti-oxidative and anti-inflammatory responses, which may be necessary to counteract harmful effects in infected tissues [326].

#### 2.11.3. Respiratory Viruses

The up-regulation of pro-inflammatory factors usually activates the expression of anti-inflammatory factors and causes homeostasis responses to inflammatory stress during viral infection [327]. The homeostasis response may be mediated by both mRNA and miRNA. Let-7 is a type of miRNA that binds to the 3′-untranslated region of IL-6 mRNA and inhibits pro-inflammatory IL-6 mRNA and protein expression. Zhang et al. [327] demonstrated that let-7e was significantly increased in THP-1 cells treated with hemagglutinin (HA) protein of the avian influenza A (H7N9) virus. However, HA may inhibit the secretion of

let-7e from THP-1 cells by activating the TLR4 pathway, and can enable the maintenance of high intracellular let-7e levels [327]. Interestingly, Zhu et al. [328] observed that patients infected with H7N9 showed decreased let-7e levels in sera, indicating that the cells of these patients attempted to trigger a protective response to avoid severe inflammatory damage in specific organs or tissues [328]. Another miR from the let-7 family also plays a role in protecting host cells from the virus: it was observed that let-7c was highly up-regulated in influenza virus-infected human lung epithelial cells (A549). This study also showed that let-7c directly targeted the 3′-UTR of the IAV matrix protein (M1) and affected the reduction of M1 levels in A549 cells at the cRNA and protein levels, where the decrease in M1 protein caused a reduction in IAV replication [329]. Interestingly, let-7c was also up-regulated within 15 days of infection with IAV (H1N1) in murine lung tissue. TargetScan analyses also revealed that let-7c is involved in targeting relevant gene functions in repair, thus limiting damage and accelerating repair after infection [269].

In RSV-infected A549 cells, let-7f is up-regulated by the RSV G protein. The results of this study also showed that let-7f regulates CCL7/MCP-3 and SOCS3, which are involved in the anti-viral cytokine response (e.g., the type I IFN response) and regulate ELF4—a known inducer of IL-8 in RSV infection [330]. Furthermore, up-regulation of let-7d has been detected in epithelial cells of the nasal mucosa in children with RSV infection [58]. Thornburg et al. [331] have demonstrated that let-7b is up-regulated in DCs, while let-7i is up-regulated in epithelial cells in a process that requires viral replication. Interestingly, researchers found that the RSV non-structural genes NS1 and NS2 antagonized the up-regulation of let-7i. In addition, they indicated that the induction of let-7b and let-7i was also enhanced by IFN-β [331]. In contrast to the above-mentioned studies, in Calu3 respiratory cells infected with RSV, let-7f was down-regulated, accompanied by the up-regulation of IFN-λ, which could induce innate anti-viral responses in epithelial cells [332].

As in the previously discussed respiratory viruses, in the course of COVID-19, the up-regulation of let-7b was obviously observed in PBMCs. In patients with post-acute COVID-19, a higher level of let-7b expression occurs compared to acute infection; this potentially suggests a role of let-7b in the immune response and repair function through the targeting of genes such as V-Rel avian reticuloendotheliosis viral oncogene homolog B (RELB), IL-6, KH-type splicing regulatory protein (KHSRP), euchromatic histone lysine methyltransferase 2 (EHMT2), lysine demethylase 2B (KDM2B), C-MYC, and LIN28B [71]. The over-expression of let-7b has also been observed in neutrophils during SARS-CoV-2 infection, which could alter their function by suppressing the TLR4/NF-κB signaling pathway [333]. Moreover, Chen et al. [333] have demonstrated, through an in vitro study, that the up-regulation of let-7b may decrease the levels of various pro-inflammatory factors, including IL-6, IL-8, and TNF-α, and up-regulate the anti-inflammatory factor IL-10. This study suggested that let-7b could reduce complications associated with COVID-19, such as viral sepsis [333]. Additionally, as in IAV infection, another miRNA from let-7 family—let-7c—may have an effect on the replication of SARS-CoV-2 by targeting ORF1ab, encoding the 5′-viral replicase [238]. An experimental study also showed that let-7d, 7e, 7f, 7g, and 7i were able to significantly suppress the expression of the S protein, while let-7b, 7c, 7g, and 7i inhibited M protein expression, thereby blocking SARS-CoV-2 replication. The researchers also showed that let-7 suppresses the expression of multiple inflammatory factors, including IL-1β, IL-6, IL-8, CCL2, GM-CSF, TNF-α, and VEGFα, by targeting the IL-6/STAT3 pathway, leading to a reduction in inflammation [334]. Interesting observations have been made by Chow et al. [74], who reported that differential expression analysis of Calu3 cells infected with SARS-CoV-2 revealed that hsa-let-7a-3p was down-regulated, in contrast to other considered miRs from the let-7 family [74].

#### 2.11.4. Human Immunodeficiency Viruses

Swaminathan et al. [335] have shown that, in infected HIV-1 cells, let-7 can repress IL-10 expression at the post-transcriptional level. They found that IL-10 was highly upregulated in HUT78 T-cells, and proposed that let-7 over-expression decreased IL-10, as the

silencing of let-7 miRNA led to a significant increase in IL-10 levels. HIV-1-infected HUT78 cells showed lower let-7 levels, accompanied by increased IL-10 levels, suggesting that the decreased let-7 level may be involved in the increased IL-10 expression seen in HIV-1 infection. They also observed reduced let-7 levels in primary $CD4^+$ T-cells retrieved from the blood samples of subjects with HIV-1 infection compared with non-infected controls, suggesting that the altered miRNA levels could be linked to increased IL-10 expression in HIV patients. They proposed that dysregulation of the let-7/IL-10 axis could result in the abnormal CTL function seen in HIV-1-infected individuals [335]. Zhang et al. [336] reported that let-7i induced gene expression in Th-cells by binding to the TATA-box of the IL-2 promoter, thus promoting the assembly of pre-initiation complexes, which are required for mRNA transcription. They observed that HIV-1 infection resulted in lower levels of mature let-7i, as well as its precursor and primary forms. Additionally, studies have shown that the function of the let-7i promoter is reduced in Th-cells following HIV-1 infection. As a result, they suggest that viral infection results in the suppression of the let-7i/IL-2 axis, contributing to Th-cell death. This is a newly described mechanism for HIV-1-induced Th-cell death, where IL-2 cytokine can enhance the survival of activated T-cells [336].

#### 2.11.5. Neurotropic Viruses

Let-7a/b may play roles in the pathogenesis of the disease caused by JEV. The studies carried out have suggested that, in microglial cells, let-7a and let-7b can activate Notch in an NF-κB-dependent manner and through the TLR7-mediated signaling pathway, inducing the production of inflammatory cytokines such as TNFα. On the other hand, the extracellular release of let-7a/b via exosomes can transfer to neurons, promoting a neurotoxic effect and neuronal damage through the activation of cPARP and caspases-3/7/9 [337].

In summary, it is possible to state that the most important role of the let-7 family involves regulation of the immune response. It has been shown that the let-7 family can reduce the expression of pro-inflammatory cytokines and promote increased expression of the anti-inflammatory cytokine (IL-10) in most of the infections discussed in our review, thus preventing an excessive inflammatory response during viral infections. Researchers have also stressed that members of the let-7 family may affect viral replication.

### *2.12. MicroRNA-10a (miR-10a)*

MiR-10a may be related to changes in immune homeostasis, and is down-regulated by many factors, such as TNF-α, IL-1β, and IL-6, as well as through promoting the production of the transcription factor YY1 [294,338]. The down-regulation of miR-10a accelerates inhibitor κB (IκB) degradation and NF-κB activation [294]. Furthermore, the reduced expression of miR-10a-5p leads to an increase in membrane-bound IL-6R [338]. Additionally, miR-10a inhibits DC expression of IL-12/IL-23p40 and NOD2, as well as inhibiting Th1 and Th17 cell functions [294].

#### 2.12.1. Hepatitis Viruses

Tan et al. [339] have observed elevated levels of miR-10a in the sera of patients with CHB. They reported that miR-10a may provide high diagnostic accuracy for CHB patients presenting persistently normal ALT with significant histological features and with no significant histological features. Additionally, miR-10a may be used to differentiate between the two units [339].

Furthermore, during HCV infection, the level of miR-10a is markedly up-regulated. Horii et al. [340] have observed that miR-10a regulates various liver metabolism genes and down-regulates the expression of the circadian rhythm gene brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1 (Bmal1) by directly suppressing the expression of RAR receptor-related orphan receptor alpha (RORA) [340]. The over-expression of miR-10a in hepatocytes inhibited the expression of lipid synthesis gene sterol-regulatory element-binding protein (SREBP1), fatty acid synthase (FANS), SREBP2, gluconeogenesis-peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α), protein

synthesis mTOR and ribosomal protein S6 kinase (S6K), and bile acid synthesis liver receptor homolog 1 (LRH1). In addition, the down-regulation of Bmal1 by miR-10a was significantly correlated with the expression of mitochondrial biogenesis-related genes, increased serum ALT, and the progression of liver fibrosis in CHC. Therefore, miR-10a is a possibly useful biomarker for estimating prognoses in liver cirrhosis. Additionally, miR-10a is significantly associated with hepatitis C-related hepatocellular carcinoma recurrence. It has been suggested that miR-10a, through Bmal1, disturbs metabolic adaptations and leads to liver damage [340].

#### 2.12.2. Respiratory Viruses

Increased levels of miR-10a expression have also been observed during RSV infection. Zhang et al. [205] showed that hsa-miR-10a-3p was elevated in children with RSV-associated pneumonia. In addition, it was reported that, in children with severe RSV-associated pneumonia, the level of miR-10 was higher than that in children with mild RSV-associated pneumonia. These results indicate that hsa-miR-10a-3p may reflect severe RSV-associated pneumonia, and may serve as a potential candidate biomarker for severe RSV-associated pneumonia [205]. GO analysis indicated that most target genes for miRNAs, including miR-10a-3p, were involved in the NF-κB and MAPK signaling pathways, crucial components of many immune response processes in humans [205,341,342]. Furthermore, NF-κB is an important anti-apoptotic transcription factor for immune cells such as neutrophils, and plays an important role in damage repair during infection and inflammation [343]. Thus, the over-activation of NF-κB signaling may result in severe complications during severe RSV infection; as such, factors regulating its activity, such as miR-10a, are important.

#### 2.12.3. Cardiotropic Viruses

Researchers have indicated that the miR-10a duplex is detectable in the cardiac tissues of suckling Balb/c mice, and can significantly up-regulate the biosynthesis of Coxsackievirus B3 (CVB3). Further research showed that the miR-10a target was located in the nt6818–nt6941 sequence of the viral 3D coding region. Taken together, these data suggest that miR-10a* can positively modulate host gene expression, and may play a role in the pathogenesis of CVB3 infection [344].

The presented studies indicate that the most important activity of miR-10a is related to regulation of the immune response. However, it is worth noting the role of miR-10a as a potential biomarker reflecting the severity of diseases of viral etiology.

Table 1 lists all of the miRNAs important in human viral infections, along with their target genes, biological functions, and modulated pathways.

**Table 1.** MicroRNAs important in human viral infections.

| MicroRNA | Virus/Disease | Target Genes | Biological Function/Modified Pathways | Reference |
|---|---|---|---|---|
| | HBV/HB | SOCS1 | Enhances the phosphorylation of STAT1 and STAT3; enhances JAK/STAT signaling pathway; suppresses HBV infection in hepatocytes; regulates IFN-γ production; inhibits Akt/mTOR pathway | [23,26,29] |
| | | C/EBP-β | Modulates HBV replication | [24] |
| | | BCL-6, SHIP-1, SOCS-1 | Induces inflammatory cytokine production | [28] |
| | HCV/HC | TNF-α | Pro-inflammatory response | [33] |
| | | Tim3/T-bet | Increases IFN-γ production; regulates Tim-3/T-bet/STAT-5 signaling and cytokine expression in NK cells; immune injury during chronic viral disease | [36] |
| | | ISG15, TLR3 | Innate and adaptive immune response | [34] |
| | | APC | Wnt pathway; apoptosis; cell proliferation | [39] |

**Table 1.** *Cont.*

| MicroRNA | Virus/Disease | Target Genes | Biological Function/Modified Pathways | Reference |
|---|---|---|---|---|
| miR-155 | DENV/dengue | Bach1 | Replication of DEVN; enhances anti-viral IFN responses | [43] |
| | | SOCS1 | Regulates cytokine signal transduction | [41] |
| | WNV/ West Nile fever | IL-13, BDNF, CCR9 | Cell survival | [45] |
| | | IL-1β, IL-12, IL-6, IL-15, GM-CSF | Anti-viral response | [47] |
| | IAV/influenza | S1PR1 | Inflammatory response; activates S1PR1/NF-κB/pro-inflammatory cytokine pathway | [51] |
| | | IFN type I | Anti-viral response | [53] |
| | RSV/upper respiratory tract infections | IFN type I | Anti-viral response | [53] |
| | | TNF-α, IL-1β, IL-6, IL-8 | Pro-inflammatory response | [59] |
| | | SHIP1, Kif1 | Antigen presentation | [61,62] |
| | | SOCS1 | Enhances activation of STAT1 and up-regulation of ISGs gene | [63] |
| | SARS-CoV-2/ COVID-19 | STAT1, STAT3, TGFB1, SMAD3, IRF1, AKT1, MYB, BCL6, TP6, HIF1A, FOXP3, JUNB, NFKB1 | Immune response; apoptosis | [71] |
| | | SOCS1, IL-6, IL-1β, CSF1R CD274, TLF6, TNF | Regulates the host immune response; miR-155-5p–IL-6/TNF/IL-1β axis | [75] |
| | | IL-1α, G-CSF, IL-9, MIP-2, IL-12-P70 VEGF, IP-10, IFN-γ, MCP-1, MIG, MIP-1α, M−CSF, TNF-α, MIP-1β | Alleviates inflammation and lung cytokine storm | [73] |
| | HAdV/respiratory infections (colds) | IFN-β | Anti-viral response | [80,81] |
| | | SOCS1 | Enhances type I IFN Anti-viral response | [82] |
| | | SHIP1 | Enhances IFN type I signaling | [20] |
| | HRV/COPD | HRV1B | Inhibits viral replication | [84] |
| miR-223 | HCV/CHC | NF-κB | Chronic liver inflammation | [94] |
| | DENV/dengue | STMN1 | DENV replication | [95] |
| | IAV/influenza | TNF-α | Pro-inflammatory response | [97,101] |
| | | PI3K, IGF1R, GPCR, PP2A, PKA and Ca2+ channel | Represses the activity of CREB; T-cell development and cell survival | [96] |
| | | IL1RN, MDA5, STAT1 | Cell death; apoptosis | [100] |
| | SARS-CoV/SARS | NF-κB | Activated CCR1, the inflammatory chemokine receptor for CCL3 and CCL5 enhances lung fibrosis | [105] |
| | SARS-CoV-2/ COVID-19 | TRAF6, FOXO1, TLR4, STMN1, PI3K/AKT, CXCL2, CCL3, IL-6, IFN-I, IL-1β, Caspase-1 and mainly NLRP3, IKKα, NF-κB | Regulates inflammatory processes; anti-oxidant and anti-viral role | [108] |
| | HIV-1/AIDS | RhoB Sp3, LIF | Inhibits HIV-1 production in resting primary CD4+ T-cells Activates the AKT–NF-κB pathway Viral replication | [110] [112] [112] |

**Table 1.** *Cont.*

| MicroRNA | Virus/Disease | Target Genes | Biological Function/Modified Pathways | Reference |
|---|---|---|---|---|
| miR-146a | HBV/CHB | NF-κB | Production of pro-inflammatory cytokines (TNF-α, IL-6, IL-8, IL-12, and IL-18) | [118] |
| | | STAT1 | Viral persistence Decreases cytotoxicity of T-cells | [117] |
| | | CFH | Regulation of the complement alternative pathway | [121] |
| | | XIAP | Regulation of HBV replication; regulation of XIAP-MDM2/p53 pathway | [118] |
| | | ZEB2 | Regulation of HBV transcription and replication | [119] |
| | | TRAF6, IRAK1 | Regulation of FEN1 by NF-κB activity; promotes HBV DNA replication | [120,124] |
| | HCV/HC | SOCS1 | STAT3 inhibition; increases IL-23, IL-10, and TGF-β expression | [125] |
| | DENV/dengue | TRAF6 | Inhibits IFN-β and IL-28A/B; increases DENV2 replication | [129] |
| | | LC3 | Autophagy; viral replication | [130] |
| | IAV/influenza | TRAF6 | Production of type I IFN; anti-viral response | [133] |
| | | IRAK1 | IL-7, VEGF and, JAK-STAT signaling pathway | [134] |
| | RSV/upper respiratory tract infections | TRAF6, IRAK1 | Viral replication | [137,138] |
| | SARS-CoV-2/ COVID-19 | TRAF6, IRAK1, IRAK2 | NF-κB pro-inflammatory signaling pathway | [139] |
| | | STAT1 | Regulates JAK/STAT pathway | [139] |
| miR-122 | HBV/HB | cyclin G1 | Replication of HBV | [149] |
| | | HO-1 | Replication of HBV; oxidative stress | [153–155] |
| | | IFN type 1 | Replication of HBV; anti-viral response | [156] |
| | | SOCS3 | JAK/STAT pathway signaling; cytokine signaling | [157] |
| | HCV/HC | viral 5′-NCR | Replication of HCV | [166] |
| | | Bach1 | HO-1 gene regulation; HCV replication | [170] |
| | | IFN | Anti-viral response | [171] |
| | | TGFBRAP1 | Promotes HCC progression induced by HCV | [172] |
| | DENV/dengue | CYP7A1, IGFR1, SFR, RAC1, RHOA, cyclin G1 | Immune response | [131] |
| | ZEBOV/RESTV/ Ebola | viral vp40 gene | Regulation of virus replication | [176,177] |
| | RSV/upper respiratory tract infections | Wnt | Inflammatory and immune response | [178] |
| | | IL1R1 | NF-κB activation, inflammatory response | [179,180] |
| | | TLR4 | Innate immune response: NF-κB activation; cytokine secretion; inflammatory response | [180] |
| | | iNOS | Anti-viral response | [184] |
| | HRV/COPD | CXCL2 | Chemotaxis | [185] |
| | | SOCS1 | Regulates cytokine signal transduction | [185] |

**Table 1.** *Cont.*

| MicroRNA | Virus/Disease | Target Genes | Biological Function/Modified Pathways | Reference |
|---|---|---|---|---|
| miR-125b | HBV/HB | LIN28B | Regulates let-7 and stimulates HBV replication | [194] |
| | | SCNN1A | Inhibits HBV core protein expression | [197] |
| | HCV/HC | TLR2 | TLR2/MyD88 signaling pathway; phosphorylation of NF-κBp65, ERK, and P38 | [198] |
| | | HuR | Viral replication | [200] |
| | RSV/upper respiratory tract infections | NF-κB, MAPK | Immune response | [205] |
| | IAV/influenza | MAPK | Cell proliferation; apoptosis | [206] |
| | SARS-CoV-2/ COVID-19 | ACE2 | Activation of immune system | [207] |
| | HIV-1/AIDS | CPSF6 | Regulation of HIV-1 nuclear entry and viral replication | [208] |
| | JEV/Japanese encephalitis | STAT3, Map2k7, Triap1 | Reduces genome replication and virus titers | [212] |
| miR-132 | HBV/HB | Akt | Development of HCC induced by HBV | [217] |
| | IAV/influenza | IRF1 | Regulation of IFN-α and IFN-β production | [219] |
| | | MAPK3 | Regulates innate immune signaling pathways | [134] |
| | HSV/orofacial and genital disease | Ras | Inflammation | [224] |
| miR-34a | HBV/HB | Wnt1 | Regulation of Wnt/β-catenin pathway; contributes to HCC induced by HBV | [229] |
| | | HMGB1 | Innate immune response; apoptosis | [227] |
| | | CCL22 | Chemotactic for monocytes, dendritic cells, natural killer cells, and T lymphocytes | [230] |
| | | Smad4 | Regulation of TGF-β/Smad3 pathway activity; liver fibrosis | [228] |
| | DENV/dengue WNV/West Nile fever | Wnt | Activates type I IFN response; regulation of WNT/β-catenin signaling; anti-viral state | [233] |
| | IAV/influenza | Bax | Apoptosis | [234] |
| | | STAT3 | Anti-inflammatory function Inhibits NF-κB gene reporters | [235] |
| | SARS-CoV-2/ COVID-19 | Bcl-2, Bax, KLF4 IL-6R | Apoptosis Inflammation | [236] |
| | HTLV-1/leukemia | SIRT1 Bax | Apoptosis | [240] |
| miR-21 | HBV/CHB | PTEN | PTEN/Akt signaling; activation of HSCs; apoptosis | [250,251] |
| | | Smad7 | Promotes α-SMA and collagen I expression in HSCs; liver fibrosis | [250,251] |
| | | TGF-β1 | Positive feedback loop; liver fibrosis | [249] |
| | | PDCD4 | Apoptosis | [252] |
| | | IL-12 | Regulates proliferation of NK and T-cells | [257] |

**Table 1.** *Cont.*

| MicroRNA | Virus/Disease | Target Genes | Biological Function/Modified Pathways | Reference |
|---|---|---|---|---|
| | HCV/HC | PTEN | Apoptosis | [260] |
| | | MyD88<br>IRAK1 | Production of IFN type I; anti-viral response | [261] |
| | | Smad7 | Fibrosis | [262] |
| | IAV/influenza | CCL1, CCL17, CCL19, IL-22, C2orf28 | Inflammation; apoptosis | [268] |
| | | TGF-β | Fibrosis | [271] |
| | Ad/respiratory infection | p53<br>TGF-β | Apoptosis | [80] |
| | SARS-CoV-2/ COVID-19 | ORF1ab<br>ORF3a<br>S protein gene | Regulation of viral entry | [273] |
| | | CCL20 | Chemotactic response<br>TGF-β and Akt signaling | [139] |
| | | IRAK1 | Participates in NF-κB pro-inflammatory signaling pathway | [139,273] |
| | | CXCL-10 | Pro-inflammatory response; chemotaxis | [273] |
| | HIV-1/AIDS | PTEN<br>PDCD4<br>CDKN1B | Apoptosis | [279] |
| | | IP-10 | Inflammation | [280] |
| | CVB3 | YOD1 | Metabolism of proteins; protein ubiquitination | [284] |
| | CHPV | PTEN | Regulation of phosphorylation of AKT and subunit p65 of NF-kB; production of pro-inflammatory cytokines (IL-6, TNF-α) | [286] |
| miR-16 | HBV/HB | CCND1 | Cell proliferation | [290] |
| | | c-Myc | Cellular metabolism and proliferation | [290] |
| | | Bcl-2 | Apoptosis | [290] |
| | HCV/HC | HGF | Hepatocyte proliferation | [291] |
| | | Smad7 | Fibrosis | [291] |
| | Human CoV (MERS, SARS, SARS-CoV-2) | polyprotein 1ab coding region | Regulation of viral replication | [272] |
| | SARS-CoV-2/ COVID-19 | MK2<br>CCND1 | Cell proliferation | [273] |
| | RSV/upper respiratory tract infections | TLR4<br>RIG-1 | Activation of NF-κB; pro-inflammatory | [58] |
| miR-181a | HBV/HB | PTEN | Cell proliferation; apoptosis; development of hepatocellular carcinoma | [300] |
| | | CLDN1 | Blocks HCV entry into cells | [306] |
| | | E2F5 | Development of HCC induced by HBV | [301] |
| | | Fas | Apoptosis and regulation of HCC cell proliferation | [302] |
| | HCV/CHC | DUSP6 | Regulation of T-cell response | [305] |
| miR-181b | HBV/HB | p27<br>PTEN | Regulation of hepatic stellate cell proliferation; pro-fibrotic role | [298,299] |
| miR-181c | HCV/HC | HOXA1 | Cell growth regulator; activates STAT3 and STAT5 | [306] |
| | | ATM | Apoptosis | [307] |
| | | CDK-2<br>cyclin-A | Cell cycle | [307] |
| | IAV/influenza | Bcl-2 | Apoptosis | [206] |
| | | IL-2, TNF-α | Immune response | [206] |

**Table 1.** *Cont.*

| MicroRNA | Virus/Disease | Target Genes | Biological Function/Modified Pathways | Reference |
|---|---|---|---|---|
| let-7a | HBV/HB | STAT3 | Immune suppression | [315,317] |
| | HCV/HC | CHUK, IKBKE, XPNPEP1 | Regulation of HCV assembly or secretion | [320] |
| | JEV/Japanese encephalitis | cPARP | Apoptosis | [337] |
| let-7b | HCV/HC | IFN type I<br>SOCS1<br>IKKα | Immune response; anti-viral response;<br>stimulation of JAK/STAT signaling pathway | [322] |
| | | GF2BP1 | Regulation of HCV replication | [323] |
| | SARS-CoV-2/ COVID-19 | RELB, IL-6, KHSRP, EHMT2, KDM2B, CMYC, LIN28B | Immune response; repair function | [71] |
| | | TLR4 | Regulation of inflammation via NF-κB | [333] |
| | | IL-6, IL-8, TNF-α, IL-10 | Regulation of pro- and anti-inflammatory cytokine production | [333] |
| | | ORF1ab (virus gene) | Regulation of viral replication | [238] |
| | HIV-1/AIDS | IL-10 | Anti-viral response | [335] |
| | JEV/Japanese encephalitis | cPARP | Apoptosis | [337] |
| let-7c | DENV/dengue | Bach1 | Regulation of HO-1; regulation of viral replication; oxidative stress; anti-inflammatory response | [326] |
| let-7f | RSV/upper respiratory tract infections | CCL7<br>SOCS3 | Anti-viral cytokine response | [330] |
| let-7i | HIV-1/AIDS | IL-2 | Th-cell death | [336] |
| miR-10a | HCV/HC | RORA | Immune response | [340] |
| | | SREBP1<br>FANS<br>SREBP2<br>PGC1α | Lipid homeostasis; cholesterol biosynthesis; fatty acid synthesis; regulation of fatty acid metabolism | [340] |
| | | Bmal1 | Liver damage | [340] |
| | RSV/upper respiratory tract infections | NF-κB<br>MAPK | Immune response | [341–343] |

The abbreviations in the table are explained in the text.

## 3. MicroRNA Signature in Animal Viral Infections

In the scope of this study, we distinguished 10 host miRNAs that are important from the point of view of their participation in viral infections in animals (Figure 2B).

### *3.1. MicroRNA-155 (miR-155)*

#### 3.1.1. Hemorrhagic Viruses

Rabbit hemorrhagic disease virus (*Lagovirus europaeus*/RHDV) belongs to the family *Caliciviridae*, with an RNA genome. *Lagovirus europaeus*/RHDV is an etiological agent of rabbit hemorrhagic disease (RHD) [345], a highly infectious and deadly disease characterized by acute necrotizing hepatitis, but hemorrhages may also be found in other organs—in particular, the lungs, heart, kidneys, and spleen—due to disseminated intravascular coagulation [346]. The expression levels of ocu-miR-155-5p in liver were significantly higher (a fold change of 5.8) in infected rabbits, compared to the healthy rabbits (see Figure 3) [347]. The increased levels of miR-155 in liver tissues may be caused by the inflammatory responses in this organ caused by RHDV 12/24 h post-infection and the infiltration of cells of the immune system. Furthermore, a functional analysis has shown that ocu-miR-155-5p can regulate the expression of genes involved in processes related to acute liver failure (ALF) in rabbits. Furthermore, STRING analysis indicated the dependences between JUN, IGFR1, KRAS, TNF-α, TGF-β, IL-1β, IL-6, and IFN, as well as TLR, among others, in monocytes/macrophages and miR-155 [347]. These results indicate the role of miR-155 in

both RHDV infection and the course of RHD, which may reflect the hepatic inflammation and impairment/dysfunction in RHD. The model used in this experiment (i.e., the infection of rabbits with *Lagovirus europaeus*/RHDV) is considered a good model for the study of viral hemorrhagic fevers in human, as well as multiple organ failure (MOF), as it exhibits biochemical and histopathological characteristics and clinical features that are remarkably similar to those in humans in the course of the disease [347].

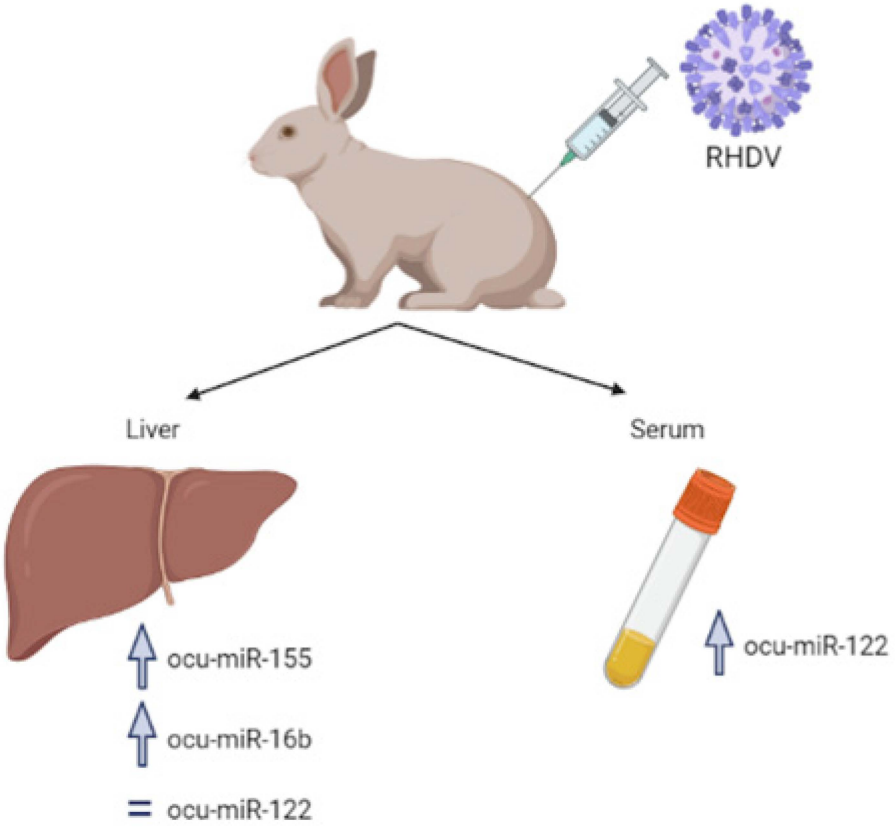


**Figure 3.** Signature of miRNAs ocu-miR-155-5p, ocu-miR-16b-5p, and ocu-miR-122-5p in the course of *Lagovirus europaeus*/RHDV infection [347].

#### 3.1.2. Respiratory Viruses

Avian influenza virus (AIV) belongs to the *Orthomyxoviridae* family of viruses, with an ssRNA genome [48]. AIV poses an ongoing risk to domestic pets, exotics, and wild birds worldwide. Disease outbreaks can threaten biodiversity due to their high morbidity and mortality in endangered species [48]. In AIV infection, researchers have shown a significant up-regulation of miR-155 [348]. Furthermore, based on target prediction, miR-155 could target the chicken anti-influenza gene MX dynamin-like GTPase 1 (MX1), as well as hemagglutinin (HA) and neuraminidase (NA), which are a major surface glycoproteins, therefore playing a role in host–AIV interactions in chickens. Additionally, up-regulated miR-155 might also activate the JNK pathway and, subsequently, induce apoptosis to eliminate virus-infected cells [348].

#### 3.1.3. Lymphotropic Viruses

Marek's disease virus (MDV) is a member of the *Herpesviridae* family, with a dsDNA genome [349]. MDV, as a lymphotropic alphaherpesvirus of chickens, causes a disease characterized by tumor formation, immunosuppression, and neurological disorders [350,351]. With the emergence of new virulent strains in the field over time, MDV remains a serious threat to the poultry industry [350,351]. In deep sequencing studies of MDV-induced splenic tumors, Burnside et al. [350] reported decreased levels of miR-155, compared to

normal spleen, resting T-cells, or activated T-cells [350]. These observations were confirmed by the in vitro study of Yao et al. [352], who observed a down-regulation of miR-155 specific to MDV-transformed tumor cells. In addition, the level of miR-155 expression was consistently reduced in all tested MDV-1-transformed lymphoblastoid cell lines, demonstrating that the down-regulation of miR-155 is a feature unique to the MDV transformation of T-cells. However, the molecular mechanisms that drives down the expression of miR-155 in MDV-transformed cell lines remains unknown [352].

In summary, it can be concluded that, in animals, the most important role of miR-155 is regulating the anti-viral response and reducing infection by inducing the apoptotic pathway in infected cells.

### *3.2. MicroRNA-223 (miR-223)*

#### 3.2.1. Respiratory Viruses

AIV sub-type H1N2 (A/H1N2) is a sub-type of the influenza A virus. It is currently endemic in the pig population and is occasionally seen in humans. The virus does not cause more severe illness than other influenza viruses, and no unusual increases in influenza activity have been associated with it. In the research of Skovgaard et al. [353], it was shown that, in the lung tissue of pigs infected with A/H1N2, the expression levels of several miRNAs were increased, including miR-223, as observed one day after infection, followed by a decrease. This result suggests that miR-223 may be involved in controlling acute influenza infection in pigs [353].

#### 3.2.2. Lymphotropic Viruses

In MDV infection, miR-223 has been found to be down-regulated in all MDV-transformed cell lines relative to the levels in normal splenocytes or CD4+ T-cells. In addition, miR-223 was decreased in retrovirus-transformed AVOL-1 cells. Although the regulatory mechanisms are not yet fully understood, recent studies have indicated a clear role for miR-223 in hematopoiesis, as well as in malignancies, suggesting that miR-223 is involved in MDV-induced lymphocyte transformation [352].

#### 3.2.3. Vector-Borne Viruses

Vesicular stomatitis virus (VSV) is a member of the *Rhabdoviridae* family, with an ssRNA genome [285]. VSV is an arthropod-borne virus that primarily affects rodents, cattle, swine, and horses, but may also infect humans and other species. VSV infection occurs primarily in domesticated cattle, horses, swine, and rarely in llamas and humans, and can cause vesiculation, epithelial cell lysis, and severe interstitial edema, which appear with the infiltration of inflammatory cells [285]. MiR-223 has a key role in the development and homeostasis of the immune system, and plays an important role in VSV infection. A study showed that VSV induced the up-regulation of miR-223 in murine macrophages. Moreover, it was found that miR-223 over-expression up-regulated IFN-1 expression levels in VSV-infected macrophages and directly targeted FOXO3 to regulate IFN-1 production. This comprises the positive feedback regulatory function of miR-223 for the regulation of IFN-1 production in VSV infection [354].

The presented studies indicate that the most important activity of miR-223 in the considered viral infections is related to the control of viral infections and the regulation of factors involved in the anti-viral response.

### *3.3. MicroRNA-146a (miR-146a)*

#### 3.3.1. Respiratory Viruses

Influenza affects the level of miR-146a in humans, but studies have indicated that influenza may also regulate this miRNA in another species [348,353,355]. AIV, outbreaks of which are worldwide threats to both poultry and humans, causes an infection of the respiratory tract, thus triggering a cascade of innate and adaptive immune responses and contributing to the up-regulation of miR-146a in chickens [348,355,356]. However, in the

lung tissue of pigs infected experimentally with influenza virus (H1N2), the level of miR-146 was significantly decreased the first day after infection, which may affect the expression of IRAK1, STAT1, and TLR2 [353].

Hendra virus (HeV) is a member of the family *Paramyxoviridae*, with an RNA genome [357], and is a relatively emerging pathogen. It was first identified as the causative agent in an outbreak of severe respiratory disease and subsequent neurological disorders in horses. In addition, HeV may cause infection in humans, with high mortality rates [358]. MiR-146a is also induced in HeV infection; in particular, miR-146a has been found to be elevated in vitro in the blood of ferrets and horses infected with HeV, as well as in human cells [359]. Stewart et al. [359] have noted that induction of miR-146a during infection may be due to RIG-I. Additionally, studies have shown that inhibition of miR-146a reduces HeV replication in vitro. This suggests a role of this miRNA in HeV replication. This effect of miR-146a is mostly mediated through its target, ring finger protein 11 (RFN11), a member of the A20 ubiquitin-editing complex that negatively regulates NF-κB activity [359]. In another study, Cowled et al. [360] showed that HeV infection altered the expression profile of many host miRNAs, including miR-146a, as demonstrated by testing whole-blood samples during infection. The authors emphasized that the development of an miRNA signature in HeV infection would provide a chance for the early identification of infected horses, ensuring the possibility of reducing human exposure to infectious secretions and, thus, reducing the risk of transmission of zoonotic infection [360].

#### 3.3.2. Lymphotropic Viruses

The down-regulation of miR-146a has also been observed in MDV-induced splenic tumors [350]. MDV is a oncogenic virus caused by Marek's disease (MD), which is a lymphoproliferative disorder in which aggressive T-cell lymphomas develop within two to six weeks following the infection of susceptible chickens [361].

#### 3.3.3. Vector-Borne Viruses

It was also found that VSV infection up-regulated miR-146a expression. MiR-146a was induced in mouse macrophages in a TLR–myeloid differentiation factor 88-independent but RIG-I/NF-κB-dependent manner during VSV. In turn, miR-146a negatively regulated VSV-triggered IFN-1 production, thus promoting VSV replication in macrophages. On the other hand, IRAK1 and IRAK2, other targets of miR-146a, also participate in VSV-induced IFN-1 production by associating with the Fas-associated death domain protein, an important adaptor in RIG-I signaling, in a VSV infection-inducible manner [116].

#### 3.3.4. Other Viruses

Foot-and-mouth disease virus (FMDV) is a member of the family *Picornaviridae*, with an RNA genome [362–364]. FMDV is the etiological agent of foot-and-mouth disease (FMD), known as one of the most contagious animal diseases. FMD targets both domestic and wild cloven-hoofed animals, and outbreaks have affected important livestock populations [363,364]. A study has shown that, during FMDV infection, one of dysregulated host miRNAs is miR-146a [365]. Stenfeldt et al. [365] have indicated that miR-146a is up-regulated in acute infection, which may modulate the immune response through induced NF-κB signaling by the targets IRAK1 and TRAF6, thus diminishing the pro-inflammatory response from TLR signaling [114,365,366].

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is a member of the family *Arteriviridae*, with an RNA genome [367], and is characterized by episodes of reproductive failure in pregnant sows and respiratory illness, particularly in young pigs [368]. PRRSV infection contributes to the up-regulation of miR-146a in macrophages. An analysis has shown that miR-146a during PRRSV infection regulates tumor necrosis factor related protein 3 (C1QTNF3) and v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) genes, the decrease in which may aid in the immune response against PRRSV infection [369].

Our review indicates that, as in humans, miR-146a plays the most important role in the regulation of genes, influencing NF-κB activity and IFN production in viral infections in animals, making it an important factor in the anti-viral response.

### 3.4. MicroRNA-145 (miR-145)

MiR-145 is a molecule that has not been studied in humans in the course of viral infections so far. Additionally, there is very limited information about the function of miR-145 in the immune response. The only information that can be found regarding miR-145 is its function as a tumor suppressor, expressed in various tumors. However, recent research has focused on the role of miR-145 in various important animal viral infections [370].

#### 3.4.1. Respiratory Viruses

A decreased level of miR-145 was observed during swine-origin influenza A (H1N1) virus infection. Additionally, it was supposed that miR-145 targets the HA gene, which has been shown to be critical for the pathogenicity of influenza virus and immunosuppression. The authors suggested that miRNA-mediated host–virus interactions may characterize the location specificity of viral replication. Further studies focusing on these interactions are needed in order to reveal the interplay mechanism between the host miRNA and viruses [371].

#### 3.4.2. Neurotropic Viruses

Rabies virus (RABV) belongs to the *Rhabdoviridae* family, with an RNA genome, and is the etiological agent of rabies. Rabies is most often transmitted through being bitten by a rabid animal. RABV infects the central nervous system of mammals, ultimately causing disease in the brain and death [372]. Zhao et al. [373] observed a decrease in the level of miR-145 in the brain of mice infected with RABV. Informatic analysis indicated that miR-145 may play a role in the Jak–STAT signaling pathway (MYC; signal transducing adaptor molecule, STAM; STAT4; and SOCS7), cytokine–cytokine receptor interactions (IL17RB and TGFBR2), Fc gamma R-mediated phagocytosis (ADP ribosylation factor 6, ARF6; cofilin 2, CFL2; linker for activation of T-cells, LAT; actin related protein 2/3 complex subunit 5, ARPC5; and CT10 regulator of kinase-like Proto-Oncogene adaptor protein, CRKL), the Wnt signaling pathway (protein phosphatase 3 catalytic subunit alpha, PPP3CA; frizzled class receptor 9, FZD9; MYC; catenin beta interacting protein 1, CTNNBIP1; SMAD3; SUMO specific peptidase 2, SENP2; and WNT5B), or the TGF-beta signaling pathway (MYC; SMAD3; inhibin beta B, INHBB; TGFBR2; and SMAD5) [373].

In related studies, miR-145 has been considered only with respect to a few viruses, which makes it impossible to provide a final comment on the specific role of this miRNA in viral infections. It can only be suggested that miR-145 is down-regulated by viral infections and may participate in various signaling pathways related to the immune system.

### 3.5. MicroRNA-21 (miR-21)

#### 3.5.1. Respiratory Viruses

During influenza virus infection in pigs and chickens, the up-regulation of miR-21 has been observed. According to the authors of several studies, miR-21 has a very important function in lymphocyte development and modulations, and may also regulate the expression of CXCL10, which is a chemoattractant for monocytes/macrophages, T-cells, and NK cells. The results of their studies demonstrate that miR-21 is an innate host immune factor, and participates in the response to viral infection [353,355,374].

#### 3.5.2. Neurotropic Viruses

A study has shown that miR-21 can be expressed differently throughout RABV infection, in a time-dependent manner. The greatest increase in miR-21 in cultured murine RABV-infected neurons occurred at 144 h of infection, reaching a fold change of about 4.0, while it reached a fold change of about 2.0 in hippocampal neurons. The results indi-

cated the participation of this miRNA in neuronal dysfunction caused by RABV infection. However, more research is needed to elucidate the role of miR-21 in RABV infection [375].

In conclusion, it should be noted that miR-21, in the above viral infections, on one hand, participates in the immune response against viral infections; on the other hand, it also potentially affects the dysfunction of the infected organs.

### *3.6. MicroRNA-16/miR-15a Cluster (miR-16/miR-15a Cluster)*

MiR-16/miR-15a inhibits cell proliferation through the regulation of a number of proliferation-related targets and apoptosis [376]. Similarly to miR-145 and miR-21, very limited research has been carried out regarding the miR-15a/miR-16 cluster in viral infections.

#### 3.6.1. Hemorrhagic Viruses

During infection with *Lagovirus europaeus*/RHDV, a significantly higher level of ocu-miR-16b-5p has been observed in the liver of infected rabbits compared to healthy rabbits (a fold change of 2.5) see Figure 3. Therefore, it has been suggested that miR-16b may play a role in the pathogenesis of RHD. Due to its role, ocu-miR-16b-5p may promote liver cell apoptosis by targeting Bcl-2 in response to viral infection, but is also crucial for an effective inflammatory response in damaged liver tissue. In addition, miR-16, by regulating HGF, is required for cell proliferation during the liver regeneration process [347].

#### 3.6.2. Respiratory Viruses

MiR-15a has been found to be significantly up-regulated after influenza A infection in lung tissues at all time points (i.e., 1 day, 3 days, and 14 days post-infection). However, target genes of miR-15a were also increased (moderately for TLR7 and strongly for CXCL10). These results indicate that miR-15a may not be involved in the observed regulation of TLR7 and CXCL10 mRNA levels, or that this miRNA acts through the repression of mRNA translation to protein, although no data yet support this notion [353].

In summary, it should be stated that the most important role of the miR-15a/miR-16 cluster concerns, on one hand, its participation in the immune response and apoptosis, and, on the other hand, organ regeneration in the course of viral infections.

### *3.7. MicroRNA-181 Family (miR-181 Family)*

The miR-181 family consists of members involved in the regulation of many relevant biological processes, including cell proliferation, apoptosis, autophagy, mitochondrial function, and immune responses [377].

#### 3.7.1. Respiratory Viruses

Low expression levels of miR-181a and miR-181b have been observed in avian influenza infection. The author suggested that miRNAs with different expression levels are important in the immune response to viral infection. The above miRNAs are involved in cell proliferation, apoptosis, and other biological processes; however, further studies are required to better understand the prevalence and functions of miR-181 family members in animal viral infections [378].

#### 3.7.2. Lymphotropic Viruses

In MDV infection, apart from miR-181b, another member—miR-181a—has also presented a lower level of expression in splenic tumors. The research suggested that the miR-181 family is very important to this virus, playing roles in immune response evasion [350].

#### 3.7.3. Other Viruses

During persistent infection with FMDV, the down-regulation of miR-181b in cow sera has been observed. MiR-181b may regulate cellular proliferation by targeting RASSF1A and NF-κB, and can also exert an immunomodulatory effect through adenylyl cyclase 9

(AC9) and inhibition of IFNα expression. However, decreased expression of miR-181b in FMDV infection may affect the increased production of IFNα, thus leading to a better anti-viral response [365].

However, in PRRSV infection, only miR-181c presented a decreased expression level in porcine alveolar macrophages (PAMs). Meanwhile, an in vitro study has indicated that both miR-181a and miR-181c inhibit viral gene expression and PRRSV production by specifically binding to a highly conserved region downstream of ORF7 in the viral genomic RNA [5]. Additionally, miR-181c can down-regulate the PRRSV receptor CD163 in blood monocytes and PAMs. Decreased CD163 levels lead to the inhibition of PRRSV entry into PAMs, subsequently suppressing PRRSV infection [379].

In summary, it can be concluded that miR-181, in the considered viral infections in animals, participates in regulation of the immune response.

### *3.8. Let-7 Family (Let-7)*

#### 3.8.1. Lymphotropic Viruses

In vitro research conducted by Tian et al. [380] has shown that the expression of let-7i is down-regulated in chicken cell lines during MDV infection. They also found that, similarly to the infected line, let-7i was decreased in MD tumors in infected chickens. Further bioinformatic analyses showed that let-7i may interact with activating transcription factor 2 (ATF2) mRNA in its coding regions, which regulates proliferation and apoptosis, and might (along with another miRNAs) be related to Marek's disease resistance/susceptibility [380].

Avian leukosis virus (ALV) belongs to the *Retroviridae* family, with an RNA genome. ALV induces myeloid leukosis (ML) primarily in broilers, leading to major economic losses in the poultry industry worldwide [381]. During ALV infection, let-7b and let-7i presented variable expression, over 120 days of infection, in liver and bone marrow tissues [382]. Meanwhile, Li et al. [383] reported only down-regulation of the tested miRNAs [383]. Through use of bioinformatic analysis, it was predicted that both gga-let-7b and gga-let-7i are involved in multiple pathways, including signaling pathways based on factors such as MAPK, TGF-β, Notch, Wnt, mTOR, cell cycle, P53, and Jak-STAT. Based on these data, the researchers suggested that let-7b and let-7i likely play critical roles in regulating tumorigenesis in the course of AVL infection [382].

#### 3.8.2. Other Viruses

Stenfeldt et al. [365] paid attention to the role of let-7g in the course of acute FMDV infection, and showed that the level of let-7g was down-regulated twice in acute infection. Furthermore, they indicated the role of let-7g in cellular proliferation by targeting lectin-like oxidized low density lipoprotein receptor-1 (LOX) or caspase-3. Additionally, it was observed that, after the treatment of FMDV infection, let-7g levels returned to the baseline levels before infection, supporting the use of serum miRNA profiling to identify infected FMDV carriers [365].

The presented studies indicate that the most important activity of the let-7 family in the considered viral infections is related to the proliferation and apoptosis of cells.

### *3.9. MicroRNA-122 (miR-122)*

#### 3.9.1. Hemorrhagic Viruses

Results for ocu-miR-122-5p were obtained in rabbits infected with *Lagovirus europaeus*/RHDV (see Figure 3), from which it was observed that the level of miR-122 was not significantly different in the liver tissue of infected rabbits compared to healthy rabbits. However, in sera from RHDV-infected rabbits, the miR-122 level was higher compared to that from controls [347]. Additionally, the results of a GO analysis for ocu-miR-122-5p indicated that its potential role in the response to RHDV infection includes regulation of the expression of genes involved in the processes of hepatic homeostasis (e.g., HGF and c-Met) and, to a lesser extent, apoptosis (e.g., STAT1 and STAT3). Based on these data, the researchers suggested that ocu-miR-122-5p in serum may potentially serve as a biomarker of liver damage from RHD [347].

#### 3.9.2. Respiratory Viruses

An increase in miR-122 has been observed in the lungs of chickens in the course of AIV infection. A comprehensive analysis, combining both gga-miR-122–1 and 122–2 with targeted mRNA gene expression for MX1, IL-8, IRF-7, and TNFRS19, indicated that they are strong candidate miRNAs and genes involved in regulation of the host response to AIV infection in the lungs of broiler chickens. Further miRNA- or gene-specific knock-down assays are thus warranted in order to elucidate the underlying mechanism of AIV infection regulation in chickens [348].

Our review indicates that the role of miR-122 in viral infections in animals may vary, depending on the tissue environment. On one hand, it can influence liver homeostasis; on the other hand, it affects the immune response in infected organs. In addition, researchers have noted the role of miR-122 in animals as a potential marker of liver damage in the course of viral infection.

Table 2 lists all of the miRNAs that are important in animal viral infections, along with their target genes, biological functions, and modulated pathways.

**Table 2.** MicroRNAs that are important in animal viral infections.

| MicroRNA | Virus/Disease | Target Genes | Biological Function/Modified Pathways | Reference |
|---|---|---|---|---|
| miR-155 | *Lagovirus europaeus*/RHDV/RHD | JUN, IGFR1, KRAS, TNF-α, TGF-β, IL-1β, IL-6, IFN | Immune response | [347] |
| | AIV/influenza | MX1 | Anti-viral response | [348] |
| | | JNK pathway | Apoptosis | [348] |
| miR-223 | VSV/vesicular stomatitis | FOXO3 | Regulation of IFN type I production | [354] |
| miR-146a | H1N2/influenza | IRAK1<br>STAT2<br>TLR2 | Innate immune response; regulation of IFN type I production | [353] |
| | HeV | RIG-I | Innate immune response | [359] |
| | | RFN11 | Regulation of viral replication | [359] |
| | VSV/vesicular stomatitis | IRAK1, IRAK2 | Regulation of IFN type I production | [116] |
| | FMDV/FMD | TRAF6<br>IRAK1 | Regulation of NF-κB activation; innate immune response | [365] |
| | PRRSV/PRRS | C1QTNF3<br>MAFB | Regulation of immune response | [369] |
| | AIV/influenza | HA | Regulation of pathogenicity of influenza virus and immunosuppression | [371] |
| | RABV/rabies | MYC, STAM, STAT4, SOCS7 | JAK/STAT signaling pathway | [373] |
| | | IL17RB<br>TGFBR2 | Receptor for the pro-inflammatory cytokines IL17B and IL17E, controlling the growth and/or differentiation of hematopoietic cells Regulation of cell cycle arrest in epithelial and hematopoietic cells; control of mesenchymal cell proliferation and differentiation; wound healing; extracellular matrix production; immunosuppression; and carcinogenesis | [373] |

**Table 2.** *Cont.*

| MicroRNA | Virus/Disease | Target Genes | Biological Function/Modified Pathways | Reference |
|---|---|---|---|---|
| miR-145 | | MYC, SMAD3, INHBB, TGFBR2, SMAD5 | TGF-beta signaling pathway | [373] |
| | | ARF6, CFL2, LAT, ARPC5, CRKL | Fc gamma R-mediated phagocytosis | [373] |
| miR-21 | H1N2/influenza | CXXL10 | Chemoattractant for monocytes/macrophages, T-cells, NK cells, and dendritic cells; may also promote T-cell adhesion to endothelial cells | [353,355] |
| miR-16 miR-15a | *Lagovirus europaeus*/RHDV/RHD | HGF Bcl-2 | Cell proliferation during the liver regeneration process Apoptosis | [347] |
| | AIV/influenza | CXCL10 | Chemoattractant for monocytes/macrophages, T-cells, NK cells, and dendritic cells; may also promote T-cell adhesion to endothelial cells | [353] |
| | | TLR7 | Activation of innate immunity | [353] |
| miR-181a | PRRSV/PRRS | ORF7 (virus gene) | Inhibits viral gene expression and virus protein production | [379] |
| miR-181b | FMDV/FMD | RASSF1A NF-κB | Regulates cellular proliferation and inflammatory response | [365] |
| | | AC9 | Immunomodulatory effect | [365] |
| | | IFNα | Anti-viral response | [365] |
| miR-181c | PRRSV/PRRS | ORF7 (virus gene) | Inhibits viral gene expression and virus protein production | [379] |
| let-7g | FMDV/FMD | LOX | Cell proliferation | [365] |
| | | caspase-3 | Apoptosis | [365] |
| let-7i | MDV/MD | ATF2 | Proliferation, apoptosis | [380] |
| miR-122 | *Lagovirus europaeus*/RHDV/RHD | HFG, c-Met STAT1, STAT3 | Hepatic homeostasis Apoptosis | [347] |
| | AIV/influenza | MX1, IL-8, IRF-7, TNFRS19 | Immune response | [348] |

The abbreviations in the table are explained in the text.

## 4. Conclusions

MiRNAs are non-coding RNAs that play key roles under pathological conditions in humans and animals, including viral infections. In this review, we summarized the available literature data, indicating that the signature miRNAs in human viral infections mainly include 12 miRNAs (i.e., miR-155, miR-223, miR-146a, miR-122, miR-125b, miR-132, miR-34a, miR -21, miR-16, miR-181 family, let-7 family, and miR-10a), while 10 key miRNAs were determined in animals (i.e., miR-155, miR-223, miR-146a, miR-145, miR-21, miR-15a/miR-16 cluster, miR-181 family, let-7 family, and miR-122).

The biological potential of host miRNA during viral infections is due, on one hand, to the fact that they can act as anti-viral microRNAs, inhibiting viral replication; on the other hand, they may also act as pro-viral microRNAs, necessary for the virus to replicate in the cellular environment. In addition, host microRNAs are essential regulators of the response to viral infections. Knowledge of host miRNAs and their roles in various viral infections can provide a useful tool for identifying the biological functions of genes and pathways that are activated in order to elicit an effective immune response, and other biological processes involved in the anti-viral response, such as apoptosis and oxidative stress.

Changes in miRNA expression during viral infections precede the detection of the viral genome in the studied material, confirming the view that determination of the miRNA

signature can be used for the early diagnosis of many viral diseases, including Hendra virus infection of horses; however, the development of miRNAs as disease-specific biomarkers likely requires more comprehensive measurement and the determination of very detailed miRNA signatures.

Knowledge of which miRNAs are involved in different viral infections and the biological roles that they play can help in understanding the pathogenesis of viral diseases, facilitating the future development of therapeutic agents for both humans and animals.

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## References

1. Winter, J.; Jung, S.; Keller, S.; Gregory, R.I.; Diederichs, S. Many roads to maturity: MicroRNA biogenesis pathways and their regulation. *Nat. Cell Biol.* **2009**, *11*, 228–234. [CrossRef] [PubMed]
2. Bartel, D.P. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* **2004**, *116*, 281–297. [CrossRef]
3. Dexheimer, P.J.; Cochella, L. MicroRNAs: From Mechanism to Organism. *Front. Cell Dev. Biol.* **2020**, *8*, 409. [CrossRef] [PubMed]
4. Leung, A.; Sharp, P.A. MicroRNA Functions in Stress Responses. *Mol. Cell* **2010**, *40*, 205–215. [CrossRef] [PubMed]
5. Rajewsky, N. MicroRNA target predictions in animals. *Nat. Genet.* **2006**, *38*, S8–S13. [CrossRef]
6. Berezikov, E.; Guryev, V.; van de Belt, J.; Wienholds, E.; Plasterk, R.H.; Cuppen, E. Phylogenetic Shadowing and Computational Identification of Human microRNA Genes. *Cell* **2005**, *120*, 21–24. [CrossRef]
7. Ignaz, P. *Circulating microRNA in Disease Diagnostics and Their Potential Biological Relevance*; Springer: Berlin/Heidelberg, Germany, 2015; Volume 106.
8. Zhang, Y.; Li, Y.-K. MicroRNAs in the regulation of immune response against infections. *J. Zhejiang Univ. Sci. B* **2013**, *14*, 1–7. [CrossRef]
9. Ardekani, A.M.; Naeini, M.M. The Role of MicroRNAs in Human Diseases. *Avicenna J. Med. Biotechnol.* **2010**, *2*, 161–179.
10. Bhaskaran, M.; Mohan, M. MicroRNAs: History, biogenesis, and their evolving role in animal development and disease. *Vet. Pathol.* **2014**, *51*, 759–774. [CrossRef]
11. Barbu, M.G.; Condrat, C.E.; Thompson, D.C.; Bugnar, O.L.; Cretoiu, D.; Toader, O.D.; Suciu, N.; Voinea, S.C. MicroRNA Involvement in Signaling Pathways During Viral Infection. *Front. Cell Dev. Biol.* **2020**, *8*, 143. [CrossRef]
12. Lee, Y.; Jeon, K.; Lee, J.-T.; Kim, S.; Kim, V.N. MicroRNA maturation: Stepwise processing and subcellular localization. *EMBO J.* **2002**, *21*, 4663–4670. [CrossRef] [PubMed]
13. O'Brien, J.; Hayder, H.; Zayed, Y.; Peng, C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front. Endocrinol.* **2018**, *9*, 402. [CrossRef] [PubMed]
14. Ha, M.; Kim, V.N. Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 509–524. [CrossRef] [PubMed]
15. Pfeffer, S.; Zavolan, M.; Grasser, F.A.; Chien, M.; Russo, J.J.; Ju, J.; John, B.; Enright, A.J.; Marks, D.; Sander, C.; et al. Identification of Virus-Encoded MicroRNAs. *Science* **2004**, *304*, 734–736. [CrossRef] [PubMed]
16. Skalsky, R.L.; Cullen, B.R. Viruses, microRNAs, and Host Interactions. *Annu. Rev. Microbiol.* **2010**, *64*, 123–141. [CrossRef]
17. Chen, M.; Wang, F.; Xia, H.; Yao, S. MicroRNA-155: Regulation of Immune Cells in Sepsis. *Mediat. Inflamm.* **2021**, *2021*, 8874854. [CrossRef]
18. Tam, W. Identification and characterization of human BIC, a gene on chromosome 21 that encodes a noncoding RNA. *Gene* **2001**, *274*, 157–167. [CrossRef]
19. Mahesh, G.; Biswas, R. MicroRNA-155: A Master Regulator of Inflammation. *J. Interf. Cytokine Res.* **2019**, *39*, 321–330. [CrossRef]
20. Vigorito, E.; Kohlhaas, S.; Lu, D.; Leyland, R. miR-155: An ancient regulator of the immune system. *Immunol. Rev.* **2013**, *253*, 146–157. [CrossRef]
21. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/reverse-transcribing-dna-and-rna-viruses/w/hepadnaviridae/1317/genus-orthohepadnavirus (accessed on 19 July 2022).

22. Rehermann, B.; Nascimbeni, M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat. Rev. Immunol.* **2005**, *5*, 215–229. [CrossRef]
23. Su, C.; Hou, Z.; Zhang, C.; Tian, Z.; Zhang, J. Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells. *Virol. J.* **2011**, *8*, 354. [CrossRef] [PubMed]
24. Sarkar, N.; Panigrahi, R.; Pal, A.; Biswas, A.; Singh, S.P.; Kar, S.K.; Bandopadhyay, M.; Das, D.; Saha, D.; Kanda, T.; et al. Expression of microRNA-155 correlates positively with the expression of Toll-like receptor 7 and modulates hepatitis B virus via C/EBP-β in hepatocytes. *J. Viral Hepat.* **2015**, *22*, 817–827. [CrossRef] [PubMed]
25. Yu, S.-L.; Deng, H.; Li, X.-H.; Huang, Y.-X.; Xie, D.-Y.; Gao, Z.-L. Expression of MicroRNA-155 is Downregulated in Peripheral Blood Mononuclear Cells of Chronic Hepatitis B Patients. *Hepat. Mon.* **2016**, *16*, e34483. [CrossRef] [PubMed]
26. Ge, J.; Huang, Z.; Liu, H.; Chen, J.; Xie, Z.; Chen, Z.; Peng, J.; Sun, J.; Hou, J.; Zhang, X. Lower Expression of MicroRNA-155 Contributes to Dysfunction of Natural Killer Cells in Patients with Chronic Hepatitis B. *Front. Immunol.* **2017**, *8*, 1173. [CrossRef]
27. Fang, J.; Zhuge, L.; Rao, H.; Huang, S.; Jin, L.; Li, J. Increased Levels of miR-155 are Related to Higher T-Cell Activation in the Peripheral Blood of Patients with Chronic Hepatitis B. *Genet. Test. Mol. Biomark.* **2019**, *23*, 118–123. [CrossRef]
28. Wang, W.; Bian, H.; Li, F.; Li, X.; Zhang, D.; Sun, S.; Song, S.; Zhu, Q.; Ren, W.; Qin, C.; et al. HBeAg induces the expression of macrophage miR-155 to accelerate liver injury via promoting production of inflammatory cytokines. *Cell. Mol. Life Sci.* **2018**, *75*, 2627–2641. [CrossRef]
29. Chen, L.; Ming, X.; Li, W.; Bi, M.; Yan, B.; Wang, X.; Yang, P.; Yang, B. The microRNA-155 mediates hepatitis B virus replication by reinforcing SOCS1 signalling–induced autophagy. *Cell Biochem. Funct.* **2020**, *38*, 436–442. [CrossRef]
30. Xiong, Y.; Chen, S.; Liu, L.; Zhao, Y.; Lin, W.; Ni, J. Increased Serum MicroRNA-155 Level Associated with Nonresponsiveness to Hepatitis B Vaccine. *Clin. Vaccine Immunol.* **2013**, *20*, 1089–1091. [CrossRef]
31. Hefzy, E.M.; Hassuna, N.A.; Shaker, O.G.; Masoud, M.; Abelhameed, T.A.; Ahmed, T.I.; Hemeda, N.F.; Abdelhakeem, M.A.; Mahmoud, R.H. miR-155 T/A (rs767649) and miR-146a A/G (rs57095329) single nucleotide polymorphisms as risk factors for chronic hepatitis B virus infection among Egyptian patients. *PLoS ONE* **2021**, *16*, e0256724. [CrossRef]
32. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/flaviviridae/362/genus-hepacivirus (accessed on 19 July 2022).
33. Bala, S.; Tilahun, Y.; Taha, O.; Alao, H.; Kodys, K.; Catalano, D.; Szabo, G. Increased microRNA-155 expression in the serum and peripheral monocytes in chronic HCV infection. *J. Transl. Med.* **2012**, *10*, 151. [CrossRef]
34. Jiang, M.; Broering, R.; Trippler, M.; Wu, J.; Zhang, E.; Zhang, X.; Gerken, G.; Lu, M.; Schlaak, J.F. MicroRNA-155 controls Toll-like receptor 3- and hepatitis C virus-induced immune responses in the liver. *J. Viral Hepat.* **2014**, *21*, 99–110. [CrossRef]
35. Sidorkiewicz, M.; Grek, M.; Piekarska, A.; Bartkowiak, J.; Fendler, W.; Kuydowicz, J.; Wroblewski, P.; Paradowski, M. Coordinated increase of miRNA-155 and miRNA-196b expression correlates with the detection of the antigenomic strand of hepatitis C virus in peripheral blood mononuclear cells. *Int. J. Mol. Med.* **2011**, *28*, 875–880. [CrossRef] [PubMed]
36. Cheng, Y.Q.; Ren, J.P.; Zhao, J.; Wang, J.M.; Zhou, Y.; Li, G.Y.; Moorman, J.P.; Yao, Z.Q. MicroRNA-155 regulates interferon-$\gamma$ production in natural killer cells via Tim-3 signalling in chronic hepatitis C virus infection. *Immunology* **2015**, *145*, 485–497. [CrossRef] [PubMed]
37. Riad, S.E.; El-Ekiaby, N.; Mekky, R.Y.; Ahmed, R.; EL Din, M.A.M.; El-Sayed, M.; Abouelkhair, M.M.; Salah, A.; Zekri, A.R.; Esmat, G.; et al. Expression signature of microRNA-155 in hepatitis C virus genotype 4 infection. *Biomed. Rep.* **2015**, *3*, 93–97. [CrossRef]
38. El-Ekiaby, N.; Hamdi, N.; Negm, M.; Ahmed, R.; Zekri, A.-R.; Esmat, G.; Abdelaziz, A. Repressed induction of interferon-related microRNAs miR-146a and miR-155 in peripheral blood mononuclear cells infected with HCV genotype 4. *FEBS Open Bio* **2012**, *2*, 179–186. [CrossRef] [PubMed]
39. Zhang, Y.; Wei, W.; Cheng, N.; Wang, K.; Li, B.; Jiang, X.; Sun, S. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology* **2012**, *56*, 1631–1640. [CrossRef]
40. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/flaviviridae/360/genus-flavivirus (accessed on 19 July 2022).
41. Arboleda, J.F.; Fernandez, G.J.; Urcuqui-Inchima, S. Vitamin D-mediated attenuation of miR-155 in human macrophages infected with dengue virus: Implications for the cytokine response. *Infect. Genet. Evol.* **2019**, *69*, 12–21. [CrossRef]
42. Costa, V.V.; Fagundes, C.T.; Souza, D.G.; Teixeira, M.M. Inflammatory and Innate Immune Responses in Dengue Infection: Protection versus disease induction. *Am. J. Pathol.* **2013**, *182*, 1950–1961. [CrossRef]
43. Su, Y.; Huang, Y.; Wu, Y.; Chen, H.; Hsu, C.; Hsu, Y.; Lee, J. MicroRNA-155 inhibits dengue virus replication by inducing heme oxygenase-1-mediated antiviral interferon responses. *FASEB J.* **2020**, *34*, 7283–7294. [CrossRef]
44. Hayes, E.B.; Komar, N.; Nasci, R.S.; Montgomery, S.P.; O'Leary, D.R.; Campbell, G.L. Epidemiology and Transmission Dynamics of West Nile Virus Disease. *Emerg. Infect. Dis.* **2005**, *11*, 1167–1173. [CrossRef]
45. Kumar, M.; Nerurkar, V.R. Integrated analysis of microRNAs and their disease related targets in the brain of mice infected with West Nile virus. *Virology* **2014**, *452–453*, 143–151. [CrossRef] [PubMed]
46. Park, K.W.; Baik, H.H.; Jin, B.K. IL-13-Induced Oxidative Stress via Microglial NADPH Oxidase Contributes to Death of Hippocampal Neurons In Vivo. *J. Immunol.* **2009**, *183*, 4666–4674. [CrossRef] [PubMed]
47. Natekar, J.P.; Rothan, H.A.; Arora, K.; Strate, P.G.; Kumar, M. Cellular microRNA-155 Regulates Virus-Induced Inflammatory Response and Protects against Lethal West Nile Virus Infection. *Viruses* **2019**, *12*, 9. [CrossRef] [PubMed]

48. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_9th_report/negative-sense-rna-viruses-2011/w/negrna_viruses/209/orthomyxoviridae (accessed on 19 July 2022).
49. Herold, S.; Becker, C.; Ridge, K.M.; Budinger, G.S. Influenza virus-induced lung injury: Pathogenesis and implications for treatment. *Eur. Respir. J.* **2015**, *45*, 1463–1478. [CrossRef] [PubMed]
50. Short, K.R.; Kroeze, E.J.B.V.; Fouchier, R.A.M.; Kuiken, T. Pathogenesis of influenza-induced acute respiratory distress syndrome. *Lancet Infect. Dis.* **2014**, *14*, 57–69. [CrossRef]
51. Shen, S.-M.; Jiang, H.; Zhao, J.-N.; Shi, Y. Down-regulation of miR-155 inhibits inflammatory response in human pulmonary microvascular endothelial cells infected with influenza A virus by targeting sphingosine-1-phosphate receptor. *Chin. Med. J.* **2020**, *133*, 2429–2436. [CrossRef]
52. Izzard, L.; Dlugolenski, D.; Xia, Y.; McMahon, M.; Middleton, D.; Tripp, R.A.; Stambas, J. Enhanced immunogenicity following miR-155 incorporation into the influenza A virus genome. *Virus Res.* **2017**, *235*, 115–120. [CrossRef]
53. Gracias, D.T.; Stelekati, E.; Hope, J.L.; Boesteanu, A.C.; Doering, T.A.; Norton, J.; Mueller, Y.M.; Fraietta, J.A.; Wherry, E.J.; Turner, M.; et al. The microRNA miR-155 controls CD8+ T cell responses by regulating interferon signaling. *Nat. Immunol.* **2013**, *14*, 593–602. [CrossRef]
54. Pociask, D.A.; Robinson, K.M.; Chen, K.; McHugh, K.J.; Clay, M.; Huang, G.T.; Benos, P.V.; Janssen-Heininger, Y.M.; Kolls, J.K.; Anathy, V.; et al. Epigenetic and Transcriptomic Regulation of Lung Repair during Recovery from Influenza Infection. *Am. J. Pathol.* **2017**, *187*, 851–863. [CrossRef]
55. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-rna-viruses/w/pneumoviridae (accessed on 19 July 2022).
56. Collins, P.L.; Murphy, B.R. Respiratory Syncytial Virus: Reverse Genetics and Vaccine Strategies. *Virology* **2002**, *296*, 204–211. [CrossRef]
57. Arroyo, M.; Salka, K.; Chorvinsky, E.; Xuchen, X.; Abutaleb, K.; Perez, G.F.; Weinstock, J.; Gaviria, S.; Gutierrez, M.J.; Nino, G. Airway mir-155 responses are associated with TH1 cytokine polarization in young children with viral respiratory infections. *PLoS ONE* **2020**, *15*, e0233352. [CrossRef] [PubMed]
58. Inchley, C.S.; Sonerud, T.; Fjærli, H.O.; Nakstad, B. Nasal mucosal microRNA expression in children with respiratory syncytial virus infection. *BMC Infect. Dis.* **2015**, *15*, 150. [CrossRef] [PubMed]
59. Xu, D.G.; Chen, Q.; Wang, R.; Xiong, T.T.; Yang, L.X. Relationship between the expression of miR-146a and miR-155 in peripheral blood mononuclear cells and inflammation response in infants with respiratory syncytial virus pneumonia. *Chin. J. Child Health Care* **2020**, *28*, 316–319. [CrossRef]
60. Oshansky, C.M.; Zhang, W.; Moore, E.; Tripp, R.A. The host response and molecular pathogenesis associated with respiratory syncytial virus infection. *Futur. Microbiol.* **2009**, *4*, 279–297. [CrossRef] [PubMed]
61. O'Connell, R.M.; Chaudhuri, A.A.; Rao, D.S.; Baltimore, D. Inositol phosphatase SHIP1 is a primary target of miR-155. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 7113–7118. [CrossRef]
62. Lu, C.; Huang, X.; Zhang, X.; Roensch, K.; Cao, Q.; Nakayama, K.I.; Blazar, B.R.; Zeng, Y.; Zhou, X. miR-221 and miR-155 regulate human dendritic cell development, apoptosis, and IL-12 production through targeting of $p27^{kip1}$, *KPC1*, and *SOCS-1*. *Blood* **2011**, *117*, 4293–4303. [CrossRef]
63. Wang, S.; Ling, Y.; Yao, Y.; Zheng, G.; Chen, W. Luteolin inhibits respiratory syncytial virus replication by regulating the MiR-155/SOCS1/STAT1 signaling pathway. *Virol. J.* **2020**, *17*, 187. [CrossRef]
64. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.* **2020**, *5*, 536–544. [CrossRef]
65. Zhou, F.; Yu, T.; Du, R.; Fan, G.; Liu, Y.; Liu, Z.; Xiang, J.; Wang, Y.; Song, B.; Gu, X.; et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet* **2020**, *395*, 1054–1062. [CrossRef]
66. Jafarzadeh, A.; Nemati, M.; Saha, B.; Bansode, Y.D.; Jafarzadeh, S. Protective Potentials of Type III Interferons in COVID-19 Patients: Lessons from Differential Properties of Type I- and III Interferons. *Viral Immunol.* **2021**, *34*, 307–320. [CrossRef]
67. Jafarzadeh, A.; Chauhan, P.; Saha, B.; Jafarzadeh, S.; Nemati, M. Contribution of monocytes and macrophages to the local tissue inflammation and cytokine storm in COVID-19: Lessons from SARS and MERS, and potential therapeutic interventions. *Life Sci.* **2020**, *257*, 118102. [CrossRef] [PubMed]
68. Jafarzadeh, A.; Nemati, M.; Jafarzadeh, S. Contribution of STAT3 to the pathogenesis of COVID-19. *Microb. Pathog.* **2021**, *154*, 104836. [CrossRef] [PubMed]
69. Haroun, R.A.-H.; Osman, W.H.; Amin, R.E.; Hassan, A.K.; Abo-Shanab, W.S.; Eessa, A.M. Circulating plasma miR-155 is a potential biomarker for the detection of SARS-CoV-2 infection. *Pathology* **2022**, *54*, 104–110. [CrossRef] [PubMed]
70. Demirci, Y.M.; Demirci, M.D.S. Circular RNA–MicroRNA–MRNA interaction predictions in SARS-CoV-2 infection. *J. Integr. Bioinform.* **2021**, *18*, 45–50. [CrossRef]
71. Donyavi, T.; Bokharaei-Salim, F.; Baghi, H.B.; Khanaliha, K.; Janat-Makan, M.A.; Karimi, B.; Nahand, J.S.; Mirzaei, H.; Khatami, A.; Garshasbi, S.; et al. Acute and post-acute phase of COVID-19: Analyzing expression patterns of miRNA-29a-3p, 146a-3p, 155–5p, and let-7b-3p in PBMC. *Int. Immunopharmacol.* **2021**, *97*, 107641. [CrossRef]
72. Soni, D.K.; Cabrera-Luque, J.; Kar, S.; Sen, C.; Devaney, J.; Biswas, R. Suppression of miR-155 attenuates lung cytokine storm induced by SARS-CoV-2 infection in human ACE2-transgenic mice. *bioRxiv* **2020**. [CrossRef]

73. Wyler, E.; Mösbauer, K.; Franke, V.; Diag, A.; Gottula, L.T.; Arsiè, R.; Klironomos, F.; Koppstein, D.; Hönzke, K.; Ayoub, S.; et al. Transcriptomic profiling of SARS-CoV-2 infected human cell lines identifies HSP90 as target for COVID-19 therapy. *iScience* **2021**, *24*, 102151. [CrossRef]
74. Chow, J.; Salmena, L. Prediction and Analysis of SARS-CoV-2-Targeting MicroRNA in Human Lung Epithelium. *Genes* **2020**, *11*, 1002. [CrossRef]
75. Qi, M.; Liu, B.; Li, S.; Ni, Z.; Li, F. Construction and Investigation of Competing Endogenous RNA Networks and Candidate Genes Involved in SARS-CoV-2 Infection. *Int. J. Gen. Med.* **2021**, *14*, 6647–6659. [CrossRef]
76. Garg, A.; Seeliger, B.; Derda, A.A.; Xiao, K.; Gietz, A.; Scherf, K.; Sonnenschein, K.; Pink, I.; Hoeper, M.M.; Welte, T.; et al. Circulating cardiovascular microRNAs in critically ill COVID-19 patients. *Eur. J. Heart Fail.* **2021**, *23*, 468–475. [CrossRef]
77. Benkő, M.; Aoki, K.; Arnberg, N.; Davison, A.J.; Echavarría, M.; Hess, M.; Jones, M.S.; Kaján, G.L.; Kajon, A.E.; Mittal, S.K.; et al. ICTV Virus Taxonomy Profile: Adenoviridae. *J. Gen. Virol.* **2022**, *103*, 001721. [CrossRef]
78. Kidd, A.H.; Garwicz, D.; Oberg, M. Human and Simian Adenoviruses: Phylogenetic Inferences from Analysis of VA RNA Genes. *Virology* **1995**, *207*, 32–45. [CrossRef]
79. Lynch, J.P., 3rd; Kajon, A.E. Adenovirus: Epidemiology, Global Spread of Novel Serotypes, and Advances in Treatment and Prevention. *Semin. Respir. Crit. Care Med.* **2016**, *37*, 586–602. [CrossRef] [PubMed]
80. Zhao, H.; Chen, M.; Tellgren-Roth, C.; Pettersson, U. Fluctuating expression of microRNAs in adenovirus infected cells. *Virology* **2015**, *478*, 99–111. [CrossRef] [PubMed]
81. O'Connell, R.M.; Taganov, K.D.; Boldin, M.P.; Cheng, G.; Baltimore, D. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 1604–1609. [CrossRef] [PubMed]
82. Wang, P.; Hou, J.; Lin, L.; Wang, C.; Liu, X.; Li, D.; Ma, F.; Wang, Z.; Cao, X. Inducible microRNA-155 Feedback Promotes Type I IFN Signaling in Antiviral Innate Immunity by Targeting Suppressor of Cytokine Signaling. *J. Immunol.* **2010**, *185*, 6226–6233. [CrossRef]
83. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/picornaviridae (accessed on 19 July 2022).
84. Bondanese, V.P.; Francisco-Garcia, A.; Bedke, N.; Davies, D.E.; Sanchez-Elsner, T. Identification of host miRNAs that may limit human rhinovirus replication. *World J. Biol. Chem.* **2014**, *5*, 437–456. [CrossRef]
85. Gutierrez, M.J.; Gómez, J.L.; Perez, G.F.; Pancham, K.; Val, S.; Pillai, D.K.; Giri, M.; Ferrante, S.; Freishtat, R.; Rose, M.C.; et al. Airway Secretory microRNAome Changes during Rhinovirus Infection in Early Childhood. *PLoS ONE* **2016**, *11*, e0162244. [CrossRef]
86. Taïbi, F.; Meuth, V.M.-L.; Massy, Z.A.; Metzinger, L. miR-223: An inflammatory oncomiR enters the cardiovascular field. *Biochim. Biophys. Acta* **2014**, *1842*, 1001–1009. [CrossRef]
87. Jiao, P.; Wang, X.-P.; Luoreng, Z.-M.; Yang, J.; Jia, L.; Ma, Y.; Wei, D.-W. miR-223: An Effective Regulator of Immune Cell Differentiation and Inflammation. *Int. J. Biol. Sci.* **2021**, *17*, 2308–2322. [CrossRef]
88. Yuan, S.; Wu, Q.; Wang, Z.; Che, Y.; Zheng, S.; Chen, Y.; Zhong, X.; Shi, F. miR-223: An Immune Regulator in Infectious Disorders. *Front. Immunol.* **2021**, *12*, 781815. [CrossRef] [PubMed]
89. Xu, J.; Wu, C.; Che, X.; Wang, L.; Yu, D.; Zhang, T.; Huang, L.; Li, H.; Tan, W.; Wang, C.; et al. Circulating MicroRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Mol. Carcinog.* **2011**, *50*, 136–142. [CrossRef] [PubMed]
90. Wong, Q.W.; Lung, R.W.; Law, P.T.; Lai, P.B.-S.; Chan, K.Y.Y.; To, K.; Wong, N. MicroRNA-223 Is Commonly Repressed in Hepatocellular Carcinoma and Potentiates Expression of Stathmin1. *Gastroenterology* **2008**, *135*, 257–269. [CrossRef] [PubMed]
91. Giray, B.G.; Emekdas, G.; Tezcan, S.; Ulger, M.; Serin, M.S.; Sezgin, O.; Altintas, E.; Tiftik, E.N. Profiles of serum microRNAs; miR-125b-5p and miR223-3p serve as novel biomarkers for HBV-positive hepatocellular carcinoma. *Mol. Biol. Rep.* **2014**, *41*, 4513–4519. [CrossRef]
92. Yu, G.; Chen, X.; Chen, S.; Ye, W.; Hou, K.; Liang, M. MiR-19a, miR-122 and miR-223 are differentially regulated by hepatitis B virus X protein and involve in cell proliferation in hepatoma cells. *J. Transl. Med.* **2016**, *14*, 122. [CrossRef] [PubMed]
93. Hyrina, A.; Olmstead, A.D.; Steven, P.; Krajden, M.; Tam, E.; Jean, F. Treatment-Induced Viral Cure of Hepatitis C Virus-Infected Patients Involves a Dynamic Interplay among three Important Molecular Players in Lipid Homeostasis: Circulating microRNA (miR)-24, miR-223, and Proprotein Convertase Subtilisin/Kexin Type 9. *eBioMedicine* **2017**, *23*, 68–78. [CrossRef]
94. El-Guendy, N.M.; Helwa, R.; El-Halawany, M.S.; Ali, S.A.R.; Aly, M.T.; Alieldin, N.H.; Fouad, S.A.H.; Saeid, H.; Abdel-Wahab, A.-H.A. The Liver MicroRNA Expression Profiles Associated With Chronic Hepatitis C Virus (HCV) Genotype-4 Infection: A Preliminary Study. *Hepat. Mon.* **2016**, *16*, e33881. [CrossRef]
95. Wu, N.; Gao, N.; Fan, D.; Wei, J.; Zhang, J.; An, J. miR-223 inhibits dengue virus replication by negatively regulating the microtubule-destabilizing protein STMN1 in EAhy926 cells. *Microbes Infect.* **2014**, *16*, 911–922. [CrossRef]
96. Li, Y.; Chan, E.Y.; Li, J.; Ni, C.; Peng, X.; Rosenzweig, E.; Tumpey, T.M.; Katze, M.G. MicroRNA Expression and Virulence in Pandemic Influenza Virus-Infected Mice. *J. Virol.* **2010**, *84*, 3023–3032. [CrossRef]
97. Vela, E.M.; Kasoji, M.D.; Wendling, M.Q.; Price, J.A.; Knostman, K.A.B.; Bresler, H.S.; Long, J.P. MicroRNA expression in mice infected with seasonal H1N1, swine H1N1 or highly pathogenic H5N. *J. Med Microbiol.* **2014**, *63*, 1131–1142. [CrossRef]
98. Wu, Z.; Hao, R.; Li, P.; Zhang, X.; Liu, N.; Qiu, S.; Wang, L.; Wang, Y.; Xue, W.; Liu, K.; et al. MicroRNA Expression Profile of Mouse Lung Infected with 2009 Pandemic H1N1 Influenza Virus. *PLoS ONE* **2013**, *8*, e74190. [CrossRef] [PubMed]

99. Tambyah, P.A.; Sepramaniam, S.; Ali, J.M.; Chai, S.C.; Swaminathan, P.; Armugam, A.; Jeyaseelan, K. microRNAs in Circulation Are Altered in Response to Influenza A Virus Infection in Humans. *PLoS ONE* **2013**, *8*, e76811. [CrossRef] [PubMed]
100. Choi, E.-J.; Kim, H.B.; Baek, Y.H.; Kim, E.-H.; Pascua, P.N.Q.; Park, S.-J.; Kwon, H.-I.; Lim, G.-J.; Kim, S.; Kim, Y.-I.; et al. Differential microRNA expression following infection with a mouse-adapted, highly virulent avian H5N2 virus. *BMC Microbiol.* **2014**, *14*, 252. [CrossRef] [PubMed]
101. Moschos, S.A.; Williams, A.E.; Perry, M.M.; Birrell, M.A.; Belvisi, M.G.; Lindsay, M.A. Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids. *BMC Genom.* **2007**, *8*, 240. [CrossRef] [PubMed]
102. Liu, C.-C.; Miao, Y.; Chen, R.-L.; Zhang, Y.-Q.; Wu, H.; Yang, S.-M.; Shang, L.-Q. STIM1 mediates IAV-induced inflammation of lung epithelial cells by regulating NLRP3 and inflammasome activation via targeting miR-223. *Life Sci.* **2021**, *266*, 118845. [CrossRef] [PubMed]
103. Micaroni, M.; Stanley, A.C.; Khromykh, T.; Venturato, J.; Wong, C.X.F.; Lim, J.P.; Marsh, B.J.; Storrie, B.; Gleeson, P.A.; Stow, J.L. Rab6a/a' Are Important Golgi Regulators of Pro-Inflammatory TNF Secretion in Macrophages. *PLoS ONE* **2013**, *8*, e57034. [CrossRef] [PubMed]
104. Song, Z.; Xu, Y.; Bao, L.; Zhang, L.; Yu, P.; Qu, Y.; Zhu, H.; Zhao, W.; Han, Y.; Qin, C. From SARS to MERS, Thrusting Coronaviruses into the Spotlight. *Viruses* **2019**, *11*, 59. [CrossRef]
105. Mallick, B.; Ghosh, Z.; Chakrabarti, J. MicroRNome Analysis Unravels the Molecular Basis of SARS Infection in Bronchoalveolar Stem Cells. *PLoS ONE* **2009**, *4*, e7837. [CrossRef]
106. Wang, Y.; Zhu, X.; Jiang, X.-M.; Guo, J.; Fu, Z.; Zhou, Z.; Yang, P.; Guo, H.; Guo, X.; Liang, G.; et al. Decreased inhibition of exosomal miRNAs on SARS-CoV-2 replication underlies poor outcomes in elderly people and diabetic patients. *Signal Transduct. Target. Ther.* **2021**, *6*, 300. [CrossRef]
107. Demiray, A.; Sarı, T.; Çalışkan, A.; Nar, R.; Aksoy, L.; Akbubak, I.H. Serum microRNA signature is capable of predictive and prognostic factor for SARS-COV-2 virulence. *Turk. J. Biochem.* **2021**, *46*, 245–253. [CrossRef]
108. Houshmandfar, S.; Saeedi-Boroujeni, A.; Rashno, M.; Khodadadi, A.; Mahmoudian-Sani, M.-R. miRNA-223 as a regulator of inflammation and NLRP3 inflammasome, the main fragments in the puzzle of immunopathogenesis of different inflammatory diseases and COVID-19. *Naunyn-Schmiedebergs Arch. Pharmakol.* **2021**, *394*, 2187–2195. [CrossRef] [PubMed]
109. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/reverse-transcribing-dna-and-rna-viruses/w/retroviridae (accessed on 19 July 2022).
110. Huang, J.; Wang, F.; Argyris, E.; Chen, K.; Liang, Z.; Tian, H.; Huang, W.; Squires, K.; Verlinghieri, G.; Zhang, H. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. *Nat. Med.* **2007**, *13*, 1241–1247. [CrossRef] [PubMed]
111. Purohit, V.; Rapaka, R.S.; Rutter, J.; Shurtleff, D. Do Opioids Activate Latent HIV-1 by Down-Regulating Anti-HIV microRNAs? *J. Neuroimmune Pharmacol.* **2012**, *7*, 519–523. [CrossRef]
112. Sun, G.; Li, H.; Wu, X.; Covarrubias, M.; Scherer, L.; Meinking, K.; Luk, B.; Chomchan, P.; Alluin, J.; Gombart, A.F.; et al. Interplay between HIV-1 infection and host microRNAs. *Nucleic Acids Res.* **2012**, *40*, 2181–2196. [CrossRef]
113. Monticelli, S.; Ansel, K.M.; Xiao, C.; Socci, N.D.; Krichevsky, A.M.; Thai, T.-H.; Rajewsky, N.; Marks, D.S.; Sander, C.; Rajewsky, K.; et al. MicroRNA profiling of the murine hematopoietic system. *Genome Biol.* **2005**, *6*, R71. [CrossRef] [PubMed]
114. Taganov, K.D.; Boldin, M.P.; Chang, K.-J.; Baltimore, D. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12481–12486. [CrossRef] [PubMed]
115. Curtale, G.; Citarella, F.; Carissimi, C.; Goldoni, M.; Carucci, N.; Fulci, V.; Franceschini, D.; Meloni, F.; Barnaba, V.; Macino, G. An emerging player in the adaptive immune response: MicroRNA-146a is a modulator of IL-2 expression and activation-induced cell death in T lymphocytes. *Blood* **2010**, *115*, 265–273. [CrossRef]
116. Hou, J.; Wang, P.; Lin, L.; Liu, X.; Ma, F.; An, H.; Wang, Z.; Cao, X. MicroRNA-146a Feedback Inhibits RIG-I-Dependent Type I IFN Production in Macrophages by Targeting TRAF6, IRAK1, and IRAK2. *J. Immunol.* **2009**, *183*, 2150–2158. [CrossRef]
117. Wang, S.; Zhang, X.; Ju, Y.; Zhao, B.; Yan, X.; Hu, J.; Shi, L.; Yang, L.; Ma, Z.; Chen, L.; et al. MicroRNA-146a Feedback Suppresses T Cell Immune Function by Targeting Stat1 in Patients with Chronic Hepatitis B. *J. Immunol.* **2013**, *191*, 293–301. [CrossRef]
118. Fu, L.; Fu, X.; Mo, J.; Li, X.; Li, R.; Peng, S. miR-146a-5p enhances hepatitis B virus replication through autophagy to promote aggravation of chronic hepatitis B. *IUBMB Life* **2019**, *71*, 1336–1346. [CrossRef]
119. Wang, Y.; Li, Y. miR-146 promotes HBV replication and expression by targeting ZEB2. *Biomed. Pharmacother.* **2018**, *99*, 576–582. [CrossRef]
120. Ji, M.; Mei, X.; Jing, X.; Xu, X.; Chen, X.; Pan, W. The cooperative complex of Argonaute-2 and microRNA-146a regulates hepatitis B virus replication through flap endonuclease. *Life Sci.* **2020**, *257*, 118089. [CrossRef] [PubMed]
121. Li, J.-F.; Dai, X.-P.; Zhang, W.; Sun, S.-H.; Zeng, Y.; Zhao, G.-Y.; Kou, Z.-H.; Guo, Y.; Yu, H.; Du, L.-Y.; et al. Upregulation of MicroRNA-146a by Hepatitis B Virus X Protein Contributes to Hepatitis Development by Downregulating Complement Factor H. *mBio* **2015**, *6*, e02459–e002473. [CrossRef] [PubMed]
122. Jiang, H.; He, X.; Li, J.; Xie, Q.; Lin, J.; Chang, Y. Association of a single-nucleotide polymorphism within the miR-146a gene with susceptibility for acute-on-chronic hepatitis B liver failure. *Immunogenetics* **2013**, *65*, 257–263. [CrossRef]
123. He, Q.; Li, W.; Ren, J.; Huang, Y.; Huang, Y.; Hu, Q.; Chen, J.; Chen, W. ZEB2 inhibits HBV transcription and replication by targeting its core promoter. *Oncotarget* **2016**, *7*, 16003–16011. [CrossRef] [PubMed]

124. Pan, W.Y.; Ge, X.; Shan, X.; Lei, X.; Ke, C.; Huang, A.; Hu, J. Effect of structure-specific nucleic acid enzyme FEN1 on HBV replication. *Third. Mil. Med. Univ.* **2012**, *34*, 1935–1938.
125. Ren, J.P.; Ying, R.S.; Cheng, Y.Q.; Wang, L.; Elgazzar, M.A.; Li, G.Y.; Ning, S.B.; Moorman, J.P.; Yao, Z.Q. HCV-induced miR146a controls SOCS1/STAT3 and cytokine expression in monocytes to promote regulatory T-cell development. *J. Viral Hepat.* **2016**, *23*, 755–766. [CrossRef]
126. Zhang, W.-J.; Wang, H.; Tong, Q.-X.; Jie, S.-H.; Yang, D.-L.; Peng, C. Involvement of TLR2-MyD88 in abnormal expression of miR-146a in peripheral blood monocytes of patients with chronic hepatitis C. *J. Huazhong Univ. Sci. Technol.* **2015**, *35*, 219–224. [CrossRef]
127. Bandiera, S.; Pernot, S.; El Saghire, H.; Durand, S.C.; Thumann, C.; Crouchet, E.; Ye, T.; Fofana, I.; Oudot, M.A.; Barths, J.; et al. Hepatitis C Virus-Induced Upregulation of MicroRNA miR-146a-5p in Hepatocytes Promotes Viral Infection and Deregulates Metabolic Pathways Associated with Liver Disease Pathogenesis. *J. Virol.* **2016**, *90*, 6387–6400. [CrossRef]
128. Srikiatkhachorn, A.; Mathew, A.; Rothman, A.L. Immune-mediated cytokine storm and its role in severe dengue. *Semin. Immunopathol.* **2017**, *39*, 563–574. [CrossRef]
129. Wu, S.; He, L.; Li, Y.; Wang, T.; Feng, L.; Jiang, L.; Zhang, P.; Huang, X. miR-146a facilitates replication of dengue virus by dampening interferon induction by targeting TRAF. *J. Infect.* **2013**, *67*, 329–341. [CrossRef]
130. Pu, J.; Wu, S.; Xie, H.; Li, Y.; Yang, Z.; Wu, X.; Huang, X. miR-146a Inhibits dengue-virus-induced autophagy by targeting TRAF6. *Arch. Virol.* **2017**, *162*, 3645–3659. [CrossRef] [PubMed]
131. de Oliveira, L.F.; de Andrade, A.A.S.; Pagliari, C.; de Carvalho, L.V.; Silveira, T.S.; Cardoso, J.F.; e Silva, A.L.T.; de Vasconcelos, J.M.; Moreira-Nunes, C.A.; Burbano, R.M.R.; et al. Differential expression analysis and profiling of hepatic miRNA and isomiRNA in dengue hemorrhagic fever. *Sci. Rep.* **2021**, *11*, 5554. [CrossRef]
132. Ouyang, X.; Jiang, X.; Gu, D.; Zhang, Y.; Kong, S.K.; Jiang, C.; Xie, W. Dysregulated Serum MiRNA Profile and Promising Biomarkers in Dengue-infected Patients. *Int. J. Med. Sci.* **2016**, *13*, 195–205. [CrossRef]
133. Zhang, F.; Sun, X.; Zhu, Y.; Qin, W. Downregulation of miR-146a inhibits influenza A virus replication by enhancing the type I interferon response in vitro and in vivo. *Biomed. Pharmacother.* **2019**, *111*, 740–750. [CrossRef] [PubMed]
134. Buggele, W.A.; Johnson, K.E.; Horvath, C.M. Influenza A Virus Infection of Human Respiratory Cells Induces Primary MicroRNA Expression. *J. Biol. Chem.* **2012**, *287*, 31027–31040. [CrossRef]
135. Terrier, O.; Textoris, J.; Carron, C.; Marcel, V.; Bourdon, J.-C.; Rosa-Calatrava, M. Host microRNA molecular signatures associated with human H1N1 and H3N2 influenza A viruses reveal an unanticipated antiviral activity for miR-146a. *J. Gen. Virol.* **2013**, *94*, 985–995. [CrossRef]
136. Deng, Y.; Yan, Y.; Tan, K.S.; Liu, J.; Chow, V.T.; Tao, Z.-Z.; Wang, D.-Y. MicroRNA-146a induction during influenza H3N2 virus infection targets and regulates TRAF6 levels in human nasal epithelial cells (hNECs). *Exp. Cell Res.* **2017**, *352*, 184–192. [CrossRef]
137. Eilam-Frenkel, B.; Naaman, H.; Brkic, G.; Veksler-Lublinsky, I.; Rall, G.; Shemer-Avni, Y.; Gopas, J. MicroRNA 146-5p, miR-let-7c-5p, miR-221 and miR-345-5p are differentially expressed in Respiratory Syncytial Virus (RSV) persistently infected HEp-2 cells. *Virus Res.* **2018**, *251*, 34–39. [CrossRef]
138. Lubkowska, A.; Pluta, W.; Strońska, A.; Lalko, A. Role of Heat Shock Proteins (HSP70 and HSP90) in Viral Infection. *Int. J. Mol. Sci.* **2021**, *22*, 9366. [CrossRef]
139. Tang, H.; Gao, Y.; Li, Z.; Miao, Y.; Huang, Z.; Liu, X.; Xie, L.; Li, H.; Wen, W.; Zheng, Y.; et al. The noncoding and coding transcriptional landscape of the peripheral immune response in patients with COVID-19. *Clin. Transl. Med.* **2020**, *10*, e200. [CrossRef] [PubMed]
140. Sabbatinelli, J.; Giuliani, A.; Matacchione, G.; Latini, S.; Laprovitera, N.; Pomponio, G.; Ferrarini, A.; Baroni, S.S.; Pavani, M.; Moretti, M.; et al. Decreased serum levels of the inflammaging marker miR-146a are associated with clinical non-response to tocilizumab in COVID-19 patients. *Mech. Ageing Dev.* **2021**, *193*, 111413. [CrossRef] [PubMed]
141. Roganović, J. Downregulation of microRNA-146a in diabetes, obesity and hypertension may contribute to severe COVID-19. *Med Hypotheses* **2021**, *146*, 110448. [CrossRef]
142. Roganović, J.R. microRNA-146a and -155, upregulated by periodontitis and type 2 diabetes in oral fluids, are predicted to regulate SARS-CoV-2 oral receptor genes. *J. Periodontol.* **2021**, *92*, e35–e43. [CrossRef] [PubMed]
143. Lagos-Quintana, M.; Rauhut, R.; Yalcin, A.; Meyer, J.; Lendeckel, W.; Tuschl, T. Identification of Tissue-Specific MicroRNAs from Mouse. *Curr. Biol.* **2002**, *12*, 735–739. [CrossRef]
144. Girard, M.; Jacquemin, E.; Munnich, A.; Lyonnet, S.; Henrion-Caude, A. miR-122, a paradigm for the role of microRNAs in the liver. *J. Hepatol.* **2008**, *48*, 648–656. [CrossRef]
145. Xu, H.; He, J.-H.; Xiao, Z.-D.; Zhang, Q.-Q.; Chen, Y.-Q.; Zhou, H.; Qu, L.-H. Liver-enriched transcription factors regulate MicroRNA-122 that targets CUTL1 during liver development. *Hepatology* **2010**, *52*, 1431–1442. [CrossRef]
146. Laudadio, I.; Manfroid, I.; Achouri, Y.; Schmidt, D.; Wilson, M.D.; Cordi, S.; Thorrez, L.; Knoops, L.; Jacquemin, P.; Schuit, F.; et al. A Feedback Loop Between the Liver-Enriched Transcription Factor Network and Mir-122 Controls Hepatocyte Differentiation. *Gastroenterology* **2012**, *142*, 119–129. [CrossRef]
147. Bandiera, S.; Pfeffer, S.; Baumert, T.F.; Zeisel, M.B. miR-122—A key factor and therapeutic target in liver disease. *J. Hepatol.* **2015**, *62*, 448–457. [CrossRef]

148. Arataki, K.; Hayes, C.N.; Akamatsu, S.; Akiyama, R.; Abe, H.; Tsuge, M.; Miki, D.; Ochi, H.; Hiraga, N.; Imamura, M.; et al. Circulating microRNA-22 correlates with microRNA-122 and represents viral replication and liver injury in patients with chronic hepatitis B. *J. Med Virol.* **2013**, *85*, 789–798. [CrossRef]
149. Wang, S.; Qiu, L.; Yan, X.; Jin, W.; Wang, Y.; Chen, L.; Wu, E.; Ye, X.; Gao, G.F.; Wang, F.; et al. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G1-modulated P53 activity. *Hepatology* **2012**, *55*, 730–741. [CrossRef] [PubMed]
150. Wu, Y.; Gao, C.; Cai, S.; Xia, M.; Liao, G.; Zhang, X.; Peng, J. Circulating miR-122 Is a Predictor for Virological Response in CHB Patients with High Viral Load Treated with Nucleos(t)ide Analogs. *Front. Genet.* **2019**, *10*, 243. [CrossRef] [PubMed]
151. Ebrahimifard, M.; Zandi, M.; Moradi, A. Evaluation of miR-122 levels in chronic HBV and liver cirrhosis patients. *Arch. Med. Lab. Sci.* **2013**, *2*, 3.
152. Van der Ree, M.H.; Jansen, L.; Kruize, Z.; van Nuenen, A.C.; van Dort, K.A.; Takkenberg, R.B.; Reesink, H.W.; Kootstra, N.A. Plasma MicroRNA Levels Are Associated with Hepatitis B e Antigen Status and Treatment Response in Chronic Hepatitis B Patients. *J. Infect. Dis.* **2017**, *215*, 1421–1429. [CrossRef]
153. Chen, S.; Ni, M.; Yu, B.; Lv, T.; Lu, M.; Gong, F. Construction and identification of a human liver specific microRNA eukaryotic expression vector. *Cell. Mol. Immunol.* **2007**, *4*, 473–477.
154. Chen, Y.; Shen, A.; Rider, P.J.; Yu, Y.; Wu, K.; Mu, Y.; Hao, Q.; Liu, Y.; Gong, H.; Zhu, Y.; et al. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. *FASEB J.* **2011**, *25*, 4511–4521. [CrossRef]
155. Li, R.-F.; Fan, C.-G.; Wang, C.-M.; Tian, C.; Wang, Y.; Li, L.; Sun, W.-S.; Liu, Y.-G. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. *Oncol. Rep.* **2011**, *26*, 1281–1286. [CrossRef]
156. Wu, D.-B.; Liu, F.-W.; Li, J.; Liu, C.; Liu, L.; Chen, E.-Q.; Zhao, L.-S.; Tang, H.; Zhou, T.-Y. Intrahepatic IFN-alpha expression in liver specimens from HBV-infected patients with different outcomes. *Eur. Rev. Med Pharmacol. Sci.* **2013**, *17*, 2474–2480.
157. Koeberlein, B.; Hausen, A.Z.; Bektas, N.; Zentgraf, H.; Chin, R.; Toan, N.L.; Kandolf, R.; Torresi, J.; Bock, C.T. Hepatitis B virus overexpresses suppressor of cytokine signaling-3 (SOCS3) thereby contributing to severity of inflammation in the liver. *Virus Res.* **2010**, *148*, 51–59. [CrossRef]
158. Li, Z.-Y.; Xi, Y.; Zhu, W.-N.; Zeng, C.; Zhang, Z.-Q.; Guo, Z.-C.; Hao, D.-L.; Liu, G.; Feng, L.; Chen, H.-Z.; et al. Positive regulation of hepatic miR-122 expression by HNF4α. *J. Hepatol.* **2011**, *55*, 602–611. [CrossRef]
159. Song, K.; Han, C.; Dash, S.; Balart, L.A.; Wu, T. MiR-122 in hepatitis B virus and hepatitis C virus dual infection. *World J. Hepatol.* **2015**, *7*, 498–506. [CrossRef] [PubMed]
160. Li, A.; Wu, J.; Zhai, A.; Qian, J.; Wang, X.; Qaria, M.A.; Zhang, Q.; Li, Y.; Fang, Y.; Kao, W.; et al. HBV triggers APOBEC2 expression through miR-122 regulation and affects the proliferation of liver cancer cells. *Int. J. Oncol.* **2019**, *55*, 1137–1148. [CrossRef] [PubMed]
161. Song, K.; Han, C.; Zhang, J.; Lu, N.; Dash, S.; Feitelson, M.; Lim, K.; Wu, T. Epigenetic regulation of MicroRNA-122 by peroxisome proliferator activated receptor-gamma and hepatitis b virus X protein in hepatocellular carcinoma cells. *Hepatology* **2013**, *58*, 1681–1692. [CrossRef] [PubMed]
162. Peng, F.; Xiao, X.; Jiang, Y.; Luo, K.; Tian, Y.; Peng, M.; Zhang, M.; Xu, Y.; Gong, G. HBx Down-Regulated Gld2 Plays a Critical Role in HBV-Related Dysregulation of miR-122. *PLoS ONE* **2014**, *9*, e92998. [CrossRef]
163. Ding, J.; Ding, X.; Ning, J.; Yi, F.; Chen, J.; Zhao, D.; Zheng, J.; Liang, Z.; Hu, Z.; Du, Q. Circulating microRNA-122 as a potential biomarker for liver injury. *Mol. Med. Rep.* **2012**, *5*, 1428–1432. [CrossRef] [PubMed]
164. Zhou, X.; Fang, S.; Wang, M.; Xiong, A.; Zheng, C.; Wang, J.; Yin, C. Diagnostic value of circulating miRNA-122 for hepatitis B virus and/or hepatitis C virus-associated chronic viral hepatitis. *Biosci. Rep.* **2019**, *39*, BSR20190900. [CrossRef]
165. Shimakami, T.; Yamane, D.; Jangra, R.K.; Kempf, B.J.; Spaniel, C.; Barton, D.J.; Lemon, S.M. Stabilization of hepatitis C virus RNA by an Ago2–miR-122 complex. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 941–946. [CrossRef]
166. Jopling, C.L.; Yi, M.; Lancaster, A.M.; Lemon, S.M.; Sarnow, P. Modulation of Hepatitis C Virus RNA Abundance by a Liver-Specific MicroRNA. *Science* **2005**, *309*, 1577–1581. [CrossRef]
167. Chang, J.; Guo, J.-T.; Jiang, D.; Guo, H.; Taylor, J.M.; Block, T.M. Liver-Specific MicroRNA miR-122 Enhances the Replication of Hepatitis C Virus in Nonhepatic Cells. *J. Virol.* **2008**, *82*, 8215–8223. [CrossRef]
168. Narbus, C.M.; Israelow, B.; Sourisseau, M.; Michta, M.L.; Hopcraft, S.E.; Zeiner, G.M.; Evans, M.J. HepG2 Cells Expressing MicroRNA miR-122 Support the Entire Hepatitis C Virus Life Cycle. *J. Virol.* **2011**, *85*, 12087–12092. [CrossRef]
169. Henke, J.I.; Goergen, D.; Zheng, J.; Song, Y.; Schüttler, C.G.; Fehr, C.; Jünemann, C.; Niepmann, M. microRNA-122 stimulates translation of hepatitis C virus RNA. *EMBO J.* **2008**, *27*, 3300–3310. [CrossRef] [PubMed]
170. Shan, Y.; Zheng, J.; Lambrecht, R.W.; Bonkovsky, H.L. Reciprocal Effects of Micro-RNA-122 on Expression of Heme Oxygenase-1 and Hepatitis C Virus Genes in Human Hepatocytes. *Gastroenterology* **2007**, *133*, 1166–1174. [CrossRef] [PubMed]
171. Pedersen, I.M.; Cheng, G.; Wieland, S.; Volinia, S.; Croce, C.M.; Chisari, F.V.; David, M. Interferon modulation of cellular microRNAs as an antiviral mechanism. *Nature* **2007**, *449*, 919–922. [CrossRef]
172. Wang, X.; Peng, J.; Wang, J.; Li, M.; Wu, D.; Wu, S.; Liao, J.; Dou, J. Hepatitis C virus core impacts expression of miR122 and miR204 involved in carcinogenic progression via regulation of TGFBRAP1 and HOTTIP expression. *OncoTargets Ther.* **2018**, *11*, 1173–1182. [CrossRef] [PubMed]

173. Bihrer, V.; Friedrich-Rust, M.; Kronenberger, B.; Forestier, N.; Haupenthal, J.; Shi, Y.; Peveling-Oberhag, J.; Radeke, H.; Sarrazin, C.; Herrmann, E.; et al. Serum miR-122 as a Biomarker of Necroinflammation in Patients With Chronic Hepatitis C Virus Infection. *Am. J. Gastroenterol.* **2011**, *106*, 1663–1669. [CrossRef]
174. Tambyah, P.A.; Ching, C.S.; Sepramaniam, S.; Ali, J.M.; Armugam, A.; Jeyaseelan, K. microRNA expression in blood of dengue patients. *Ann. Clin. Biochem.* **2016**, *53*, 466–476. [CrossRef]
175. Lee, T.-C.; Lin, Y.-L.; Liao, J.-T.; Su, C.-M.; Lin, C.-C.; Lin, W.-P.; Liao, C.-L. Utilizing liver-specific microRNA-122 to modulate replication of dengue virus replicon. *Biochem. Biophys. Res. Commun.* **2010**, *396*, 596–601. [CrossRef]
176. Diallo, I.; Ho, J.; Laffont, B.; Laugier, J.; Benmoussa, A.; Lambert, M.; Husseini, Z.; Soule, G.; Kozak, R.; Kobinger, G.; et al. Altered microRNA Transcriptome in Cultured Human Liver Cells upon Infection with Ebola Virus. *Int. J. Mol. Sci.* **2021**, *22*, 3792. [CrossRef]
177. Madara, J.J.; Han, Z.; Ruthel, G.; Freedman, B.D.; Harty, R.N. The multifunctional Ebola virus VP40 matrix protein is a promising therapeutic target. *Futur. Virol.* **2015**, *10*, 537–546. [CrossRef]
178. Wang, S.; Liu, P.; Yang, P.; Zheng, J.; Zhao, D. Peripheral blood microRNAs expression is associated with infant respiratory syncytial virus infection. *Oncotarget* **2017**, *8*, 96627–96635. [CrossRef]
179. Ioannidis, I.; McNally, B.; Willette, M.; Peeples, M.E.; Chaussabel, D.; Durbin, J.E.; Ramilo, O.; Mejias, A.; Flaño, E. Plasticity and Virus Specificity of the Airway Epithelial Cell Immune Response during Respiratory Virus Infection. *J. Virol.* **2012**, *86*, 5422–5436. [CrossRef] [PubMed]
180. Kurt-Jones, E.A.; Popova, L.; Kwinn, L.A.; Haynes, L.M.; Jones, L.P.; Tripp, R.; Walsh, E.E.; Freeman, M.W.; Golenbock, D.T.; Anderson, L.J.; et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol.* **2000**, *1*, 398–401. [CrossRef]
181. Haynes, L.M.; Moore, D.D.; Kurt-Jones, E.A.; Finberg, R.W.; Anderson, L.J.; Tripp, R.A. Involvement of Toll-Like Receptor 4 in Innate Immunity to Respiratory Syncytial Virus. *J. Virol.* **2001**, *75*, 10730–10737. [CrossRef]
182. Thornburg, N.J.; Shepherd, B.; Crowe, J.E., Jr. Transforming Growth Factor Beta Is a Major Regulator of Human Neonatal Immune Responses following Respiratory Syncytial Virus Infection. *J. Virol.* **2010**, *84*, 12895–12902. [CrossRef] [PubMed]
183. Esposito, S.; Salice, P.; Bosis, S.; Ghiglia, S.; Tremolati, E.; Tagliabue, C.; Gualtieri, L.; Barbier, P.; Galeone, C.; Marchisio, P.; et al. Altered cardiac rhythm in infants with bronchiolitis and respiratory syncytial virus infection. *BMC Infect. Dis.* **2010**, *10*, 305. [CrossRef] [PubMed]
184. Mohamed, M.A.E.-G.; Zayed, K.M. Impact of Acute Bronchiolitis on Cardiac Functions and Serum microRNA-122 and 499. *Am. J. Infect. Dis.* **2016**, *12*, 11–19. [CrossRef]
185. Collison, A.M.; Sokulsky, L.A.; Kepreotes, E.; de Siqueira, A.P.; Morten, M.; Edwards, M.R.; Walton, R.P.; Bartlett, N.W.; Yang, M.; Nguyen, T.H.; et al. miR-122 promotes virus-induced lung disease by targeting SOCS1. *JCI Insight* **2021**, *6*, e127933. [CrossRef] [PubMed]
186. Wang, Y.; Zeng, G.; Jiang, Y. The Emerging Roles of miR-125b in Cancers. *Cancer Manag. Res.* **2020**, *12*, 1079–1088. [CrossRef]
187. Tili, E.; Michaille, J.-J.; Cimino, A.; Costinean, S.; Dumitru, C.D.; Adair, B.; Fabbri, M.; Alder, H.; Liu, C.G.; Calin, G.; et al. Modulation of miR-155 and miR-125b Levels following Lipopolysaccharide/TNF-α Stimulation and Their Possible Roles in Regulating the Response to Endotoxin Shock. *J. Immunol.* **2007**, *179*, 5082–5089. [CrossRef]
188. Murphy, A.J.; Guyre, P.M.; Pioli, P.A. Estradiol Suppresses NF-κB Activation through Coordinated Regulation of let-7a and miR-125b in Primary Human Macrophages. *J. Immunol.* **2010**, *184*, 5029–5037. [CrossRef]
189. Busch, S.; Auth, E.; Scholl, F.; Huenecke, S.; Koehl, U.; Suess, B.; Steinhilber, D. 5-Lipoxygenase Is a Direct Target of miR-19a-3p and miR-125b-5p. *J. Immunol.* **2015**, *194*, 1646–1653. [CrossRef] [PubMed]
190. Chaudhuri, A.A.; So, A.Y.-L.; Sinha, N.; Gibson, W.S.J.; Taganov, K.D.; O'Connell, R.M.; Baltimore, D. MicroRNA-125b Potentiates Macrophage Activation. *J. Immunol.* **2011**, *187*, 5062–5068. [CrossRef] [PubMed]
191. Sui, X.; Kong, N.; Ye, L.; Han, W.; Zhou, J.; Zhang, Q.; He, C.; Pan, H. p38 and JNK MAPK pathways control the balance of apoptosis and autophagy in response to chemotherapeutic agents. *Cancer Lett.* **2014**, *344*, 174–179. [CrossRef] [PubMed]
192. Gong, J.; Zhang, J.-P.; Li, B.; Zeng, C.; You, K.; Chen, M.-X.; Yuan, Y.; Zhuang, S.-M. MicroRNA-125b promotes apoptosis by regulating the expression of Mcl-1, Bcl-w and IL-6R. *Oncogene* **2013**, *32*, 3071–3079. [CrossRef] [PubMed]
193. Maes, M.E.; Schlamp, C.L.; Nickells, R.W. BAX to basics: How the BCL2 gene family controls the death of retinal ganglion cells. *Prog. Retin. Eye Res.* **2017**, *57*, 1–25. [CrossRef]
194. Deng, W.; Zhang, X.; Ma, Z.; Lin, Y.; Lu, M. MicroRNA-125b-5p mediates post-transcriptional regulation of hepatitis B virus replication via the LIN28B/*let-7* axis. *RNA Biol.* **2017**, *14*, 1389–1398. [CrossRef]
195. Li, F.; Zhou, P.; Deng, W.; Wang, J.; Mao, R.; Zhang, Y.; Li, J.; Yu, J.; Yang, F.; Huang, Y.; et al. Serum microRNA-125b correlates with hepatitis B viral replication and liver necroinflammation. *Clin. Microbiol. Infect.* **2016**, *22*, 384.e1–384.e10. [CrossRef]
196. Chen, S.; Chen, H.; Gao, S.; Qiu, S.; Zhou, H.; Yu, M.; Tu, J. Differential expression of plasma microRNA-125b in hepatitis B virus-related liver diseases and diagnostic potential for hepatitis B virus-induced hepatocellular carcinoma. *Hepatol. Res.* **2017**, *47*, 312–320. [CrossRef]
197. Zhang, Z.; Chen, J.; He, Y.; Zhan, X.; Zhao, R.; Huang, Y.; Xu, H.; Zhu, Z.; Liu, Q. miR-125b inhibits hepatitis B virus expression in vitro through targeting of the SCNN1A gene. *Arch. Virol.* **2014**, *159*, 3335–3343. [CrossRef]
198. Peng, C.; Wang, H.; Zhang, W.-J.; Jie, S.-H.; Tong, Q.-X.; Lu, M.-J.; Yang, D.-L. Inhibitory effect of miR-125b on hepatitis C virus core protein-induced TLR2/MyD88 signaling in THP-1 cells. *World J. Gastroenterol.* **2016**, *22*, 4354–4361. [CrossRef]

199. Dai, C.-Y.; Tsai, Y.-S.; Chou, W.-W.; Liu, T.; Huang, C.-F.; Wang, S.-C.; Tsai, P.-C.; Yeh, M.-L.; Hsieh, M.-Y.; Huang, C.-I.; et al. The IL-6/STAT3 pathway upregulates microRNA-125b expression in hepatitis C virus infection. *Oncotarget* **2018**, *9*, 11291–11302. [CrossRef] [PubMed]
200. Shwetha, S.; Sharma, G.; Raheja, H.; Goel, A.; Aggarwal, R.; Das, S. Interaction of miR-125b-5p with Human antigen R mRNA: Mechanism of controlling HCV replication. *Virus Res.* **2018**, *258*, 1–8. [CrossRef] [PubMed]
201. Sultana, C.; Rosca, A.; Ruta, S. Correlation Between miR-125b Expression and Liver Fibrosis in Patients with Chronic Hepatitis C. *Hepat. Mon.* **2019**, *19*, e84615. [CrossRef]
202. Liu, W.; Hu, J.; Zhou, K.; Chen, F.; Wang, Z.; Liao, B.; Dai, Z.; Cao, Y.; Fan, J.; Zhou, J. Serum exosomal miR-125b is a novel prognostic marker for hepatocellular carcinoma. *OncoTargets Ther.* **2017**, *10*, 3843–3851. [CrossRef]
203. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/hepeviridae (accessed on 19 July 2022).
204. Harms, D.; Choi, M.; Allers, K.; Wang, B.; Pietsch, H.; Papp, C.-P.; Hanisch, L.; Kurreck, J.; Hofmann, J.; Bock, C.-T. Specific circulating microRNAs during hepatitis E infection can serve as indicator for chronic hepatitis E. *Sci. Rep.* **2020**, *10*, 5337. [CrossRef]
205. Zhang, X.; Huang, F.; Yang, D.; Peng, T.; Lu, G. Identification of miRNA-mRNA Crosstalk in Respiratory Syncytial Virus-(RSV-) Associated Pediatric Pneumonia through Integrated miRNAome and Transcriptome Analysis. *Mediat. Inflamm.* **2020**, *2020*, 8919534. [CrossRef]
206. Makkoch, J.; Poomipak, W.; Saengchoowong, S.; Khongnomnan, K.; Praianantathavorn, K.; Jinato, T.; Poovorawan, Y.; Payungporn, S. Human microRNAs profiling in response to influenza A viruses (subtypes pH1N1, H3N2, and H5N1). *Exp. Biol. Med.* **2016**, *241*, 409–420. [CrossRef]
207. Chen, L.; Liu, Y.; Wu, J.; Deng, C.; Tan, J.; Liu, H.; Zhong, L. Lung adenocarcinoma patients have higher risk of SARS-CoV-2 infection. *Aging* **2021**, *13*, 1620–1632. [CrossRef]
208. Chaudhuri, E.; Dash, S.; Balasubramaniam, M.; Padron, A.; Holland, J.; Sowd, G.A.; Villalta, F.; Engelman, A.N.; Pandhare, J.; Dash, C. The HIV-1 capsid-binding host factor CPSF6 is post-transcriptionally regulated by the cellular microRNA miR-125b. *J. Biol. Chem.* **2020**, *295*, 5081–5094. [CrossRef]
209. Mantri, C.K.; Dash, J.P.; Mantri, J.V.; Dash, C.C.V. Cocaine Enhances HIV-1 Replication in CD4+ T Cells by Down-Regulating MiR-125b. *PLoS ONE* **2012**, *7*, e51387. [CrossRef]
210. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/flaviviridae (accessed on 19 July 2022).
211. Ghosh, D.; Basu, A. Japanese Encephalitis—A Pathological and Clinical Perspective. *PLOS Negl. Trop. Dis.* **2009**, *3*, e437. [CrossRef] [PubMed]
212. Huang, C.-W.; Tsai, K.-N.; Chen, Y.-S.; Chang, R.-Y. Differential miRNA Expression Profiling Reveals Correlation of miR125b-5p with Persistent Infection of Japanese Encephalitis Virus. *Int. J. Mol. Sci.* **2021**, *22*, 4218. [CrossRef] [PubMed]
213. Mziaut, H.; Henniger, G.; Ganss, K.; Hempel, S.; Wolk, S.; McChord, J.; Chowdhury, K.; Ravassard, P.; Knoch, K.-P.; Krautz, C.; et al. MiR-132 controls pancreatic beta cell proliferation and survival through Pten/Akt/Foxo3 signaling. *Mol. Metab.* **2020**, *31*, 150–162. [CrossRef] [PubMed]
214. Li, D.; Wang, A.; Liu, X.; Meisgen, F.; Grünler, J.; Botusan, I.R.; Narayanan, S.; Erikci, E.; Blomqvist, L.; Du, L.; et al. MicroRNA-132 enhances transition from inflammation to proliferation during wound healing. *J. Clin. Investig.* **2015**, *125*, 3008–3026. [CrossRef] [PubMed]
215. Liu, X.; Tong, Z.; Chen, K.; Hu, X.; Jin, H.; Hou, M. The Role of miRNA-132 against Apoptosis and Oxidative Stress in Heart Failure. *BioMed Res. Int.* **2018**, *2018*, 3452748. [CrossRef] [PubMed]
216. Strum, J.C.; Johnson, J.H.; Ward, J.; Xie, H.; Feild, J.; Hester, A.; Alford, A.; Waters, K.M. MicroRNA 132 Regulates Nutritional Stress-Induced Chemokine Production through Repression of SirT1. *Mol. Endocrinol.* **2009**, *23*, 1876–1884. [CrossRef]
217. Wei, X.; Tan, C.; Tang, C.; Ren, G.; Xiang, T.; Qiu, Z.; Liu, R.; Wu, Z. Epigenetic repression of miR-132 expression by the hepatitis B virus x protein in hepatitis B virus-related hepatocellular carcinoma. *Cell. Signal.* **2013**, *25*, 1037–1043. [CrossRef]
218. Liu, B.; Yang, X.-F.; Liang, X.-P.; Wang, L.; Shao, M.-M.; Han, W.-X.; Wu, Y.-H. Expressions of MiR-132 in patients with chronic hepatitis B, posthepatitic cirrhosis and hepatitis B virus-related hepatocellular carcinoma. *Eur. Rev. Med. Pharmacol. Sci.* **2018**, *22*, 8431–8437.
219. Zhang, F.; Lin, X.; Yang, X.; Lu, G.; Zhang, Q.; Zhang, C. MicroRNA-132-3p suppresses type I IFN response through targeting IRF1 to facilitate H1N1 influenza A virus infection. *Biosci. Rep.* **2019**, *39*, BSR20192769. [CrossRef]
220. Hsu, A.C.-Y.; Parsons, K.; Moheimani, F.; Knight, D.A.; Hansbro, P.M.; Fujita, T.; Wark, P.A. Impaired Antiviral Stress Granule and IFN-β Enhanceosome Formation Enhances Susceptibility to Influenza Infection in Chronic Obstructive Pulmonary Disease Epithelium. *Am. J. Respir. Cell Mol. Biol.* **2016**, *55*, 117–127. [CrossRef]
221. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/dsdna-viruses/w/herpesviridae (accessed on 19 July 2022).
222. Tognarelli, E.I.; Palomino, T.F.; Corrales, N.; Bueno, S.M.; Kalergis, A.; González, P.A. Herpes Simplex Virus Evasion of Early Host Antiviral Responses. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 127. [CrossRef] [PubMed]
223. Pepose, J.S. Herpes simplex keratitis: Role of viral infection versus immune response. *Surv. Ophthalmol.* **1991**, *35*, 345–352. [CrossRef]

224. Mulik, S.; Xu, J.; Reddy, P.B.; Rajasagi, N.K.; Gimenez, F.; Sharma, S.; Lu, P.Y.; Rouse, B.T. Role of miR-132 in Angiogenesis after Ocular Infection with Herpes Simplex Virus. *Am. J. Pathol.* **2012**, *181*, 525–534. [CrossRef]
225. Taheri, F.; Ebrahimi, S.O.; Shareef, S.; Reiisi, S. Regulatory and immunomodulatory role of miR-34a in T cell immunity. *Life Sci.* **2020**, *262*, 118209. [CrossRef] [PubMed]
226. Hermeking, H. The miR-34 family in cancer and apoptosis. *Cell Death Differ.* **2010**, *17*, 193–199. [CrossRef] [PubMed]
227. Zhang, Y.; Ren, H.; Li, J.; Xue, R.; Liu, H.; Zhu, Z.; Pan, C.; Lin, Y.; Hu, A.; Gou, P.; et al. Elevated HMGB1 expression induced by hepatitis B virus X protein promotes epithelial-mesenchymal transition and angiogenesis through STAT3/miR-34a/NF-kappaB in primary liver cancer. *Am. J. Cancer Res.* **2021**, *11*, 479–494.
228. FeiLi, X.; Wu, S.; Ye, W.; Tu, J.; Lou, L. MicroRNA-34a-5p inhibits liver fibrosis by regulating TGF-β1/Smad3 pathway in hepatic stellate cells. *Cell Biol. Int.* **2018**, *42*, 1370–1376. [CrossRef]
229. Rana, M.A.; Ijaz, B.; Daud, M.; Tariq, S.; Nadeem, T.; Husnain, T. Interplay of Wnt β-catenin pathway and miRNAs in HBV pathogenesis leading to HCC. *Clin. Res. Hepatol. Gastroenterol.* **2019**, *43*, 373–386. [CrossRef]
230. Yang, P.; Li, Q.-J.; Feng, Y.; Zhang, Y.; Markowitz, G.J.; Ning, S.; Deng, Y.; Zhao, J.; Jiang, S.; Yuan, Y.; et al. TGF-β-miR-34a-CCL22 Signaling-Induced Treg Cell Recruitment Promotes Venous Metastases of HBV-Positive Hepatocellular Carcinoma. *Cancer Cell* **2012**, *22*, 291–303. [CrossRef]
231. Cermelli, S.; Ruggieri, A.; Marrero, J.A.; Ioannou, G.N.; Beretta, L. Circulating MicroRNAs in Patients with Chronic Hepatitis C and Non-Alcoholic Fatty Liver Disease. *PLoS ONE* **2011**, *6*, e23937. [CrossRef]
232. Rossi, A.D.; Higa, L.M.; Herlinger, A.L.; Ribeiro-Alves, M.; de Menezes, M.T.; Giannini, A.L.M.; Cardoso, C.C.; Da Poian, A.T.; Tanuri, A.; Aguiar, R.S. Differential Expression of Human MicroRNAs During Dengue Virus Infection in THP-1 Monocytes. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 714088. [CrossRef] [PubMed]
233. Smith, J.L.; Jeng, S.; McWeeney, S.K.; Hirsch, A.J. A MicroRNA Screen Identifies the Wnt Signaling Pathway as a Regulator of the Interferon Response during Flavivirus Infection. *J. Virol.* **2017**, *91*, e02388–e02404. [CrossRef]
234. Fan, N.; Wang, J. MicroRNA 34a contributes to virus-mediated apoptosis through binding to its target gene Bax in influenza A virus infection. *Biomed. Pharmacother.* **2016**, *83*, 1464–1470. [CrossRef] [PubMed]
235. Othumpangat, S.; Beezhold, D.H.; Umbright, C.M.; Noti, J.D. Influenza Virus-Induced Novel miRNAs Regulate the STAT Pathway. *Viruses* **2021**, *13*, 967. [CrossRef]
236. Centa, A.; Fonseca, A.S.; Ferreira, S.G.D.S.; Azevedo, M.L.V.; de Paula, C.B.V.; Nagashima, S.; Machado-Souza, C.; Miggiolaro, A.F.R.D.S.; Baena, C.P.; de Noronha, L.; et al. Deregulated miRNA expression is associated with endothelial dysfunction in post-mortem lung biopsies of COVID-19 patients. *Am. J. Physiol. Cell. Mol. Physiol.* **2021**, *320*, L405–L412. [CrossRef] [PubMed]
237. Bartoszewski, R.; Dabrowski, M.; Jakiela, B.; Matalon, S.; Harrod, K.S.; Sanak, M.; Collawn, J.F. SARS-CoV-2 may regulate cellular responses through depletion of specific host miRNAs. *Am. J. Physiol. Cell. Mol. Physiol.* **2020**, *319*, L444–L455. [CrossRef] [PubMed]
238. Demirci, M.D.S.; Adan, A. Computational analysis of microRNA-mediated interactions in SARS-CoV-2 infection. *PeerJ* **2020**, *8*, e9369. [CrossRef]
239. Verdonck, K.; González, E.; Van Dooren, S.; Vandamme, A.-M.; Vanham, G.; Gotuzzo, E. Human T-lymphotropic virus 1: Recent knowledge about an ancient infection. *Lancet Infect. Dis.* **2007**, *7*, 266–281. [CrossRef]
240. Sharma, V.K.; Raimondi, V.; Ruggero, K.; Pise-Masison, C.A.; Cavallari, I.; Silic-Benussi, M.; Ciminale, V.; D'Agostino, D.M. Expression of miR-34a in T-Cells Infected by Human T-Lymphotropic Virus. *Front. Microbiol.* **2018**, *9*, 832. [CrossRef]
241. Kumarswamy, R.; Volkmann, I.; Thum, T. Regulation and function of miRNA-21 in health and disease. *RNA Biol.* **2011**, *8*, 706–713. [CrossRef]
242. Jenike, A.E.; Halushka, M.K. miR-21: A non-specific biomarker of all maladies. *Biomark. Res.* **2021**, *9*, 18. [CrossRef] [PubMed]
243. Sheetz, M. A Tale of Two States: Normal and Transformed, With and Without Rigidity Sensing. *Annu. Rev. Cell Dev. Biol.* **2019**, *35*, 169–190. [CrossRef] [PubMed]
244. Zhang, K.; Shi, Z.; Zhang, M.; Dong, X.; Zheng, L.; Li, G.; Han, X.; Yao, Z.; Han, T.; Hong, W. Silencing lncRNA Lfar1 alleviates the classical activation and pyoptosis of macrophage in hepatic fibrosis. *Cell Death Dis.* **2020**, *11*, 132. [CrossRef] [PubMed]
245. El–Serag, H.B.; Rudolph, K.L. Hepatocellular Carcinoma: Epidemiology and Molecular Carcinogenesis. *Gastroenterology* **2007**, *132*, 2557–2576. [CrossRef]
246. Lee, Y.-H.; Yun, Y. HBx Protein of Hepatitis B Virus Activates Jak1-STAT Signaling. *J. Biol. Chem.* **1998**, *273*, 25510–25515. [CrossRef] [PubMed]
247. Lee, Y.I.; Kang-Park, S.; Do, S.-I.; Lee, Y.I. The Hepatitis B Virus-X Protein Activates a Phosphatidylinositol 3-Kinase-dependent Survival Signaling Cascade. *J. Biol. Chem.* **2001**, *276*, 16969–16977. [CrossRef]
248. Wu, H.-C.; Huang, C.-L.; Wang, H.-W.; Hsu, W.-F.; Tsai, T.-Y.; Chen, S.-H.; Peng, C.-Y. Serum miR-21 correlates with the histological stage of chronic hepatitis B-associated liver fibrosis. *Int. J. Clin. Exp. Pathol.* **2019**, *12*, 3819–3829.
249. Li, W.; Yu, X.; Chen, X.; Wang, Z.; Yin, M.; Zhao, Z.; Zhu, C. HBV induces liver fibrosis via the TGF-β1/miR-21-5p pathway. *Exp. Ther. Med.* **2021**, *21*, 169. [CrossRef]
250. Wei, J.; Feng, L.; Li, Z.; Xu, G.; Fan, X. MicroRNA-21 activates hepatic stellate cells via PTEN/Akt signaling. *Biomed. Pharmacother.* **2013**, *67*, 387–392. [CrossRef]
251. Fu, R.-Q.; Hu, D.-P.; Hu, Y.-B.; Hong, L.; Sun, Q.-F.; Ding, J.-G. miR-21 promotes α-SMA and collagen I expression in hepatic stellate cells via the Smad7 signaling pathway. *Mol. Med. Rep.* **2017**, *16*, 4327–4333. [CrossRef]

252. Qiu, X.; Dong, S.; Qiao, F.; Lu, S.; Song, Y.; Lao, Y.; Li, Y.; Zeng, T.; Hu, J.; Zhang, L.; et al. HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene* **2013**, *32*, 3296–3305. [CrossRef] [PubMed]
253. Damania, P.; Sen, B.; Dar, S.B.; Kumar, S.; Kumari, A.; Gupta, E.; Sarin, S.K.; Venugopal, S.K. Hepatitis B Virus Induces Cell Proliferation via HBx-Induced microRNA-21 in Hepatocellular Carcinoma by Targeting Programmed Cell Death Protein4 (PDCD4) and Phosphatase and Tensin Homologue (PTEN). *PLoS ONE* **2014**, *9*, e91745. [CrossRef]
254. Li, C.H.; Xu, F.; Chow, S.; Feng, L.; Yin, D.; Ng, T.B.; Chen, Y. Hepatitis B virus X protein promotes hepatocellular carcinoma transformation through interleukin-6 activation of microRNA-21 expression. *Eur. J. Cancer* **2014**, *50*, 2560–2569. [CrossRef] [PubMed]
255. Li, C.H.; Chow, S.C.; Yin, D.L.; Ng, T.B.; Chen, Y.C. Functional characterisation of hepatitis B viral X protein/microRNA-21 interaction in HBV-associated hepatocellular carcinoma. *Hong Kong Med. J.* **2016**, *22*, 37–42.
256. Bandopadhyay, M.; Banerjee, A.; Sarkar, N.; Panigrahi, R.; Datta, S.; Pal, A.; Singh, S.P.; Biswas, A.; Chakrabarti, S.; Chakravarty, R. Tumor suppressor micro RNA miR-145 and onco micro RNAs miR-21 and miR-222 expressions are differentially modulated by Hepatitis B virus X protein in malignant hepatocytes. *BMC Cancer* **2014**, *14*, 721. [CrossRef]
257. Yin, D.; Wang, Y.; Sai, W.; Zhang, L.; Miao, Y.; Cao, L.; Zhai, X.; Feng, X.; Yang, L. HBx-induced miR-21 suppresses cell apoptosis in hepatocellular carcinoma by targeting interleukin-12. *Oncol. Rep.* **2016**, *36*, 2305–2312. [CrossRef]
258. Otania, T.; Nakamuraa, S.; Tokia, M.; Motodaa, R.; Kurimotob, M.; Oritaa, K. Identification of IFN-γ-Producing Cells in IL-12/IL-18-Treated Mice. *Cell. Immunol.* **1999**, *198*, 111–119. [CrossRef]
259. Zeh, H.J.; Hurd, S.; Storkus, W.J.; Lotze, M.T. Interleukin-12 Promotes the Proliferation and Cytolytic Maturation of Immune Effectors: Implications for the immunotherapy of cancer. *J. Immunother.* **1993**, *14*, 155–161. [CrossRef]
260. Clément, S.; Sobolewski, C.; Gomes, D.; Rojas, A.; Goossens, N.; Conzelmann, S.; Calo, N.; Negro, F.; Foti, M. Activation of the oncogenic miR-21-5p promotes HCV replication and steatosis induced by the viral core 3a protein. *Liver Int.* **2019**, *39*, 1226–1236. [CrossRef]
261. Chen, Y.; Chen, J.; Wang, H.; Shi, J.; Wu, K.; Liu, S.; Liu, Y.; Wu, J. HCV-Induced miR-21 Contributes to Evasion of Host Immune System by Targeting MyD88 and IRAK1. *PLOS Pathog.* **2013**, *9*, e1003248. [CrossRef]
262. Marquez, R.T.; Bandyopadhyay, S.; Wendlandt, E.B.; Keck, K.; Hoffer, B.A.; Icardi, M.S.; Christensen, R.N.; Schmidt, W.N.; McCaffrey, A.P. Correlation between microRNA expression levels and clinical parameters associated with chronic hepatitis C viral infection in humans. *Lab. Investig.* **2010**, *90*, 1727–1736. [CrossRef] [PubMed]
263. Kanokudom, S.; Vilaivan, T.; Wikan, N.; Thepparit, C.; Smith, D.R.; Assavalapsakul, W. miR-21 promotes dengue virus serotype 2 replication in HepG2 cells. *Antivir. Res.* **2017**, *142*, 169–177. [CrossRef] [PubMed]
264. Duy, J.; Koehler, J.; Honko, A.; Schoepp, R.; Wauquier, N.; Gonzalez, J.-P.; Pitt, M.L.; Mucker, E.; Johnson, J.; O'Hearn, A.; et al. Circulating microRNA profiles of Ebola virus infection. *Sci. Rep.* **2016**, *6*, 24496. [CrossRef] [PubMed]
265. Shi, J.; Feng, P.; Gu, T. MicroRNA-21-3p modulates FGF2 to facilitate influenza A virus H5N1 replication by refraining type I interferon response. *Biosci. Rep.* **2020**, *40*, BSR20200158. [CrossRef]
266. Xia, B.; Lu, J.; Wang, R.; Yang, Z.; Zhou, X.; Huang, P. miR-21-3p Regulates Influenza A Virus Replication by Targeting Histone Deacetylase-8. *Front. Cell. Infect. Microbiol.* **2018**, *8*, 175. [CrossRef]
267. Chew, C.L.; Conos, S.A.; Unal, B.; Tergaonkar, V. Noncoding RNAs: Master Regulators of Inflammatory Signaling. *Trends Mol. Med.* **2018**, *24*, 66–84. [CrossRef]
268. Lam, W.-Y.; Yeung, A.C.-M.; Ngai, K.L.-K.; Li, M.-S.; To, K.-F.; Tsui, S.K.-W.; Chan, P.K.-S. Effect of avian influenza A H5N1 infection on the expression of microRNA-141 in human respiratory epithelial cells. *BMC Microbiol.* **2013**, *13*, 104. [CrossRef]
269. Tan, K.S.; Choi, H.; Jiang, X.; Yin, L.; Seet, J.E.; Patzel, V.; Engelward, B.P.; Chow, V.T. Micro-RNAs in regenerating lungs: An integrative systems biology analysis of murine influenza pneumonia. *BMC Genom.* **2014**, *15*, 587. [CrossRef]
270. Zhong, Z.; Dong, Z.; Yang, L.; Gong, Z. miR-21 induces cell cycle at S phase and modulates cell proliferation by down-regulating hMSH2 in lung cancer. *J. Cancer Res. Clin. Oncol.* **2012**, *138*, 1781–1788. [CrossRef]
271. Vaporidi, K.; Vergadi, E.; Kaniaris, E.; Hatziapostolou, M.; Lagoudaki, E.; Georgopoulos, D.; Zapol, W.M.; Bloch, K.D.; Iliopoulos, D. Pulmonary microRNA profiling in a mouse model of ventilator-induced lung injury. *Am. J. Physiol. Cell. Mol. Physiol.* **2012**, *303*, L199–L207. [CrossRef]
272. Nersisyan, S.; Engibaryan, N.; Gorbonos, A.; Kirdey, K.; Makhonin, A.; Tonevitsky, A. Potential role of cellular miRNAs in coronavirus-host interplay. *PeerJ* **2020**, *8*, e9994. [CrossRef] [PubMed]
273. Jafarinejad-Farsangi, S.; Jazi, M.M.; Rostamzadeh, F.; Hadizadeh, M. High affinity of host human microRNAs to SARS-CoV-2 genome: An in silico analysis. *Non-Coding RNA Res.* **2020**, *5*, 222–231. [CrossRef] [PubMed]
274. Chahar, H.S.; Corsello, T.; Kudlicki, A.S.; Komaravelli, N.; Casola, A. Respiratory Syncytial Virus Infection Changes Cargo Composition of Exosome Released from Airway Epithelial Cells. *Sci. Rep.* **2018**, *8*, 387. [CrossRef] [PubMed]
275. Boyapalle, S.; Wong, T.; Garay, J.; Teng, M.; Vergara, H.S.J.; Mohapatra, S.; Mohapatra, S. Respiratory Syncytial Virus NS1 Protein Colocalizes with Mitochondrial Antiviral Signaling Protein MAVS following Infection. *PLoS ONE* **2012**, *7*, e29386. [CrossRef]
276. Kim, R.Y.; Horvat, J.C.; Pinkerton, J.W.; Starkey, M.R.; Essilfie, A.T.; Mayall, J.R.; Nair, P.M.; Hansbro, N.G.; Jones, B.; Haw, T.J.; et al. MicroRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying phosphoinositide 3-kinase–mediated suppression of histone deacetylase 2. *J. Allergy Clin. Immunol.* **2017**, *139*, 519–532. [CrossRef]
277. Liu, Z.; Fan, P.; Chen, M.; Xu, Y.; Zhao, D. miRNAs and Leukotrienes in Respiratory Syncytial Virus Infection. *Front. Pediatr.* **2021**, *9*, 602195. [CrossRef]

278. Ruiz-De-León, M.J.; Jiménez-Sousa, M.A.; Moreno, S.; García, M.; Gutiérrez-Rivas, M.; León, A.; Montero-Alonso, M.; González-García, J.; Resino, S.; Rallón, N.; et al. Lower expression of plasma-derived exosome miR-21 levels in HIV-1 elite controllers with decreasing CD4 T cell count. *J. Microbiol. Immunol. Infect.* **2019**, *52*, 667–671. [CrossRef]
279. Cojo, M.S.-D.; López-Huertas, M.R.; Díez-Fuertes, F.; Rodríguez-Mora, S.; Bermejo, M.; López-Campos, G.; Mateos, E.; Jiménez-Tormo, L.; Gómez-Esquer, F.; Díaz-Gil, G.; et al. Changes in the cellular microRNA profile by the intracellular expression of HIV-1 Tat regulator: A potential mechanism for resistance to apoptosis and impaired proliferation in HIV-1 infected CD4+ T cells. *PLoS ONE* **2017**, *12*, e0185677. [CrossRef]
280. Jiao, Y.; Zhang, T.; Wang, R.; Zhang, H.; Huang, X.; Yin, J.; Zhang, L.; Xu, X.; Wu, H. Plasma IP-10 Is Associated with Rapid Disease Progression in Early HIV-1 Infection. *Viral Immunol.* **2012**, *25*, 333–337. [CrossRef]
281. Liu, P.P.; Mason, J.W. Advances in the Understanding of Myocarditis. *Circulation* **2001**, *104*, 1076–1082. [CrossRef]
282. He, F.; Yao, H.; Xiao, Z.; Han, J.; Zou, J.; Liu, Z. Inhibition of IL-2 inducible T-cell kinase alleviates T-cell activation and murine myocardial inflammation associated with CVB3 infection. *Mol. Immunol.* **2014**, *59*, 30–38. [CrossRef]
283. He, F.; Xiao, Z.; Yao, H.; Li, S.; Feng, M.; Wang, W.; Liu, Z.; Liu, Z.; Wu, J. The protective role of microRNA-21 against coxsackievirus B3 infection through targeting the MAP2K3/P38 MAPK signaling pathway. *J. Transl. Med.* **2019**, *17*, 335. [CrossRef] [PubMed]
284. Ye, X.; Zhang, H.M.; Qiu, Y.; Hanson, P.J.; Hemida, M.; Wei, W.; Hoodless, P.A.; Chu, F.; Yang, D. Coxsackievirus-Induced miR-21 Disrupts Cardiomyocyte Interactions via the Downregulation of Intercalated Disk Components. *PLOS Pathog.* **2014**, *10*, e1004070. [CrossRef] [PubMed]
285. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-rna-viruses/w/rhabdoviridae/805/genus-vesiculovirus (accessed on 19 July 2022).
286. Pandey, N.; Rastogi, M.; Singh, S.K. Chandipura virus dysregulates the expression of hsa-miR-21-5p to activate NF-κB in human microglial cells. *J. Biomed. Sci.* **2021**, *28*, 52. [CrossRef]
287. Aqeilan, R.I.; Calin, G.A.; Croce, C.M. miR-15a and miR-16-1 in cancer: Discovery, function and future perspectives. *Cell Death Differ.* **2010**, *17*, 215–220. [CrossRef] [PubMed]
288. Yan, X.; Liang, H.; Deng, T.; Zhu, K.; Zhang, S.; Wang, N.; Jiang, X.; Wang, X.; Liu, R.; Zen, K.; et al. The identification of novel targets of miR-16 and characterization of their biological functions in cancer cells. *Mol. Cancer* **2013**, *12*, 92. [CrossRef]
289. Yan, L.; Liang, M.; Hou, X.; Zhang, Y.; Zhang, H.; Guo, Z.; Jinyu, J.; Feng, Z.; Mei, Z. The role of microRNA-16 in the pathogenesis of autoimmune diseases: A comprehensive review. *Biomed. Pharmacother.* **2019**, *112*, 108583. [CrossRef]
290. Wu, G.; Yu, F.; Xiao, Z.; Xu, K.; Xu, J.; Tang, W.; Wang, J.; Song, E. Hepatitis B virus X protein downregulates expression of the miR-16 family in malignant hepatocytes in vitro. *Br. J. Cancer* **2011**, *105*, 146–153. [CrossRef]
291. Zhu, B.; Wei, X.-X.; Wang, T.-B.; Zhou, Y.-C.; Liu, A.-M.; Zhang, G.-W. Increased miR-16 expression induced by hepatitis C virus infection promotes liver fibrosis through downregulation of hepatocyte growth factor and Smad7. *Arch. Virol.* **2015**, *160*, 2043–2050. [CrossRef]
292. Wang, H.; Tian, Z.; Xu, Y.; Wang, Q.; Ding, S.-W.; Li, Y. Altering Intracellular Localization of the RNA Interference Factors by Influenza A Virus Non-structural Protein 1. *Front. Microbiol.* **2020**, *11*, 590904. [CrossRef]
293. Sun, X.; Sit, A.; Feinberg, M.W. Role of miR-181 family in regulating vascular inflammation and immunity. *Trends Cardiovasc. Med.* **2014**, *24*, 105–112. [CrossRef] [PubMed]
294. Tahamtan, A.; Teymoori-Rad, M.; Nakstad, B.; Salimi, V. Anti-Inflammatory MicroRNAs and Their Potential for Inflammatory Diseases Treatment. *Front. Immunol.* **2018**, *9*, 1377. [CrossRef] [PubMed]
295. Williams, A.; Henao-Mejia, J.; Harman, C.C.D.; Flavell, R.A. miR-181 and Metabolic Regulation in the Immune System. *Cold Spring Harb. Symp. Quant. Biol.* **2013**, *78*, 223–230. [CrossRef] [PubMed]
296. Liu, X.-Y.; Zhang, F.-R.; Shang, J.-Y.; Liu, Y.-Y.; Lv, X.-F.; Yuan, J.-N.; Zhang, T.-T.; Li, K.; Lin, X.-C.; Liu, X.; et al. Renal inhibition of miR-181a ameliorates 5-fluorouracil-induced mesangial cell apoptosis and nephrotoxicity. *Cell Death Dis.* **2018**, *9*, 610. [CrossRef] [PubMed]
297. Yu, F.; Zhou, G.; Li, G.; Chen, B.; Dong, P.; Zheng, J. Serum miR-181b Is Correlated with Hepatitis B Virus Replication and Disease Progression in Chronic Hepatitis B Patients. *Dig. Dis. Sci.* **2015**, *60*, 2346–2352. [CrossRef]
298. Wang, B.; Li, W.; Guo, K.; Xiao, Y.; Wang, Y.; Fan, J. miR-181b Promotes hepatic stellate cells proliferation by targeting p27 and is elevated in the serum of cirrhosis patients. *Biochem. Biophys. Res. Commun.* **2012**, *421*, 4–8. [CrossRef]
299. Zheng, J.; Wu, C.; Xu, Z.; Xia, P.; Dong, P.; Chen, B.; Yu, F. Hepatic stellate cell is activated by microRNA-181b via PTEN/Akt pathway. *Mol. Cell. Biochem.* **2015**, *398*, 1–9. [CrossRef]
300. Zou, C.; Li, Y.; Cao, Y.; Zhang, J.; Jiang, J.; Sheng, Y.; Wang, S.; Huang, A.; Tang, H. Up-regulated MicroRNA-181a induces carcinogenesis in Hepatitis B virus-related hepatocellular carcinoma by targeting E2F5. *BMC Cancer* **2014**, *14*, 97. [CrossRef]
301. Tian, Y.; Xiao, X.; Gong, X.; Peng, F.; Xu, Y.; Jiang, Y.; Gong, G. HBx promotes cell proliferation by disturbing the cross-talk between miR-181a and PTEN. *Sci. Rep.* **2017**, *7*, 40089. [CrossRef]
302. Zou, C.; Chen, J.; Chen, K.; Wang, S.; Cao, Y.; Zhang, J.; Sheng, Y.; Huang, A.; Tang, H. Functional analysis of miR-181a and Fas involved in hepatitis B virus-related hepatocellular carcinoma pathogenesis. *Exp. Cell Res.* **2015**, *331*, 352–361. [CrossRef]
303. Elhelw, D.S.; Mekky, R.Y.; El-Ekiaby, N.; Ahmed, R.; Eldin, M.A.M.; El-Sayed, M.; AbouElKhair, M.; Salah, A.; Zekri, A.R.; Esmat, G.; et al. Predictive prognostic role of miR-181a with discrepancy in the liver and serum of genotype 4 hepatitis C virus patients. *Biomed. Rep.* **2014**, *2*, 843–848. [CrossRef] [PubMed]

304. Boštjančič, E.; Bandelj, E.; Luzar, B.; Poljak, M.; Glavač, D. Hepatic expression of miR-122, miR-126, miR-136 and miR-181a and their correlation to histopathological and clinical characteristics of patients with hepatitis C. *J. Viral Hepat.* **2015**, *22*, 146–157. [CrossRef] [PubMed]
305. Li, G.Y.; Zhou, Y.; Ying, R.S.; Shi, L.; Cheng, Y.Q.; Ren, J.P.; Griffin, J.W.; Jia, Z.S.; Li, C.F.; Moorman, J.P.; et al. Hepatitis C virus-induced reduction in miR-181a impairs $CD4^+$T-cell responses through overexpression of DUSP6. *Hepatology* **2015**, *61*, 1163–1173. [CrossRef] [PubMed]
306. Mukherjee, A.; Shrivastava, S.; Chowdhury, J.B.; Ray, R.; Ray, R.B. Transcriptional Suppression of miR-181c by Hepatitis C Virus Enhances Homeobox A1 Expression. *J. Virol.* **2014**, *88*, 7929–7940. [CrossRef]
307. Patra, T.; Meyer, K.; Ray, R.B.; Ray, R. Hepatitis C Virus Mediated Inhibition of miR-181c Activates ATM Signaling and Promotes Hepatocyte Growth. *Hepatology* **2020**, *71*, 780–793. [CrossRef]
308. Lim, J.; Byun, J.; Guk, K.; Hwang, S.G.; Bae, P.K.; Jung, J.; Kang, T.; Lim, E.-K. Highly Sensitive in Vitro Diagnostic System of Pandemic Influenza A (H1N1) Virus Infection with Specific MicroRNA as a Biomarker. *ACS Omega* **2019**, *4*, 14560–14568. [CrossRef]
309. Peng, F.; He, J.; Loo, J.F.C.; Yao, J.; Shi, L.; Liu, C.; Zhao, C.; Xie, W.; Shao, Y.; Kong, S.K.; et al. Identification of microRNAs in Throat Swab as the Biomarkers for Diagnosis of Influenza. *Int. J. Med. Sci.* **2016**, *13*, 77–84. [CrossRef]
310. Atherton, L.J.; Jorquera, P.A.; Bakre, A.A.; Tripp, R.A. Determining Immune and miRNA Biomarkers Related to Respiratory Syncytial Virus (RSV) Vaccine Types. *Front. Immunol.* **2019**, *10*, 2323. [CrossRef]
311. Su, J.-L.; Chen, P.-S.; Johansson, G.; Kuo, M.-L. Function and Regulation of Let-7 Family microRNAs. *MicroRNA* **2012**, *1*, 34–39. [CrossRef]
312. Thammaiah, C.K.; Jayaram, S. Role of let-7 family microRNA in breast cancer. *Non-Coding RNA Res.* **2016**, *1*, 77–82. [CrossRef]
313. Jiang, S. Recent findings regarding let-7 in immunity. *Cancer Lett.* **2018**, *434*, 130–131. [CrossRef] [PubMed]
314. Letafati, A.; Najafi, S.; Mottahedi, M.; Karimzadeh, M.; Shahini, A.; Garousi, S.; Abbasi-Kolli, M.; Nahand, J.S.; Zadeh, S.S.T.; Hamblin, M.R.; et al. MicroRNA let-7 and viral infections: Focus on mechanisms of action. *Cell. Mol. Biol. Lett.* **2022**, *27*, 1–47. [CrossRef]
315. Wang, Y.; Lu, Y.; Toh, S.T.; Sung, W.-K.; Tan, P.; Chow, P.; Chung, A.Y.; Jooi, L.L.; Lee, C.G.L. Lethal-7 is down-regulated by the hepatitis B virus x protein and targets signal transducer and activator of transcription. *J. Hepatol.* **2010**, *53*, 57–66. [CrossRef] [PubMed]
316. Wu, G.; Huang, P.; Ju, X.; Li, Z.; Wang, Y. Lin28B over-expression mediates the repression of let-7 by hepatitis B virus X protein in hepatoma cells. *Int. J. Clin. Exp. Med.* **2015**, *8*, 15108–15116.
317. Al Zaid Siddiquee, K.; Turkson, J. STAT3 as a target for inducing apoptosis in solid and hematological tumors. *Cell Res.* **2008**, *18*, 254–267. [CrossRef] [PubMed]
318. Qiu, D.; Chen, J.; Liu, J.; Luo, Z.; Jiang, W.; Huang, J.; Qiu, Z.; Yue, W.; Wu, L. Expression of microRNA let-7a positively correlates with hepatitis B virus replication in hepatocellular carcinoma tissues. *Exp. Biol. Med.* **2017**, *242*, 939–944. [CrossRef] [PubMed]
319. Takata, A.; Otsuka, M.; Ohno, M.; Kishikawa, T.; Yoshikawa, T.; Koike, K. Mutual antagonism between hepatitis B viral mRNA and host microRNA let-7. *Sci. Rep.* **2016**, *6*, 23237. [CrossRef] [PubMed]
320. Li, Q.; Lowey, B.; Sodroski, C.; Krishnamurthy, S.; Alao, H.; Cha, H.; Chiu, S.; El-Diwany, R.; Ghany, M.G.; Liang, T.J. Cellular microRNA networks regulate host dependency of hepatitis C virus infection. *Nat. Commun.* **2017**, *8*, 1789. [CrossRef]
321. Cheng, J.-C.; Yeh, Y.-J.; Tseng, C.-P.; Hsu, S.-D.; Chang, Y.-L.; Sakamoto, N.; Huang, H.-D. Let-7b is a novel regulator of hepatitis C virus replication. *Cell. Mol. Life Sci.* **2012**, *69*, 2621–2633. [CrossRef]
322. Yeh, Y.-J.; Tseng, C.-P.; Hsu, S.-D.; Huang, H.-Y.; Lai, M.M.C.; Huang, H.-D.; Cheng, J.-C. Dual Effects of Let-7b in the Early Stage of Hepatitis C Virus Infection. *J. Virol.* **2021**, *95*, e01800–e01820. [CrossRef]
323. Cheng, M.; Si, Y.; Niu, Y.; Liu, X.; Li, X.; Zhao, J.; Jin, Q.; Yang, W. High-Throughput Profiling of Alpha Interferon- and Interleukin-28B-Regulated MicroRNAs and Identification of let-7s with Anti-Hepatitis C Virus Activity by Targeting IGF2BP. *J. Virol.* **2013**, *87*, 9707–9718. [CrossRef] [PubMed]
324. Matsuura, K.; Aizawa, N.; Enomoto, H.; Nishiguchi, S.; Toyoda, H.; Kumada, T.; Iio, E.; Ito, K.; Ogawa, S.; Isogawa, M.; et al. Circulating let-7 Levels in Serum Correlate with the Severity of Hepatic Fibrosis in Chronic Hepatitis C. *Open Forum Infect. Dis.* **2018**, *5*, ofy268. [CrossRef] [PubMed]
325. Matsuura, K.; De Giorgi, V.; Schechterly, C.; Wang, R.Y.; Farci, P.; Tanaka, Y.; Alter, H.J. Circulating let-7 levels in plasma and extracellular vesicles correlate with hepatic fibrosis progression in chronic hepatitis C. *Hepatology* **2016**, *64*, 732–745. [CrossRef] [PubMed]
326. Escalera-Cueto, M.; Medina-Martínez, I.; del Angel, R.; Berumen, J.; Gutiérrez-Escolano, A.L.; Yocupicio-Monroy, M. Let-7c overexpression inhibits dengue virus replication in human hepatoma Huh-7 cells. *Virus Res.* **2015**, *196*, 105–112. [CrossRef]
327. Zhang, S.; Gu, D.; Ouyang, X.; Xie, W. Proinflammatory effects of the hemagglutinin protein of the avian influenza A (H7N9) virus and microRNA-mediated homeostasis response in THP-1 cells. *Mol. Med. Rep.* **2015**, *12*, 6241–6246. [CrossRef]
328. Zhu, Z.; Qi, Y.; Ge, A.; Zhu, Y.; Xu, K.; Ji, H.; Shi, Z.; Cui, L.; Zhou, M. Comprehensive Characterization of Serum MicroRNA Profile in Response to the Emerging Avian Influenza A (H7N9) Virus Infection in Humans. *Viruses* **2014**, *6*, 1525–1539. [CrossRef]
329. Ma, Y.-J.; Yang, J.; Fan, X.-L.; Zhao, H.-B.; Hu, W.; Li, Z.-P.; Yu, G.; Ding, X.-R.; Wang, J.-Z.; Bo, X.-C.; et al. Cellular microRNA let-7c inhibits M1 protein expression of the H1N1 influenza A virus in infected human lung epithelial cells. *J. Cell. Mol. Med.* **2012**, *16*, 2539–2546. [CrossRef]

330. Bakre, A.; Mitchell, P.; Coleman, J.K.; Jones, L.P.; Saavedra, G.; Teng, M.; Tompkins, S.; Tripp, R.A. Respiratory syncytial virus modifies microRNAs regulating host genes that affect virus replication. *J. Gen. Virol.* **2012**, *93*, 2346–2356. [CrossRef]
331. Thornburg, N.; Hayward, S.L.; Crowe, J.E. Respiratory Syncytial Virus Regulates Human MicroRNAs by Using Mechanisms Involving Beta Interferon and NF-κB. *mBio* **2012**, *3*, e00220–e00232. [CrossRef]
332. Bakre, A.A.; Harcourt, J.L.; Haynes, L.M.; Anderson, L.J.; Tripp, R.A. The Central Conserved Region (CCR) of Respiratory Syncytial Virus (RSV) G Protein Modulates Host miRNA Expression and Alters the Cellular Response to Infection. *Vaccines* **2017**, *5*, 16. [CrossRef]
333. Chen, B.; Han, J.; Chen, S.; Xie, R.; Yang, J.; Zhou, T.; Zhang, Q.; Xia, R. MicroLet-7b Regulates Neutrophil Function and Dampens Neutrophilic Inflammation by Suppressing the Canonical TLR4/NF-κB Pathway. *Front. Immunol.* **2021**, *12*, 653344. [CrossRef] [PubMed]
334. Xie, C.; Chen, Y.; Luo, D.; Zhuang, Z.; Jin, H.; Zhou, H.; Li, X.; Lin, H.; Zheng, X.; Zhang, J.; et al. Therapeutic potential of C1632 by inhibition of SARS-CoV-2 replication and viral-induced inflammation through upregulating let-7. *Signal Transduct. Target. Ther.* **2021**, *6*, 84. [CrossRef] [PubMed]
335. Swaminathan, S.; Suzuki, K.; Seddiki, N.; Kaplan, W.; Cowley, M.J.; Hood, C.L.; Clancy, J.L.; Murray, D.D.; Méndez, C.; Gelgor, L.; et al. Differential Regulation of the Let-7 Family of MicroRNAs in CD4$^{+}$ T Cells Alters IL-10 Expression. *J. Immunol.* **2012**, *188*, 6238–6246. [CrossRef] [PubMed]
336. Zhang, Y.; Yin, Y.; Zhang, S.; Luo, H.; Zhang, H. HIV-1 Infection-Induced Suppression of the Let-7i/IL-2 Axis Contributes to CD4+ T Cell Death. *Sci. Rep.* **2016**, *6*, 25341. [CrossRef] [PubMed]
337. Mukherjee, S.; Akbar, I.; Kumari, B.; Vrati, S.; Basu, A.; Banerjee, A. Japanese Encephalitis Virus-induced *let-7a/b* interacted with the NOTCH—TLR 7 pathway in microglia and facilitated neuronal death via caspase activation. *J. Neurochem.* **2019**, *149*, 518–534. [CrossRef]
338. Bin, S.; Xin, L.; Lin, Z.; Jinhua, Z.; Rui, G.; Xiang, Z. Targeting miR-10a-5p/IL-6R axis for reducing IL-6-induced cartilage cell ferroptosis. *Exp. Mol. Pathol.* **2021**, *118*, 104570. [CrossRef]
339. Tan, Y.; Ge, G.; Pan, T.; Wen, D.; Gan, J. Serum MiRNA panel as potential biomarkers for chronic hepatitis B with persistently normal alanine aminotransferase. *Clin. Chim. Acta* **2015**, *451*, 232–239. [CrossRef]
340. Horii, R.; Honda, M.; Shirasaki, T.; Shimakami, T.; Shimizu, R.; Yamanaka, S.; Murai, K.; Kawaguchi, K.; Arai, K.; Yamashita, T.; et al. MicroRNA-10a Impairs Liver Metabolism in Hepatitis C Virus-Related Cirrhosis Through Deregulation of the Circadian Clock Gene Brain and Muscle Aryl Hydrocarbon Receptor Nuclear Translocator-Like 1. *Hepatol. Commun.* **2019**, *3*, 1687–1703. [CrossRef]
341. Lawrence, T. The Nuclear Factor NF-kappa B Pathway in Inflammation. *Cold Spring Harb. Perspect. Biol.* **2009**, *1*, a001651. [CrossRef]
342. Arthur, J.S.C.; Ley, S.C. Mitogen-activated protein kinases in innate immunity. *Nat. Rev. Immunol.* **2013**, *13*, 679–692. [CrossRef]
343. Li, Q.; Verma, I.M. NF-κB regulation in the immune system. *Nat. Rev. Immunol.* **2002**, *2*, 725–734. [CrossRef] [PubMed]
344. Tong, L.; Lin, L.; Wu, S.; Guo, Z.; Wang, T.; Qin, Y.; Wang, R.; Zhong, X.; Wu, X.; Wang, Y.; et al. MiR-10a* up-regulates coxsackievirus B3 biosynthesis by targeting the 3D-coding sequence. *Nucleic Acids Res.* **2013**, *41*, 3760–3771. [CrossRef] [PubMed]
345. Le Pendu, J.; Abrantes, J.; Bertagnoli, S.; Guitton, J.-S.; Le Gall-Reculé, G.; Lopes, A.M.; Marchandeau, S.; Alda, F.; Almeida, T.; Célio, A.P.; et al. Proposal for a unified classification system and nomenclature of lagoviruses. *J. Gen. Virol.* **2017**, *98*, 1658–1666. [CrossRef] [PubMed]
346. Abrantes, J.; Van Der Loo, W.; Le Pendu, J.; Esteves, P.J. Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): A review. *Vet. Res.* **2012**, *43*, 12. [CrossRef] [PubMed]
347. Hukowska-Szematowicz, B.; Maciejak-Jastrzębska, A.; Blatkiewicz, M.; Maciak, K.; Góra, M.; Janiszewska, J.; Burzyńska, B. Changes in MicroRNA Expression during Rabbit Hemorrhagic Disease Virus (RHDV) Infection. *Viruses* **2020**, *12*, 965. [CrossRef] [PubMed]
348. Wang, Y.; Brahmakshatriya, V.; Lupiani, B.; Reddy, S.M.; Soibam, B.; Benham, A.L.; Gunaratne, P.; Liu, H.-C.; Trakooljul, N.; Ing, N.; et al. Integrated analysis of microRNA expression and mRNA transcriptome in lungs of avian influenza virus infected broilers. *BMC Genom.* **2012**, *13*, 278. [CrossRef]
349. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/dsdna-viruses/w/herpesviridae/1611/genus-mardivirus (accessed on 19 July 2022).
350. Burnside, J.; Morgan, R. Emerging roles of chicken and viral microRNAs in avian disease. *BMC Proc.* **2011**, *5*, S2. [CrossRef]
351. Burnside, J.; Morgan, R.W. Genomics and Marek's disease virus. *Cytogenet. Genome Res.* **2007**, *117*, 376–387. [CrossRef]
352. Yao, Y.; Zhao, Y.; Smith, L.P.; Lawrie, C.; Saunders, N.; Watson, M.; Nair, V. Differential expression of microRNAs in Marek's disease virus-transformed T-lymphoma cell lines. *J. Gen. Virol.* **2009**, *90*, 1551–1559. [CrossRef]
353. Skovgaard, K.; Cirera, S.; Vasby, D.; Podolska, A.; Breum, S.; Dürrwald, R.; Schlegel, M.; Heegaard, P.M. Expression of innate immune genes, proteins and microRNAs in lung tissue of pigs infected experimentally with influenza virus (H1N2). *Innate Immun.* **2013**, *19*, 531–544. [CrossRef]
354. Chen, L.; Song, Y.; He, L.; Wan, X.; Lai, L.; Dai, F.; Liu, Y.; Wang, Q. MicroRNA-223 Promotes Type I Interferon Production in Antiviral Innate Immunity by Targeting Forkhead Box Protein O3 (FOXO3). *J. Biol. Chem.* **2016**, *291*, 14706–14716. [CrossRef] [PubMed]

355. Wang, Y.; Brahmakshatriya, V.; Zhu, H.; Lupiani, B.; Reddy, S.M.; Yoon, B.-J.; Gunaratne, P.H.; Kim, J.H.; Chen, R.; Wang, J.; et al. Identification of differentially expressed miRNAs in chicken lung and trachea with avian influenza virus infection by a deep sequencing approach. *BMC Genom.* **2009**, *10*, 512. [CrossRef] [PubMed]
356. Li, D.; Xue, M.; Wang, C.; Wang, J.; Chen, P. Bursopentine as a Novel Immunoadjuvant Enhances both Humoral and Cell-Mediated Immune Responses to Inactivated H9N2 Avian Influenza Virus in Chickens. *Clin. Vaccine Immunol.* **2011**, *18*, 1497–1502. [CrossRef] [PubMed]
357. Rima, B.; Balkema-Buschmann, A.; Dundon, W.G.; Duprex, P.; Easton, A.; Fouchier, R.; Kurath, G.; Lamb, R.; Lee, B.; Rota, P.; et al. ICTV Virus Taxonomy Profile: Paramyxoviridae. *J. Gen. Virol.* **2019**, *100*, 1593–1594. [CrossRef] [PubMed]
358. Murray, K.; Selleck, P.; Hooper, P.; Hyatt, A.; Gould, A.; Gleeson, L.; Westbury, H.; Hiley, L.; Selvey, L.; Rodwell, B.; et al. A Morbillivirus that Caused Fatal Fisease in Horses and Humans. *Science* **1995**, *268*, 94–97. [CrossRef] [PubMed]
359. Stewart, C.R.; Marsh, G.A.; Jenkins, K.A.; Gantier, M.P.; Tizard, M.L.; Middleton, D.; Lowenthal, J.W.; Haining, J.; Izzard, L.; Gough, T.J.; et al. Promotion of Hendra Virus Replication by MicroRNA 146a. *J. Virol.* **2013**, *87*, 3782–3791. [CrossRef]
360. Cowled, C.; Foo, C.-H.; Deffrasnes, C.; Rootes, C.L.; Williams, D.T.; Middleton, D.; Wang, L.-F.; Bean, A.G.D.; Stewart, C.R. Circulating microRNA profiles of Hendra virus infection in horses. *Sci. Rep.* **2017**, *7*, 7431. [CrossRef]
361. Witter, R.L.S.; Schat, K. Diseases of Poultry. In *Marek's Disease*; Agricultural Research Service US Department of Agriculture: Urbana, IL, USA, 2003; pp. 407–465.
362. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/picornaviridae/707/genus-aphthovirus (accessed on 19 July 2022).
363. Mason, P.W.; Grubman, M.J.; Baxt, B. Molecular basis of pathogenesis of FMDV. *Virus Res.* **2003**, *91*, 9–32. [CrossRef]
364. Mason, P.W.; Chinsangaram, J.; Moraes, M.P.; Mayr, G.A.; Grubman, M.J. Engineering better vaccines for foot-and-mouth disease. *Dev. Biol.* **2003**, *114*, 79–88.
365. Stenfeldt, C.; Arzt, J.; Smoliga, G.; LaRocco, M.; Gutkoska, J.; Lawrence, P. Proof-of-concept study: Profile of circulating microRNAs in Bovine serum harvested during acute and persistent FMDV infection. *Virol. J.* **2017**, *14*, 71. [CrossRef]
366. Quinn, S.R.; O'Neill, L. A trio of microRNAs that control Toll-like receptor signalling. *Int. Immunol.* **2011**, *23*, 421–425. [CrossRef] [PubMed]
367. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/arteriviridae/1590/genus-betaarterivirus (accessed on 19 July 2022).
368. Chand, R.J.; Trible, B.R.; Rowland, R.R. Pathogenesis of porcine reproductive and respiratory syndrome virus. *Curr. Opin. Virol.* **2012**, *2*, 256–263. [CrossRef] [PubMed]
369. Hicks, J.A.; Yoo, D.; Liu, H.-C. Characterization of the microRNAome in Porcine Reproductive and Respiratory Syndrome Virus Infected Macrophages. *PLoS ONE* **2013**, *8*, e82054. [CrossRef]
370. Ye, D.; Shen, Z.; Zhou, S. Function of microRNA-145 and mechanisms underlying its role in malignant tumor diagnosis and treatment. *Cancer Manag. Res.* **2019**, *11*, 969–979. [CrossRef]
371. He, T.; Feng, G.; Chen, H.; Wang, L.; Wang, Y. Identification of host encoded microRNAs interacting with novel swine-origin influenza A (H1N1) virus and swine influenza virus. *Bioinformation* **2009**, *4*, 112–118. [CrossRef]
372. International Committee on Taxonomy of Viruses (ICTV). Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-rna-viruses/w/rhabdoviridae/795/genus-lyssavirus (accessed on 19 July 2022).
373. Zhao, P.; Zhao, L.; Zhang, K.; Feng, H.; Wang, H.; Wang, T.; Xu, T.; Feng, N.; Wang, C.; Gao, Y.; et al. Infection with street strain rabies virus induces modulation of the microRNA profile of the mouse brain. *Virol. J.* **2012**, *9*, 159. [CrossRef]
374. Booth, V.; Keizer, D.; Kamphuis, M.B.; Clark-Lewis, A.I.; Sykes, B.D. The CXCR3 Binding Chemokine IP-10/CXCL10: Structure and Receptor Interactions. *Biochemistry* **2002**, *41*, 10418–10425. [CrossRef]
375. Shi, N.; Zhang, X.Y.; Dong, C.; Hou, J.L.; Zhang, M.L.; Guan, Z.H.; Li, Z.Y.; Duan, M. Alterations in microRNA expression profile in rabies virus-infected mouse neurons. *Acta Virol.* **2014**, *58*, 120–127. [CrossRef]
376. Liu, T.; Xu, Z.; Ou, D.; Liu, J.; Zhang, J. The miR-15a/16 gene cluster in human cancer: A systematic review. *J. Cell. Physiol.* **2019**, *234*, 5496–5506. [CrossRef]
377. Indrieri, A.; Carrella, S.; Carotenuto, P.; Banfi, S.; Franco, B. The Pervasive Role of the miR-181 Family in Development, Neurodegeneration, and Cancer. *Int. J. Mol. Sci.* **2020**, *21*, 2092. [CrossRef]
378. Peng, X.; Gao, Q.S.; Zhou, L.; Chen, Z.H.; Lu, S.; Huang, H.J.; Zhan, C.Y.; Xiang, M. microRNAs in avian influenza virus H9N2-infected and non-infected chicken embryo fibroblasts. *Genet. Mol. Res.* **2015**, *14*, 9081–9091. [CrossRef] [PubMed]
379. Guo, X.-K.; Zhang, Q.; Gao, L.; Li, N.; Chen, X.-X.; Feng, W.-H. Increasing Expression of MicroRNA 181 Inhibits Porcine Reproductive and Respiratory Syndrome Virus Replication and Has Implications for Controlling Virus Infection. *J. Virol.* **2013**, *87*, 1159–1171. [CrossRef] [PubMed]
380. Tian, F.; Luo, J.; Zhang, H.; Chang, S.; Song, J. MiRNA expression signatures induced by Marek's disease virus infection in chickens. *Genomics* **2012**, *99*, 152–159. [CrossRef]
381. Payne, L.N.; Nair, V. The long view: 40 years of avian leukosis research. *Avian Pathol.* **2012**, *41*, 11–19. [CrossRef]
382. Ji, J.; Shang, H.; Zhang, H.; Li, H.; Ma, J.; Bi, Y.; Xie, Q. Temporal changes of microRNA gga-let-7b and gga-let-7i expression in chickens challenged with subgroup J avian leukosis virus. *Vet. Res. Commun.* **2017**, *41*, 219–226. [CrossRef] [PubMed]
383. Li, H.; Ji, J.; Xie, Q.; Shang, H.; Zhang, H.; Xin, X.; Chen, F.; Sun, B.; Xue, C.; Ma, J.; et al. Aberrant expression of liver microRNA in chickens infected with subgroup avian leukosis virus. *Virus Res.* **2012**, *169*, 268–271. [CrossRef] [PubMed]

# Publikacja 2 [P-2]

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# MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes

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MicroRNAs (miRs) are a group of small, 17–25 nucleotide, non-coding RNA that regulate gene expression at the post-transcriptional level. To date, little is known about the molecular signatures of regulatory interactions between miRs and apoptosis and oxidative stress in viral diseases. *Lagovirus europaeus* is a virus that causes severe disease in rabbits (*Oryctolagus cuniculus*) called Rabbit Hemorrhagic Disease (RHD) and belongs to the *Caliciviridae* family, *Lagovirus* genus. Within *Lagovirus europaeus* associated with RHD, two genotypes (GI.1 and GI.2) have been distinguished, and the GI.1 genotype includes four variants (GI.1a, GI.1b, GI.1c, and GI.1d). The study aimed to assess the expression of miRs and their target genes involved in apoptosis and oxidative stress, as well as their potential impact on the pathways during *Lagovirus europaeus*—two genotypes (GI.1 and GI.2) infection of different virulences in four tissues (liver, lung, kidneys, and spleen). The expression of miRs and target genes related to apoptosis and oxidative stress was determined using quantitative real-time PCR (qPCR). In this study, we evaluated the expression of miR-21 (*PTEN*, *PDCD4*), miR-16b (*Bcl-2*, *CXCL10*), miR-34a (*p53*, *SIRT1*), and miRs—related to oxidative stress—miR-122 (*Bach1*) and miR-132 (*Nfr-2*). We also examined the biomarkers of both processes (*Bax*, *Bax/Bcl-2* ratio, *Caspase-3*, *PARP*) and *HO-1* as biomarkers of oxidative stress. Our report is the first to present the regulatory effects of miRs on apoptosis and oxidative stress genes in rabbit infection with *Lagovirus europaeus*—two genotypes (GI.1 and GI.2) in four tissues (liver, lungs, kidneys, and spleen). The regulatory effect of miRs indicates that, on the one hand, miRs can intensify apoptosis (miR-16b, miR-34a) in the examined organs in response to a viral stimulus and, on the other hand, inhibit (miR-21), which in both cases may be a determinant of the pathogenesis of RHD and tissue damage. Biomarkers of the *Bax* and *Bax*/*Bcl-2* ratio promote more intense apoptosis after infection with the *Lagovirus europaeus* GI.2 genotype. Our findings demonstrate that miR-122 and miR-132 regulate oxidative stress in the pathogenesis of RHD, which is associated with tissue damage. The *HO-1* biomarker in the course of rabbit hemorrhagic disease

indicates oxidative tissue damage. Our findings show that miR-21, miR-16b, and miR-34a regulate three apoptosis pathways. Meanwhile, miR-122 and miR-132 are involved in two oxidative stress pathways.

KEYWORDS

***Lagovirus europaeus*, RHDV, rabbit hemorrhagic disease (RHD), miRs, apoptosis, oxidative stress, rabbits, biomarker**

## 1 Introduction

MicroRNA molecules (miRs) are single-stranded, non-coding ribonucleic acid sequences of 17–25 nucleotides in length, playing an essential role in the post-transcriptional regulation of gene expression. They are created from double-stranded precursors produced by RNA polymerase II (Bartel, 2004).

Our recent studies indicate the role of miRs as micro-players of great importance in viral infections in humans and animals (Hukowska-Szematowicz et al., 2020; Ostrycharz and Hukowska-Szematowicz, 2022; Hukowska-Szematowicz et al., 2023). miRs can regulate gene expression related to apoptosis (Su et al., 2015) and oxidative stress (Banerjee et al., 2017; Fioravanti et al., 2022) during diseases, including viral ones (Hukowska-Szematowicz et al., 2020; Fioravanti et al., 2022; Ostrycharz and Hukowska-Szematowicz, 2022), and thus affect the activity status of signaling pathways (Chen, 2015; Sadri Nahand et al., 2021).

Apoptosis and oxidative balance maintain cell homeostasis and play an essential role in viral infections (Thomson, 2001; Santus et al., 2022). Many cells undergo apoptosis in response to viral infection, reducing the release of progeny virus. During viral infection, the relative expression of virus-related genes and the activation of innate antiviral response systems lead to an increase in reactive oxygen species (ROS), reactive nitrogen species (RNS), and toxic of by products energy metabolism. The imbalance between ROS and antioxidants in the body leads to oxidative stress, cell death, and tissue/organ damage in the host. ROS and the resulting changes in cellular redox status become one of the inducing factors for cell apoptosis (Valyi-Nagy and Dermody, 2005). Tissue damage related to apoptosis and oxidative stress is an essential element in the pathogenesis of infection with coronaviruses (Gain et al., 2022), herpesviruses (Sun et al., 2023), rhabdoviruses (Verma et al., 2018), and paramyxoviruses (Yang et al., 2023). So far, little is known about the molecular signatures of regulatory interactions between specific miRs and apoptosis and oxidative stress in diseases (Su et al., 2015; Banerjee et al., 2017; Klieser et al., 2019; Sadri Nahand et al., 2021; Fioravanti et al., 2022; Abolfathi et al., 2023). No studies are addressing this scientific problem in viral diseases. Understanding these interactions has diagnostic (searching for potential disease biomarkers) and therapeutic (modulating miR-dependent pathways) potential in the course of acute liver failure (ALF) and organ dysfunction in multi-organ failure (MOF) of viral etiology, which we encounter during *Lagovirus europaeus* infection (Tunon et al., 2003; Esteves et al., 2018; Asim et al., 2020).

*Lagovirus europaeus* (*L. europaeus*) genotypes GI.1 and GI.2 are etiological factors for Rabbit Hemorrhagic Disease (RHD), belonging to the *Caliciviridae* family, *Lagovirus* genus (Le Pendu et al., 2017). *Lagovirus europaeus* is a single-stranded RNA virus (Le Pendu et al., 2017). The *L. europaeus* GI.1 genotype includes four variants (GI.1a/GI.1b, GI.1c, and GI.1d), with the GI.1a variant being highly pathogenic and most often causing an acute form of the disease, with mortality rates of 90%–100%. However, the *L. europaeus* GI.2 genotype evokes per-acute, acute, subacute chronic, and subclinical forms of the disease with variable mortality of 50%–80%, depending on the strain (Rocchi and Dagleish, 2018).

Although the first RHD epidemic occurred 39 years ago, its pathogenesis mechanisms are still not fully understood (Liu et al., 1984). During RHD, many pathological changes occur in the rabbit's organs, especially the liver (the site of virus replication), lung, spleen, and kidney (Abrantes et al., 2012). The highest titer of *L. europaeus* is found in the liver, lung, spleen, kidneys, trachea, and bone marrow, which are the most frequently assessed organs in the diagnostic process (Abrantes et al., 2012; Rocchi and Dagleish, 2018). The main changes in RHD are acute liver, spleen, lung, and kidney inflammation. Pathological changes include enlargement of the liver, spleen (splenomegaly), and kidneys, as well as hyperemia and pulmonary edema (results in severe failure manifesting as shortness of breath, worsening by death; Abrantes et al., 2012; Rocchi and Dagleish, 2018). In these organs, inflammatory foci rich in neutrophils and T and B lymphocytes are observed, as well as an increase in the expression of the inflammatory biomarker—miR-155-5p—in the liver, lungs, and kidneys and a decrease in the spleen (Hukowska-Szematowicz et al., 2023). It is known that systemic hemorrhagic diathesis and intravascular coagulation syndrome (DIC) can lead to death in rabbits (Trzeciak-Ryczek et al., 2015). Innate and adaptive immunity also play an essential role in the pathogenesis of RHD (Hukowska-Szematowicz, 2013), including peripheral blood leukocytes (Trzeciak-Ryczek et al., 2016, 2017; Semerjyan et al., 2019). After *L. europaeus* infection, the level of pro-inflammatory and anti-inflammatory cytokines increases in the serum, liver, and spleen (Teixeira et al., 2012; Trzeciak-Ryczek et al., 2016; Ostrycharz et al., 2021; O'Toole et al., 2022).

It has also been shown that apoptosis (Alonso et al., 1998; Jung et al., 2000; San-Miguel et al., 2006; Ni et al., 2009; Garcia-Lastra et al., 2010; Marques et al., 2010; Tunon et al., 2011; Chen et al., 2018), necrosis (Park et al., 1995), and oxidative stress (San-Miguel et al., 2006; Hu et al., 2020) are elements in the pathogenesis of RHD. The progression of the disease correlates with increased apoptosis not only of liver cells (Alonso et al., 1998; Jung et al., 2000; Garcia-Lastra et al., 2010; Tunon et al., 2011; Vallejo et al., 2014; Chen et al., 2018;

Bebnowska et al., 2024) but also of lung, kidney, heart, and spleen cells (Alonso et al., 1998; San-Miguel et al., 2006; Ni et al., 2009; Bebnowska et al., 2023), as well as necrosis (Park et al., 1995). Further studies showed apoptosis of T and B lymphocytes in the liver, spleen, and granulocytes and lymphocytes in peripheral blood (Marques et al., 2010; Niedzwiedzka-Rystwej and Deptula, 2012; Hukowska-Szematowicz, 2013; Niedzwiedzka-Rystwej et al., 2013a,b). Hukowska-Szematowicz et al. (2020) showed that after infection of rabbits with the *L. europaeus* GI.1 genotype, the expression of proapoptotic miR-16-5p increased in the liver. The same studies did not show changes in the liver's expression of miR-122-5p (potentially involved in oxidative stress) but were detected in the serum. In the same year, Hu et al. (2020) showed that oxidative stress plays a crucial role in liver damage, especially in early RHDV infection (18 h p.i.). The nuclear translocation of Keap1-NF-kB is crucial for suppressing the NFR-2-ARE pathway in hepatocytes. Upregulation of Nrf-2 protein levels in liver cell nuclei by tert-butylhydroquinone (tBHQ) delayed the death of rabbits with RHDV infection. Earlier in 2006, San-Miguel et al. (2006) showed that oxidative stress is the main apoptosis pathway in RHD.

The above findings indicate that apoptosis and oxidative stress play an essential role in the pathogenesis of RHD. However, so far [apart from the research of Hukowska-Szematowicz et al. (2020) and Ostrycharz and Hukowska-Szematowicz (2022)], nothing is known about the molecular signatures of regulatory interactions between miRs and apoptosis and oxidative stress in *L. europaeus* infection/pathogenesis of RHD.

Therefore, we undertook research aimed at assessing the expression of miRs and their target genes related to apoptosis and oxidative stress and assessing their potential impact on the pathways in infection with *L. europaeus*—two genotypes (GI.1 and GI.2) with different virulence in four tissues (liver, lungs, kidneys, and spleen). Based on *in silico* analysis and our previous literature data (Hukowska-Szematowicz et al., 2020; Ostrycharz and Hukowska-Szematowicz, 2022), we selected miRs/target genes in the apoptosis and oxidative stress processes. For apoptosis: miR-21 (target genes—phosphatase and tensin homolog [*PTEN*] and programmed cell death factor 4 [*PDCD4*]), miR-16b (antiapoptotic B cell lymphoma 2 [*Bcl-2*] and C-X-C motif chemokine ligand 10 [*CXCL10*]), miR-34a (p53 protein gene (*p53*), and silent information regulator 1 [*SIRT1*]). For oxidative stress: miR-122 (BTB domain and CNC homolog 1 [*Bach1*]); miR-132 (nuclear factor erythroid 2-related factor 2 [*Nfr-2*]). We also examined biomarkers of both processes in rabbits infected with *L. europaeus* GI.1 and GI.2 genotypes: *Bax*, *Bax/Bcl-2* ratio, poly (ADP-ribose) polymerase (*PARP*), *Caspase-3*, and heme oxygenase-1 (*HO-I*) as a biomarker of oxidative stress.

# 2 Materials and methods

## 2.1 Ethical statements

The experiment was conducted in the experimental animal facility of the Pomeranian Medical University (PUM) in Szczecin. The experiment was approved by the Local Ethical Committee for Animal Experiments in Poznań, Poland (no. 51/2022). Rabbits were maintained according to European Union and national guidelines for animal experimentation.

## 2.2 Viruses

Two viruses were used to provoke infection in rabbits—*L. europaeus* (Fitzner et al., 2021). *Lagovirus europaeus* genotype GI.1, variant GI.1a, was named BBI (Poland, 2017; GenBank accession no. MG602005) and *L. europaeus* genotype GI.2 was named PIN (Poland, 2018; GenBank accession no. MN853660). Both viruses were titer-determined by the hemagglutination (HA) assay. The infectious titer of the *L. europaeus* GI.1 genotype inoculum (1 mL) was determined to be 0.5 u/mL (1 HA unit corresponds to $10^4$ particles per ml). The infectious titer of the *L. europaeus* GI.2 genotype inoculum (1 mL) was determined to be 2.048 u/mL (1 HA unit corresponds to $10^4$ particles per ml). The viruses have been prepared at the National Reference Laboratory for Rabbit Hemorrhagic Disease (RHD) and the Department of Foot and Mouth Disease, the National Veterinary Research Institute—State Research Institute in Zduńska Wola, Poland.

## 2.3 Experimental model

The study involved 30 European rabbits, *Oryctolagus cuniculus*—Crl:KBL (NZW)/052, both sexes (50:50 ratio), 6-month-old, body weights 4.0–4.5 kg, purchased from AnimaLab Limited Liability Company (branch in Poland, Poznań), and a 3-week adaptation period occurred after the animals were delivered to the university's experimental facility. The animals had constant access to entertainment in their living environment, such as wooden chews and tunnels filled with hay. The recommended national guideline standards developed per the European Union Directive during the experiment ensured appropriate zoo-technical conditions. Autonomous air conditioning provided a temperature of 22°C (±1°C), 50%–60% humidity, and 15–20 air changes per hour. The animals stayed in rooms with artificial lighting, automatically controlled (12 h of light/12 h of darkness), and red night lighting. Both water and food were available to the animals *ad libitum*. Animals were randomly divided into three groups. The control group ($n = 10$) was injected intramuscularly with PBS (phosphate-buffered saline; 1 mL) as a placebo. Rabbits in the infection groups were injected intramuscularly with 1 mL virus. Group 2 received the *L. europaeus* GI.1 genotype (named BBI strain, Poland 2017). Group 3 received the *L. europaeus* GI.2 genotype (PIN strain, Poland 2018). Administration of the antigen in the infected rabbit group and PBS in the control group was marked as the beginning of the experiment. After this time, the animals' health was monitored by measuring body temperature and registering clinical signs. After the infected animals showed severe symptoms of the disease, confirmed by a doctor, they were subjected to a euthanasia procedure as a result of intravenous administration of an anesthetic agent (ketamine 35–50 mg/kg; xylazine 5–10 mg/kg), followed by administration of the drug causing cardiac arrest, sodium pentobarbital (at 240 mg/kg).

## 2.4 Tissue sample collection

Tissue samples from the liver, lungs, spleen, and kidneys were obtained from the infected animals (20 rabbits) post-mortem or euthanasia, clinically defined. The organs were taken from the healthy control animals (10 rabbits) after euthanasia (as described above).

Each tissue sample was washed in cold PBS and immediately placed in liquid nitrogen. Tissue samples were stored at −80°C until mRNA/miRs extraction.

## 2.5 Selection and *in silico* prediction of miRs target genes involved in the apoptosis and oxidative stress in *Oryctolagus cuniculus*

The process included several stages. In the first stage, miRs involved in apoptosis and oxidative stress processes were selected (Hukowska-Szematowicz et al., 2020; Ostrycharz and Hukowska-Szematowicz, 2022). At this stage, the miRTarBase database (Chou et al., 2018) was used to select miRs using various search strategies (by miRs, by target gene, by pathway/process, by validated methods, by disease). The criterion for selecting miRs was validation methods: strong evidence and our previous literature data (Hukowska-Szematowicz et al., 2020; Ostrycharz and Hukowska-Szematowicz, 2022). *Homo sapiens* miRs with a described role in the apoptosis process (miR-21, miR-16b, miR-34a) and oxidative stress (miR-122, miR-132) were selected (Chou et al., 2018; Hukowska-Szematowicz et al., 2020; Ostrycharz and Hukowska-Szematowicz, 2022). In the second stage, the miRTarBase database (Chou et al., 2018) and miRDB database (Chen and Wang, 2020) were used to select target genes (for chosen miRs) previously validated by RT-qPCR, Western blot, or a reporter assay in other species (validation methods: strong evidence). Next, the set of genes was used to conduct a gene ontology (GO) analysis via a GO enrichment analysis powered by protein annotation through evolutionary relationship (PANTHER; Ashburner et al., 2000). The analysis included: analysis type: PANTHER overrepresentation test; reference list: all *Homo sapiens* genes in the database; annotation data set: GO biological process complete; test type: Fisher's exact; and correction: calculate false discovery rate (FDR). From all the processes with FDR $p < 0.05$, those correlated with apoptosis, oxidative stress, liver diseases, and multi-organ dysfunction in humans and animals were used for further steps. The 3′-UTR sequences of the *Oryctolagus cuniculus* genes involved in the selected processes were assessed to determine if they featured binding sites for miR-21-5p, miR-16-5p, miR-34a-5p, miR-122-5p, and miR-132-5p using the TargetScan database (Agarwal et al., 2015). In the third stage, to verify the importance of miR-21-5p, miR-16-5p, miR-34a-5p, miR-122-5p, and miR-132-5p in *L. europaeus* infection, an *in silico* analysis of putative target genes was conducted. Due to the inability to use one database to demonstrate the miR–mRNA interactions in *Oryctolagus cuniculus,* the following approach was selected: (i) mature sequences of these miRs in *Oryctolagus cuniculus* and *Homo sapiens* were compared, and no differences were found. We decided to use miRTarBase (Chou et al., 2018), which lists genes with validated miR–mRNA interactions by RT-qPCR or luciferase assays in *Homo sapiens*. Ultimately, five lists containing 53 for miR-21-5p, 36 for miR-16b-5p, 80 for miR-34a-5p, 18 target genes for miR-122-5p, and 18 for miR-132-5p were created; (ii) there was an attempt to determine the processes related to RHD that miR-21-5p, miR-16-5p, miR-34a-5p, miR-122-5p, and miR-132-5p might regulate. For this purpose, a GO analysis was conducted on the putative target genes for every miR separately. Thus, 52 processes for miR-21-5p, 1,152 processes for miR-16b-5p, 53 processes for miR-34a-5p, 80 processes for miR-122-5p, and 50 processes for miR-132-5p were identified. From these three groups, processes that correlated with RHD pathogenesis, ALF, and MOF were chosen for further analysis: 12 for miR-21-5p, 14 for miR-16b-5p, 14 for miR-34-5p, 7 for miR-122-5p, and 8 for miR-132-5p. At this step, all analyses were performed based on miR–mRNA interactions in *Homo sapiens*. The TargetScan database confirmed whether these regulations might also occur in *Oryctolagus cuniculus*. This tool enabled us to verify if the predicted binding sites were conserved in *Oryctolagus cuniculus*. Genes engaged in RHD, ALF, and MOF processes were selected. Each miR–3′-UTR interaction was checked independently. The TargetScan analysis revealed that 25 out of 40 genes for ocu-miR-21-5p, −30 out of 46 genes for ocu-miR-16b-5p, 12 out of 36 genes for ocu-miR-34-5p, 11 out of 38 genes for ocu-miR-122-5p, and 10 out of 36 genes for ocu-miR-132-5p have binding sites in 3′-UTR in *Oryctolagus cuniculus* genes. Selected miRs/target genes are presented in Table 1.

TABLE 1 Selected studied miRs/target genes involved in apoptosis and oxidative stress.

| miRs | Target genes | Gene product | References used to select target genes |
|---|---|---|---|
| **Apoptosis** | | | |
| miR-21 | *PTEN* | Phosphatase and tensin homolog | Buscaglia and Li (2011), Wei et al. (2013), Ghafouri-Fard et al. (2021), and He et al. (2021) |
| | *PDCD4* | Programmed cell death factor 4 | Qiu et al. (2013) and He et al. (2021) |
| miR-16b | *Bcl-2* | B-cell lymphoma 2 | Cimmino et al. (2005), Wu et al. (2011), Gao and Zhao (2020) and Hukowska-Szematowicz et al. (2020) |
| | *CXCL10* | C-X-C motif chemokine ligand 10 | Gao and Zhao (2020) and Lai et al. (2021) |
| miR-34a | *SIRT1* | Silent information regulator 1 | Yamakuchi and Lowenstein (2009), Hermeking (2010), Sharma et al. (2018), and Jiang et al. (2019) |
| | *p53* | Protein *p53* | |
| **Oxidative stress** | | | |
| miR-122 | *Bach1* | BTB domain and CNC homolog 1 | Shan et al. (2007), Klieser et al. (2019), and Hukowska-Szematowicz et al. (2020) |
| miR-132 | *Nrf-2* | Nuclear factor erythroid 2-related factor 2 | Wasik et al. (2017), Zhou et al. (2020), Herengt et al. (2021), Xu et al. (2021), and Mao et al. (2022) |

## 2.6 miRs and mRNA isolation from tissues

Total RNA, including miRs, was extracted from 50 mg of the infected and healthy rabbits' liver, lung, kidney, and spleen tissues using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. RNA concentration and quality were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States).

## 2.7 miRs polyadenylation and reverse transcription reaction

The reverse transcription (RT) reaction was performed using a miRCURY LNA RT Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. In the tissue samples, 5 ng/μL of total RNA was used for cDNA synthesis. The cycling conditions for the RT reaction were as follows: incubation for 60 min at 42°C, heat inactivation of the reverse transcriptase for 5 min at 95°C, and immediate cooling to 4°C. The obtained cDNA was stored at −20°C until further experiments.

## 2.8 mRNA polyadenylation and reverse transcription reaction

cDNA synthesis was carried out using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, United States) according to the manufacturer's protocol. Oligo(dT) and random hexamers were used to perform the reaction to increase its yield. The cycling conditions for the RT reaction were as follows: incubation for 5 min at 25°C followed by 60 min at 42°C, termination of the reaction by heating at 70°C for 5 min, and immediate cooling to 4°C. The obtained cDNA was stored at −20°C until further experiments.

## 2.9 Quantification of miRs in tissue samples using quantitative real-time PCR and data analysis

The expression of miRs (Table 2) was determined by the quantitative real-time PCR (qPCR) reaction in tissue samples using the miRCURY LNA miRNA PCR Assay (Qiagen, Hilden, Germany) and the miRCURY LNA SYBR Green PCR Kit, according to the manufacturer's instructions. cDNA templates were diluted 60-fold in RNase-free water. The amplification of the selected miRs was performed using a real-time PCR system. The qPCR data were normalized using ocu-miR-103a-3p as a stable reference gene. This gene was chosen based on a previous study (Hukowska-Szematowicz et al., 2020) and evaluation under experimental conditions using the geNorm, NormFinder, and BestKeeper algorithms (Hukowska-Szematowicz et al., 2020). Fluorescence data were analyzed using a real-time PCR system, and the expression of miRs, normalized to an endogenous reference, was determined using the $2^{-\Delta\Delta Ct}$ formula.

TABLE 2 Sequences of the tested miRs of *Oryctolagus cuniculus* (ocu).

| miRNAs | Sequence |
|---|---|
| **miRNAs tested** | |
| ocu-miR-21-5p | 5′-UAGCUUAUCAGACUGAUGUUGACU-3′ |
| ocu-miR-16b-5p | 5′-UAGCAGCACGUAAAUAUUGGCGU-3′ |
| ocu-miR-34a-5p | 5′-UGGCAGUGUCUUAGCUGGUUGU-3′ |
| ocu-miR-122-5p | 5′-UGGAGUGUGACAAUGGUGUUUG-3′ |
| ocu-miR-132-5p | 5′-ACCAUGGCUUUCGAUUGUUACU-3′ |
| **reference miRNAs** | |
| ocu-miR-103a-3p | 5′-AGCAGCAUUGUACAGGGCUAUGA-3′ |

## 2.10 Quantification of mRNAs in tissue samples using quantitative real-time PCR and data analysis

Computational tools for online Primer-BLAST (2024) and Beacon Designer (2024) were used to design specific primers for qPCR analysis for the tested target genes (Table 3). To validate the designed primers, temperature gradient PCR was performed using the Color OptiTaq PCR Master Mix (2×) kit (Euryx, Poland), followed by agarose gel electrophoresis. It allowed us to select the optimal annealing temperature of the primers and check whether non-specific products were formed. After checking the primers, optimization of the real-time PCR reaction was performed. An optimized approach was used to sequentially optimize primer sequences, annealing temperatures, primer concentrations, and a range of cDNA concentrations for each gene tested. Using the calibration method and the standard curve allowed us to obtain a standard curve. Using the optimal annealing temperature and primer concentration for each primer pair, we used serial dilutions of the same cDNA (1:2, 1:4, 1:8, 1:16, and 1:32 dilutions). Dilutions were made from a 10 ng/μL concentration, which was recommended as the highest possible concentration by the manufacturing protocol (HOT FIREPol® EvaGreen® qPCR Supermix, 5×; Solis BioDyne, Estonia). We noticed that different primer pairs had different optimal cDNA concentration ranges for each gene, giving rise to the most significant coefficient of determination (R2) and optimal efficiencies (100% ± 5%). In addition, the specificity of the primers was verified experimentally by melting curve analysis. According to the manufacturer's instructions, the qPCR reaction in tissue samples determined the mRNA expression using the HOT FIREPol® EvaGreen® qPCR Supermix, 5× (Solis BioDyne, Estonia). The amplification of the selected mRNAs was performed using the Quant Studio 5 Real-Time PCR system (Applied Biosystems, United States). The qPCR data were normalized using the reference gene 18S. Fluorescence data were analyzed using a real-time PCR system, and the expression of mRNA, normalized to an endogenous reference, was determined using the $2^{-\Delta\Delta Ct}$ formula. A melting curve analysis was performed each time.

## 2.11 Statistical analysis

All results were statistically analyzed using STATISTICA PL Version 13. The Shapiro–Wilk test was performed to determine the

TABLE 3 qPCR primers used in the study target gene expression.

| Gene | GenBank accession no. | Primers | | $T_a$ (°C) | Amplicon length (bp) | $T_m$ of the amplification products (°C) |
|---|---|---|---|---|---|---|
| | | Forward | Reverse | | | |
| *PTEN* | XM_008270133.3 | 5′-GCGGAACTTGCAATCCTCAG-3′ | 5′-TCGTGTGGGTCCTGAATTGG-3′ | 60 | 77 | 81 |
| *PDCD4* | XM_017348553.2 | 5′-GAATAACCGTGCCAACCAGTCC-3′ | 5′-CTTTCCCTCCTGCACCACCTTTC-3′ | 60 | 102 | 85 |
| *Bcl-2* | XM_008261439.2 | 5′-TGTGTGTGGAGAGCGTCAAC-3′ | 5′-AGTTCCACGAAGGCATCCCAG-3′ | 62 | 133 | 87.5 |
| *CXCL10* | XM_002717106.4 | 5′-AGCATTTAGCAAGGAAAGGTCCAG-3′ | 5′-AGAAGGGAAGTGTGGCAGAGG-3′ | 60 | 110 | 83.8 |
| *p53* | XM_008270660.3 | 5′-TGACGGAAGTTGTCAGACGC-3′ | 5′-TACAGTCAGAGCCAACCTCGG-3′ | 60 | 183 | 89.8 |
| *SIRT1* | XM_002718460.4 | 5′-AGTAAGCGGCTCGATGGTAATCAG-3′ | 5′-TCCAGTTCCTCCAGGTCTCTCTG-3′ | 63 | 249 | 85.5 |
| *Bach1* | XM_002716782.4 | 5′-ACTCTACCAGAAGAGGTGACAG-3′ | 5′-TGAGAAACTGAAAGCAGGACTC-3′ | 60 | 160 | 78 |
| *Nrf-2* | XM_051849401.1 | 5′-AGAAACAGAACACAAGGACATGG-3′ | 5′-TTGGGCTGGCTGAATTGGG-3′ | 60 | 241 | 85.7 |
| *Bax* | XM_008252361.3 | 5′-ACATGGAGCTACAGAGGATGATCG-3′ | 5′-AGCGTCCAGCCCATAATAGTCC-3′ | 61 | 205 | 86 |
| *Caspase-3* | NM_001082117.1 | 5′-AACTTTTCATTATTCAGGCTTGCCG-3′ | 5′-TCAACCCCACTGTCTGTCTCG-3′ | 58 | 70 | 84.2 |
| *PARP* | XM_008268352.3 | 5′-CGGACAAGCTCTACCGAGTG-3′ | 5′-CATCGAACATGGGCGACTGC-3′ | 60 | 123 | 88 |
| *HO-1* | XM_051846030.1 | 5′-ACTGCCGAGGGTTTTAAGCTGG-3′ | 5′-ACCGGGTTCTCCTTGTTGTGC-3′ | 60 | 92 | 85 |
| *18S* | NR_033238.1 | 5′-ATCAGATACCGTCGTAGTTC-3′ | 5′-TTCCGTCAATTCCTTTAAG-3′ | 60 | 167 | 88 |



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distribution of the analyzed variables. Depending on the obtained distribution, the Student's *t*-test for data with a normal distribution and the Mann–Whitney *U* test for non-parametric data were used. The one-way ANOVA was performed for parametric data to determine possible changes in all miRs or mRNAs; however, the Kruskal–Wallis test was used for non-parametric data. The data are presented in graphs as average values ± standard error of the mean (SEM). The values for which the *p*-value does not exceed 5% ($p \leq 0.05$) were considered significant statistical differences. Correlation analyses were performed using the non-parametric Spearman's rank method. Results were considered statistically significant if $p < 0.05$.

# 3 Results

## 3.1 Clinical signs of disease and post-mortem analysis

Animals infected with both *L. europaeus* genotypes—GI.1 and GI.2—showed clinical signs consistent with RHD (apathy, dyspnea, body temperature > 41°C, anorexia, and neurological symptoms). Two rabbits after the *L. europaeus* GI.2 infection died asymptomatically. Mortality after infection with *L. europaeus* in both genotypes was 90% to 60 h p.i. The *L. europaeus* GI.2 genotype was more virulent, causing 90% mortality in rabbits within 32 h p.i. and a fulminant course of the disease. The disease ranged from per-acute to acute in animals infected with this *L. europaeus* genotype. Whereas, after *L. europaeus* GI.1 infection, the mortality rate was −10% to 32 h p.i., 40% to 36 h p.i., and 40% between 56 and 60 h p.i.

## 3.2 miRs expression levels and its downstream targets involved in apoptosis during *Lagovirus europaeus* GI.1 and GI.2 genotype infection in rabbits

We analyzed the expression of miRs involved in apoptosis (miR-21, miR-34a, and miR-16b) in four tissues (liver, lung, spleen, and kidney) in rabbits infected with *L. europaeus* GI.1 and GI.2 genotypes and its downstream targets.

Increased miR-21 expression was observed after infection with *L. europaeus*—two genotypes (GI.1 and GI.2) in all four tissues examined. In the liver, miR-21 expression was substantially increased in both *L. europaeus* GI.1 (13.2-fold changes vs. control, $p = 0.0002$) and GI.2 p.i. (8.4-fold changes vs. control, $p = 0.0002$; Figure 1A). In cases of infection with GI.1 and GI.2, upregulation of miR-21 was accompanied by decreased levels of *PTEN* (8.5-fold reduction, 88% reduction, vs. control, $p = 0.001$ and 6-fold reduction, 84% reduction, vs. control, $p = 0.002$, respectively; Figure 1B) and *PDCD4* (71.4-fold reduction, 98.6% reduction, $p = 0.0002$ and 273.4-fold reduction, 99.6% reduction, $p = 0.0001$, respectively; Figure 1C). In the lung, we also observed the upregulation of miR-21 for both genotypes compared to the control group (3.2-fold change, $p < 0.0001$ for GI.1 and 2.6-fold change, $p = 0.0003$ for GI.2; Figure 1D). In contrast to the liver, in lung tissue compared to the control, the level of *PTEN* was upregulated in GI.1 and GI.2 (1.4-fold change, $p = 0.04$ and 1.5-fold change, $p = 0.03$, respectively; Figure 1E). While no changes in *PDCD4* expression levels were noted (Figure 1F). The miR-21 level was significantly increased also in the kidneys (Figure 1G) and spleen (Figure 1J) of the rabbits infected with both *L. europaeus* genotypes, with a 3.6-fold ($p = 0.002$) and 6.1-fold ($p = 0.0006$) increase, respectively, vs. control in kidneys and a 6.1-fold change ($p = 0.0002$) and a 4.5-fold change ($p = 0.0002$), respectively, in spleen. Simultaneously, in both tissues, downregulation of *PTEN* [15.6-fold reduction, 94% reduction, $p = 0.0002$ in kidney (Figure 1H) and 3.4-fold reduction, 70.5% reduction, $p = 0.002$ in spleen (Figure 1K)] and *PDCD4* [30.4-fold reduction, 97% reduction, $p = 0.0002$ in kidney (Figure 1I) and 4.4-fold reduction, 23% reduction, $p = 0.002$ in spleen (Figure 1L)] was detected during GI.1 infection. Furthermore, during infection with the second *L. europaeus* GI.2 genotype, the expression of *PTEN* and *PDCD4* was downregulated in the kidneys and spleen compared to healthy rabbits. *PTEN* [4.6-fold reduction, 78% reduction, $p = 0.002$ in kidney (Figure 1H)] and 3.7-fold reduction, 73% reduction, $p = 0.0003$ in spleen (Figure 1K). *PDCD4* [3.3-fold reduction, 70% reduction, $p = 0.01$ in kidney (Figure 1I) and 18.7-fold reduction, 95% reduction, $p = 0.0002$ in spleen (Figure 1L)].

The expression level of miR-16b was significantly higher in tissues (liver, kidney, and spleen) in both infected groups of rabbits, excluding the lungs. In the liver, we observed a 3.5-fold change, $p = 0.002$ for GI.1, and a 2.3-fold change, $p = 0.01$ for GI.2 (Figure 2A). The expression of miR-16b in the lung was unchanged (Figure 2D). In the kidneys, the expression of miR-16b for GI.1 and GI.2 increased 1.3-fold, $p = 0.023$, and 1.9-fold, $p = 0.19$, respectively (Figure 2G). Whereas in the spleen during *L. europaeus* GI.1 infection, we observed the highest increase in miR-16b levels, which was a 5-fold change ($p = 0.0002$), while during GI.2, expression was 2.2-fold higher ($p = 0.0003$) compared to the control (Figure 2J).

Upregulation of miR-16b was associated with downregulation of Bcl-2 in both infected groups compared to controls in the liver (Figure 2B), kidneys (Figure 2H), and spleen (Figure 2K). However, the expression of *Bcl-2* mRNA in the lung was unchanged (Figure 2E). In liver tissue, the reduction of *Bcl-2* was 16.2-fold, 94% reduction, $p = 0.02$ for GI.1 and 49.4-fold, 98% reduction, $p = 0.004$ for GI.2 (Figure 2B). For kidney tissue, the reduction was accordingly 16.7-fold reduction, 94% reduction, $p = 0.0004$ and 4.6-fold reduction, 78% reduction, $p = 0.005$ for GI.1 and GI.2 (Figure 2H). The minor decrease in *Bcl-2* expression was observed in the spleen and amounted to a 1.6-fold reduction (39% reduction, $p = 0.03$) for GI.1 and a 5.7-fold reduction (82% reduction, $p = 0.001$) for GI.2 compared to the control (Figure 2K). In the case of *CXCL10*, another target gene for miR-16b, significantly enhanced expression was noted in all tested tissues (Figures 2C,F,I,L). The highest expression was observed in the kidney during the GI.2 infection, and it amounted to a 162.6-fold change, $p = 0.0002$ (Figure 2I). On the other hand, the lowest increase in expression was also in the kidney, but in the *L. europaeus* GI.1-infected group, it amounted to a 6.2-fold change, $p = 0.03$ compared to the control (Figure 2I).

In the case of miR-34a in the liver, only a change in expression was demonstrated during *L. europaeus* GI.1 genotype infection and was

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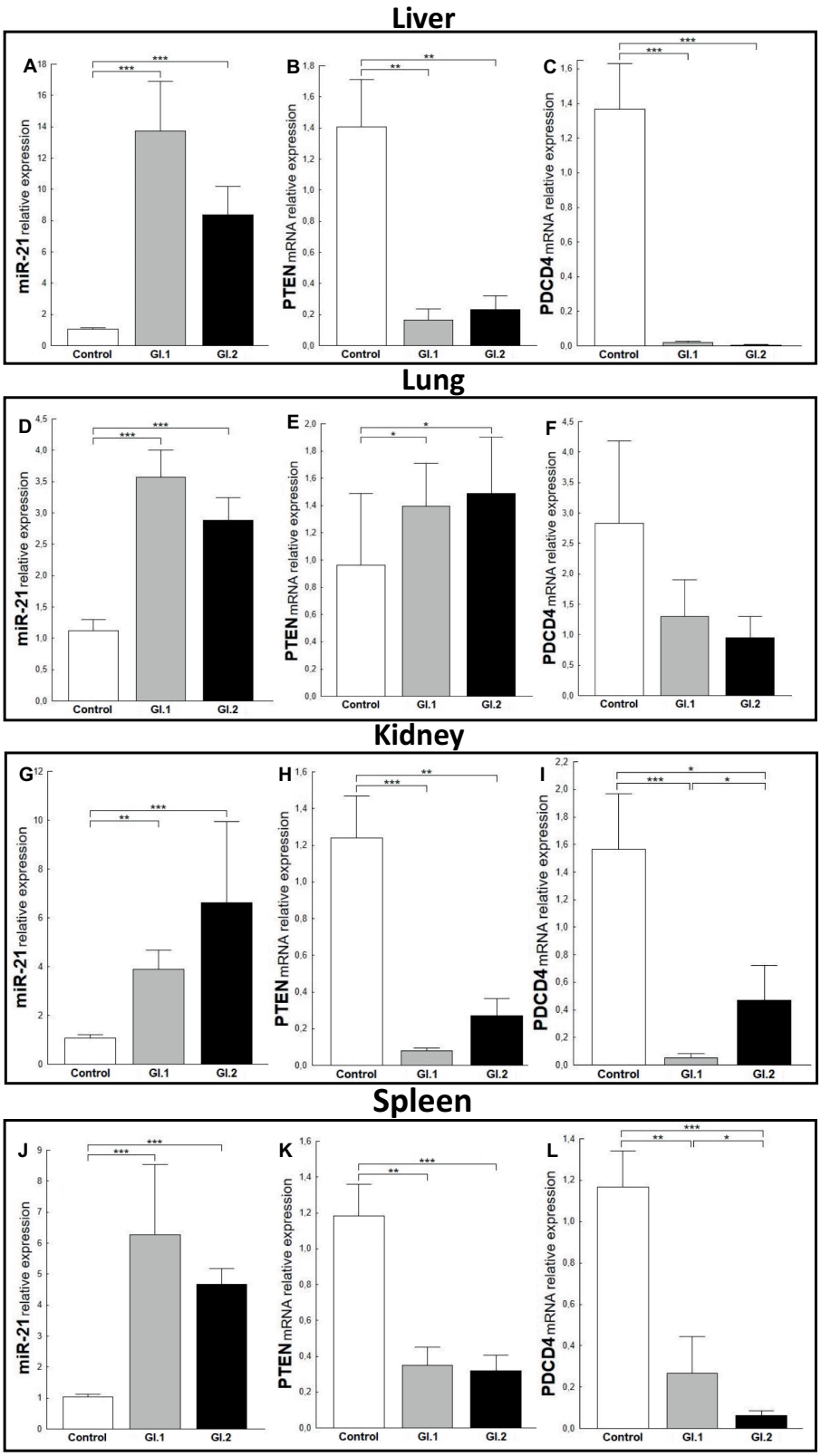


FIGURE 1
Expression of miR-21, *PTEN*, and *PDCD4* mRNA in four tested tissues during infection with *Lagovirus europaeus*—two genotypes (GI.1 and GI.2). Relative expression levels of miR-21 **(A,D,G,J)**, *PTEN* **(B,E,H,K)**, and *PDCD4* **(C,F,I,L)** in the liver **(A–C)**, lung **(D–F)**, kidney **(G–I)**, and spleen **(J–L)** of controls ($n = 10$), GI.1 ($n = 10$), and GI.2 ($n = 10$). The expression of all genes is normalized to an endogenous reference (miR-103a for miR-21 and 18S rRNA for other genes) and presented as a relative fold change to controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney *U* test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), $*p < 0.5$, $**p < 0.01$, and $***p < 0.001$.

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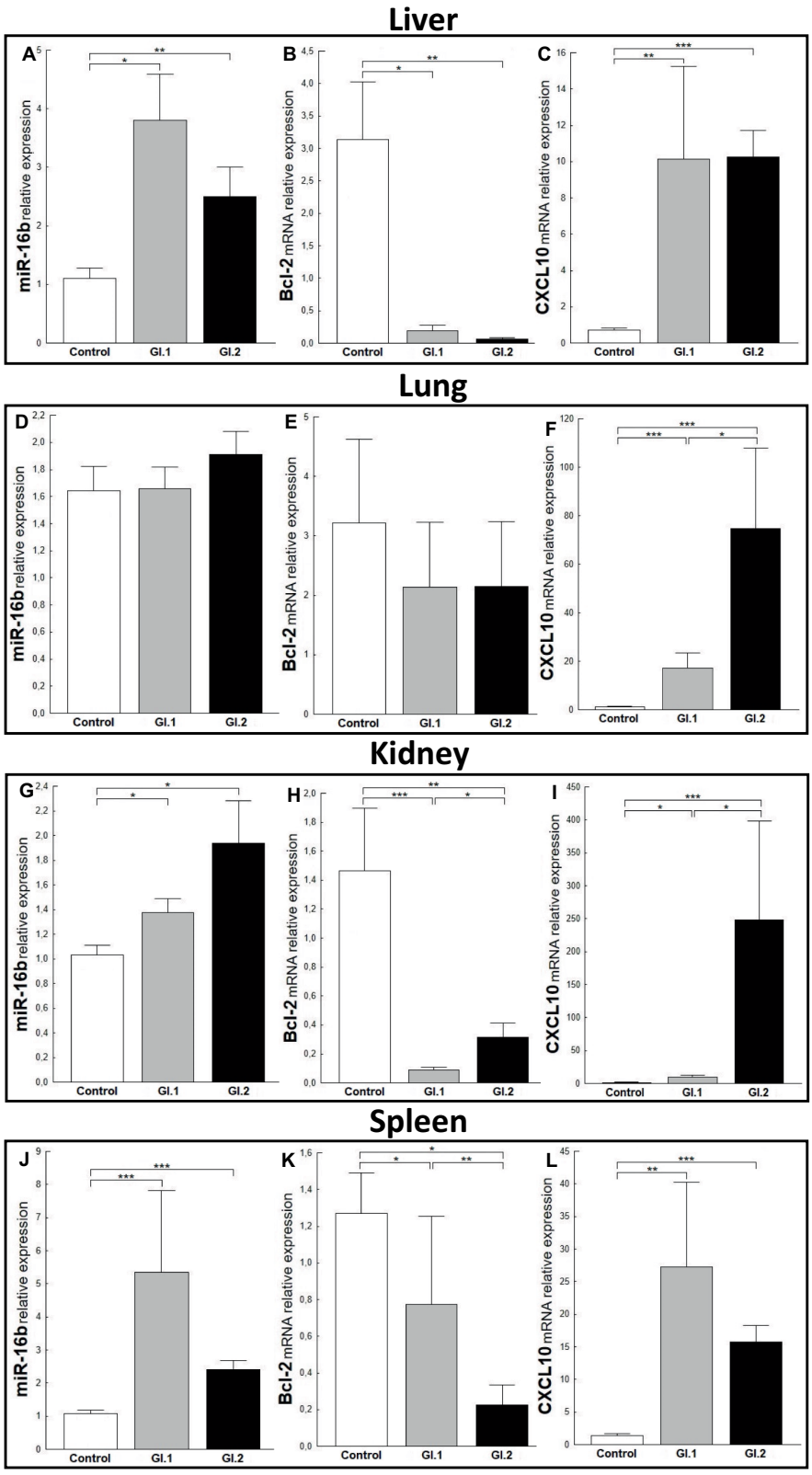


FIGURE 2
Expression of miR-16b, *Bcl-2*, and *CXCL10* mRNA in four tissues during infection with *Lagovirus europaeus*—two genotypes (GI.1 and GI.2). Relative expression levels of miR-16b **(A,D,G,J)**, *Bcl-2* **(B,E,H,K)**, and *CXCL10* **(C,F,I,L)** in the liver **(A–C)**, lung **(D–F)**, kidney **(G–I)**, and spleen **(J–L)** of controls ($n = 10$), GI.1 ($n = 10$), and GI.2 ($n = 10$). The expression of all genes is normalized to an endogenous reference (miR-103a for miR-16b and 18S rRNA for other genes) and presented as a relative fold change to controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney *U* test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), $*p < 0.5$, $**p < 0.01$, and $***p < 0.001$.

upregulated by a 1.6-fold change, $p = 0.04$ (Figure 3A). Whereas, no statistically significant change was observed in the expression level of *SIRT1*, a downstream target of miR-34a, in both of the infected groups of rabbits (Figure 3B). However, a significant increase in *p53*, a transcriptional activator of miR-34a, was observed in infected rabbits compared to controls [2.9-fold change, $p = 0.02$ for GI.1 vs. control and 2.1-fold change, $p = 0.01$ for GI.2 vs. control (Figure 3C)]. Our research demonstrated that in the lung, miR-34a was significantly suppressed in the GI.1 (1.4-fold reduction, 29% reduction, $p = 0.003$) and GI.2 (1.8-fold reduction, 43% reduction, $p = 0.003$) groups (Figure 3D). In both of the infected groups of rabbits (GI.1 and GI.2), downregulation of miR-34a was accompanied by an increase in *SIRT1* (1.6-fold change, $p = 0.03$ and 1.8-fold change, $p = 0.029$, respectively; Figure 3E). However, no change in *p53* expression was observed in either group (Figure 3F). Compared to the control, the level of miR-34a and *SIRT1* was unchanged in the kidney tissue (Figures 3G,H) from both of the infected groups of rabbits. The only change in the kidneys was observed in the level of *p53* during infection with *L. europaeus* GI.1 (Figure 3I). The level of *p53* decreased 1.3-fold (22% reduction, $p = 0.04$ vs. control). In the spleen, it was noted that there was an increase in miR-34a levels in both *L. europaeus* genotypes (6.8-fold change, $p = 0.0002$ in the GI.1 group and 3.9-fold change, $p < 0.0001$ in the GI.2 group; Figure 3J) and increased expression of *p53* (1.9-fold change, $p = 0.04$ for the GI.1 vs. control and 2.2-fold change, $p = 0.004$ for the GI.2 vs. control; Figure 3L). However, no change in expression was detected for the miR-34a target gene—*SIRT1* (Figure 3K).

## 3.3 Biomarkers of apoptosis *Bax*, *Bax*/*Bcl-2* ratio, *Caspase-3*, and *PARP* during *Lagovirus europaeus* GI.1 and GI.2 genotype infection in rabbits

The study aimed to determine the relative level of mRNA expression of proapoptotic biomarkers of apoptosis—*Bax*, *Bax*/*Bcl-2* ratio, *Caspase-3*, and *PARP*—to assess the degree of apoptosis in the liver, lung, kidney, and spleen.

The relative level of mRNA expression of *Bax* was unchanged in most examined tissues except liver tissue during *L. europaeus* GI.2 infection and was 12.3-fold higher compared to control ($p = 0.03$; Figures 4A,E,I,M). However, a *Bax*/*Bcl-2* ratio mRNA was largely changed and was the highest in the liver, where upregulation was 181-fold ($p = 0.006$ vs. control) and 535-fold ($p = 0.0003$ vs. control) for GI.1 and GI.2, respectively (Figure 4B). In the lungs, an increase in the *Bax*/*Bcl-2* ratio was found only in the GI.2 group (7.9-fold, $p = 0.01$ vs. control; Figure 4F). Compared to the control, the *Bax*/*Bcl-2* ratio was 35.6-fold enhanced for GI.1 ($p = 0.0007$) and 11.2-fold enhanced for GI.2 ($p = 0.005$) in the kidneys (Figure 4J). In turn, in the spleen, we observed an upregulation of *Bax*/*Bcl-2* ratio of 15.8-fold level growth ($p = 0.02$) in the GI.1 group and 28.3-fold level growth ($p = 0.003$) in the GI.2 group compared to tissues from healthy rabbits (Figure 4N).

Caspases are cysteine proteases that play fundamental roles in the apoptotic responses of cells to different stimuli. Our research indicated downregulation of relative levels of *Caspase-3* mRNA in the liver in the GI.2-infected group (2.2-fold reduction, 54% reduction, $p = 0.02$; Figure 4C) and the kidney in the GI.2-infected group (2.9-fold reduction, 66% reduction, $p = 0.02$; Figure 4K). Whereas, in the lung and spleen, we found an upregulation of the expression of Caspase-3 for both viruses. During infection with the *L. europaeus* GI.1 genotype, we noted a similar increase in the expression level of *Caspase-3* in the lung and in the spleen, which was a 2.1-fold change ($p = 0.003$ for the lung; Figure 4G $p = 0.007$ for spleen; Figure 4O) compared to control. Whereas during *L. europaeus* GI.2 infection, in the lung level of *Caspase-3* enhanced 2.3-fold change ($p = 0.016$ vs. control; Figure 4G) and in the spleen 1.4-fold change ($p = 0.04$ vs. control; Figure 4O). In other cases (liver in the GI.1 group (Figure 4C) and kidney in the GI.2 group (Figure 4K), the level of *Caspase-3* was unchanged).

Furthermore, we examined the level of *PARP* mRNA expression. We observed downregulation of *PARP* in the liver of rabbits infected with *L. europaeus* GI.1 (5.4-fold reduction, 81% reduction, $p = 0.004$ vs. control; Figure 4D). While in the liver in the GI.2 group, expression of *PARP* was unchanged (Figure 4D). We also noted unchanged *PARP* levels in the lungs during infection with both *L. europaeus* genotypes (Figure 4H). However, the expression of *PARP* was significantly decreased in the kidneys and spleen of the rabbits infected with the *L. europaeus* GI.1 and GI.2 genotypes, with a 22.6-fold reduction (95% reduction, $p = 0.0004$ vs. control) and a 4.4-fold reduction (77% reduction, $p = 0.006$ vs. control), respectively, in the kidney (Figure 4L), and a 4.1-fold reduction (76% reduction, $p = 0.002$ vs. control) and a 6.7-fold reduction (85% reduction, $p = 0.0006$ vs. control), respectively, in the spleen (Figure 4P).

## 3.4 miR expression levels and its downstream targets involved in oxidative stress during *Lagovirus europaeus* GI.1 and GI.2 infection in rabbits

We further investigated the expression of miRs and target genes involved in oxidative stress—miR-122 (*Bach1*) and miR-132 (*Nrf-2*).

The relative expression of miR-122 was significantly different in the liver tissue of the infected rabbits compared to the healthy rabbits only during infection with *L. europaeus* GI.1 (2.3-fold change, $p = 0.002$; Figure 5A). While in liver tissue, a downregulation of *Bach1* was noted for both infected groups (27.6-fold reduction, 96% reduction, $p = 0.0002$ for GI.1 and 98-fold reduction, 99% reduction, $p = 0.00018$ for GI.2; Figure 5B). In the lung, we observed the upregulation of miR-122 for both *L. europaeus* genotypes compared to the control group (17.1-fold change, $p = 0.001$ for GI.1 and 49.4-fold change, $p = 0.0007$ for GI.2; Figure 5C) with a simultaneous increase in expression of *Bach1* (2.3-fold change; $p = 0.04$ and 7.1-fold change, $p = 0.02$ for GI.1 and GI.2, respectively; Figure 5D). The miR-122 level was significantly increased in the kidneys of the rabbits infected with GI.1 and GI.2, with a 34.8-fold ($p < 0.0001$) and 64.3-fold ($p = 0.0001$) increase, respectively (Figure 5E). The upregulation of miR-122 was accompanied by significantly decreased *Bach1* in both infected groups (13.6-fold reduction, 93% reduction, $p = 0.0003$ in the GI.1 group and 1.4-fold reduction, 26% reduction, $p = 0.04$ in the GI.2 group; Figure 5F). While the highest increase in miR-122 expression occurred in the spleen and was, respectively, 554.3-fold change ($p = 0.0002$) for GI.1

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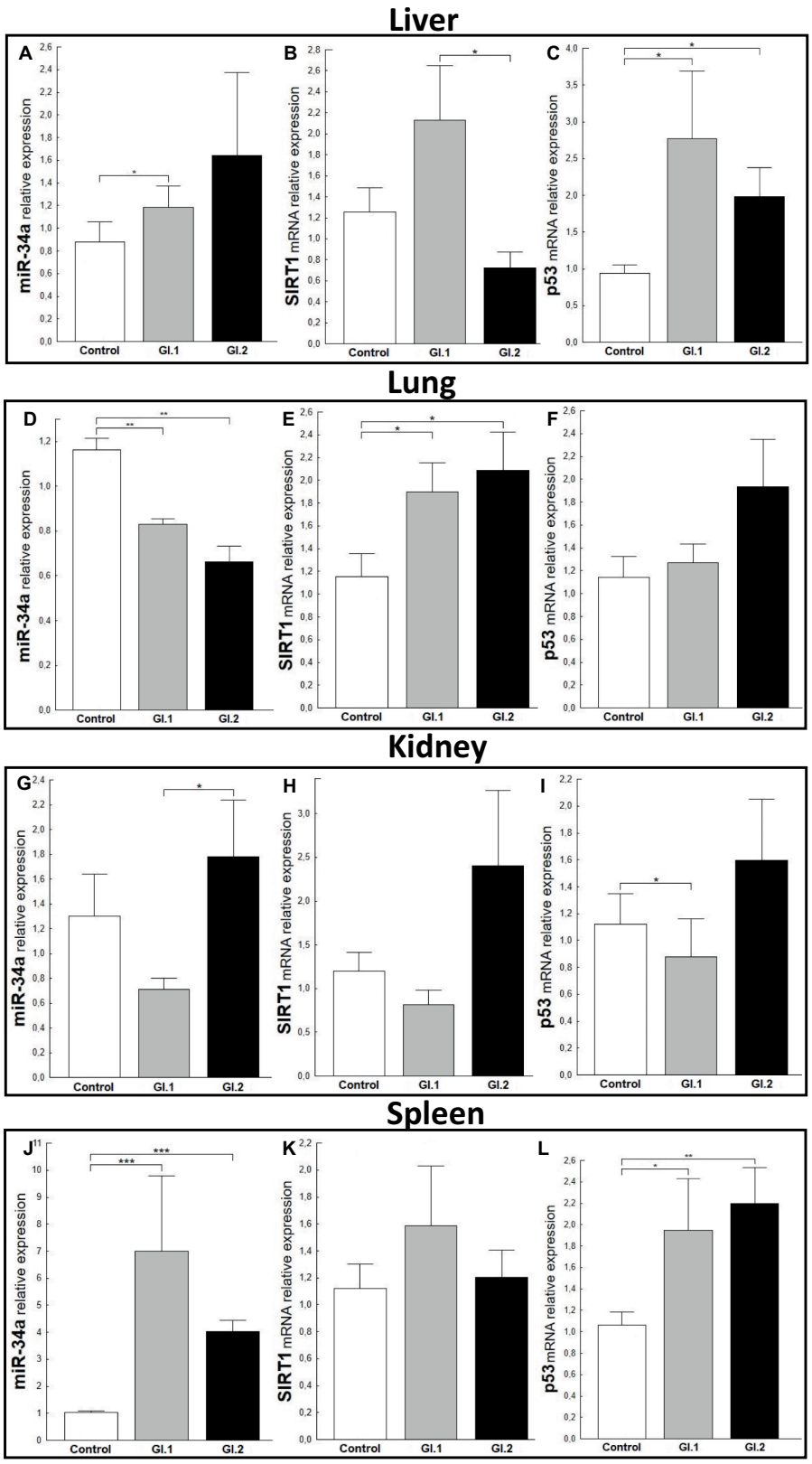


FIGURE 3
Expression of miR-34a, *SIRT1*, and *p53* mRNA in four tissues during infection with *Lagovirus europaeus*—two genotypes (GI.1 and GI.2). Relative expression levels of miR-34a **(A,D,G,J)**, *SIRT1* **(B,E,H,K)**, and *p53* **(C,F,I,L)** in the liver **(A–C)**, lung **(D–F)**, kidney **(G–I)**, and spleen **(J–L)** of controls ($n = 10$), GI.1 ($n = 10$), and GI.2 ($n = 10$). The expression of all genes is normalized to an endogenous reference (miR-103a for miR-34a and 18S rRNA for other genes) and presented as a relative fold change to controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney *U* test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), $*p < 0.5$, $**p < 0.01$, and $***p < 0.001$.

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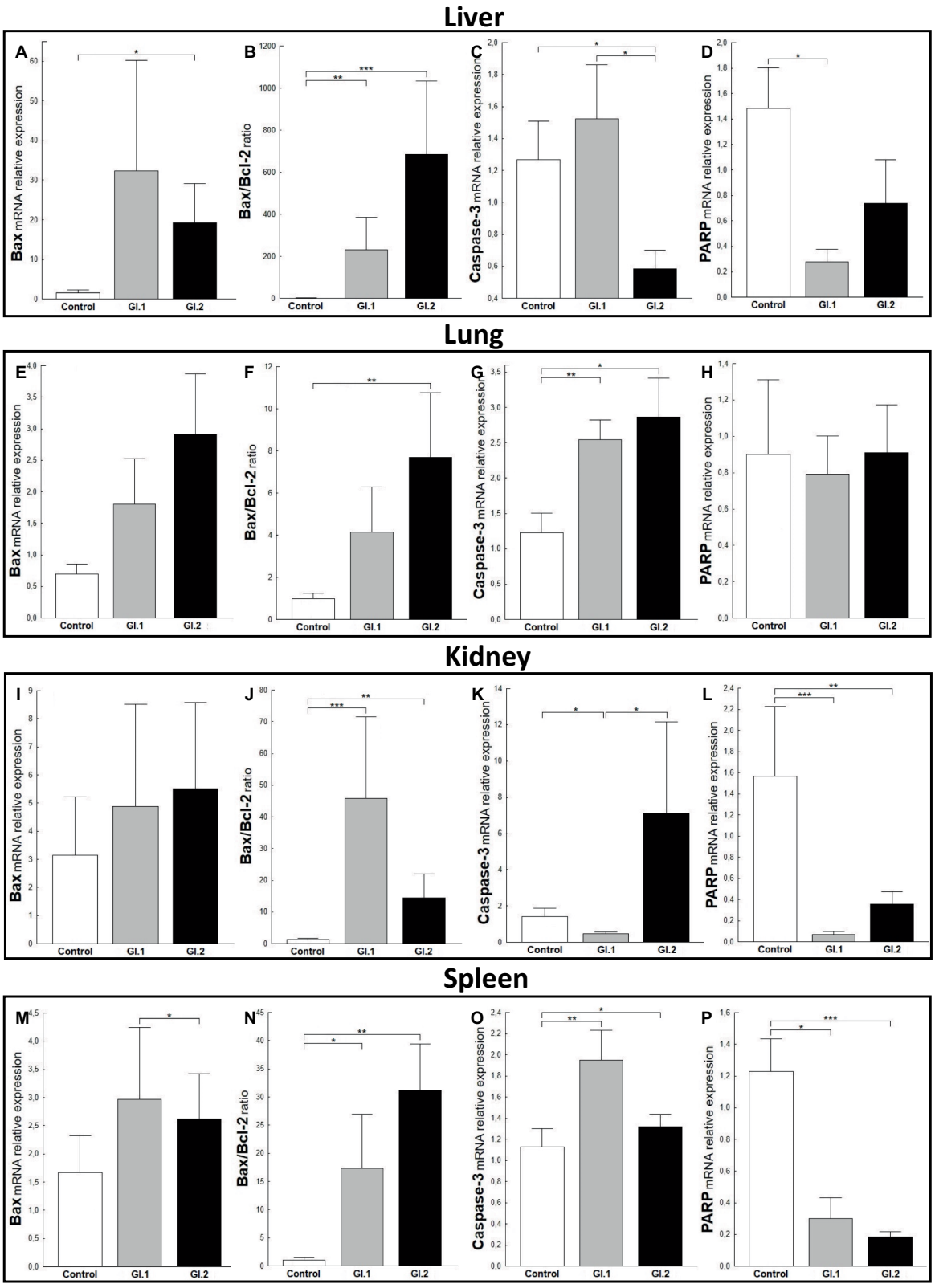


FIGURE 4
Expression levels of biomarkers *Bax* mRNA, *Bax/Bcl-2* ratio, Caspase-3, and *PARP* mRNA in four tissues during infection with *L. europaeus*—two genotypes (GI.1 and GI.2). Relative expression levels of *Bax* **(A,E,I,M)**, *Bax/Bcl-2* ratio **(B,F,J,N)**, Caspase-3 **(C,G,K,O)**, and PARP **(D,H,L,P)** in the liver **(A–D)**, lung **(E–H)**, kidney **(I–L)**, and spleen **(M–P)** of controls ($n = 10$), GI.1 ($n = 10$), and GI.2 ($n = 10$). The expression of the gene is normalized to an endogenous reference 18S rRNA and presented as a relative fold change to controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The mRNA levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney *U* test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), $*p < 0.5$, $**p < 0.01$, and $***p < 0.001$.

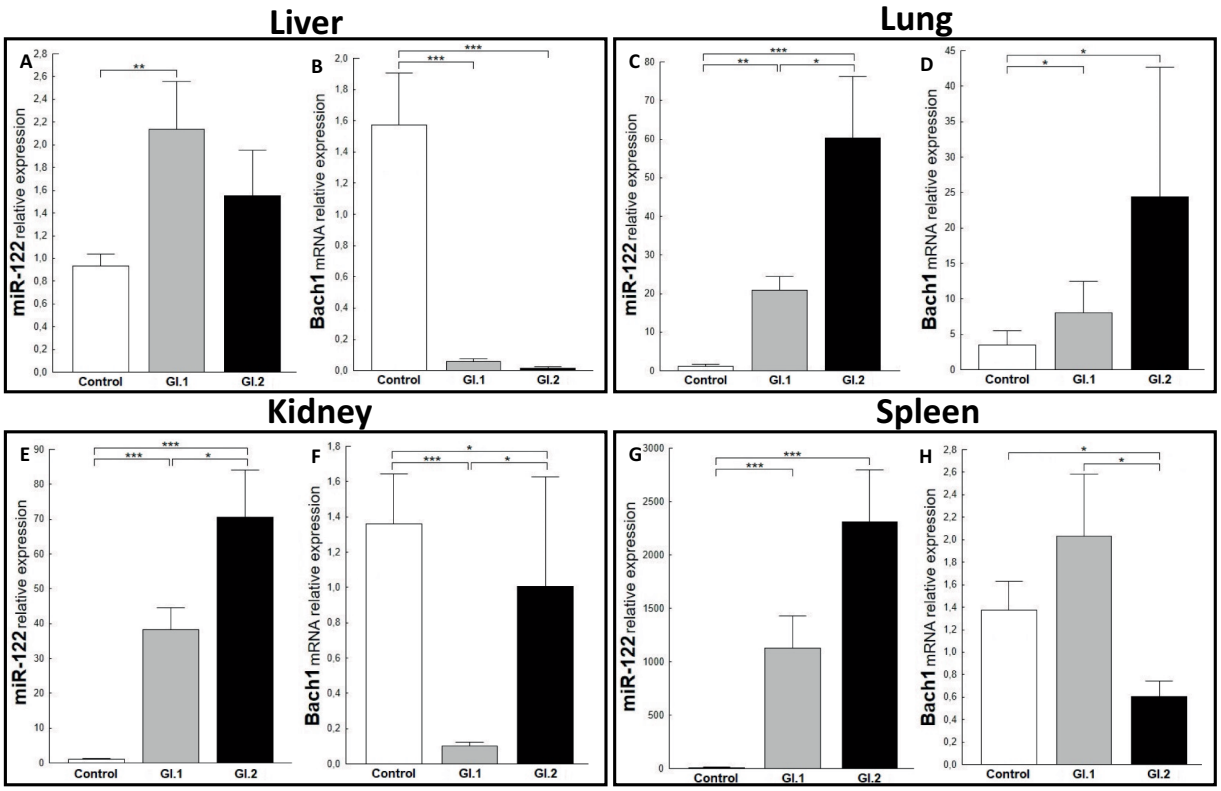


FIGURE 5
Expression of miR-122 and *Bach1* mRNA in four tissues during infection with *L. europaeus*—two genotypes (GI.1 and GI.2). Relative expression levels of miR-122 **(A,C,E,G)** and *Bach1* **(B,D,F,H)** in the liver **(A,B)**, lung **(C,D)**, kidney **(E,F)**, and spleen **(G,H)** of controls ($n = 10$), GI.1 ($n = 10$), and GI.2 ($n = 10$). The expression of all genes is normalized to an endogenous reference (miR-103a for miR-122 and 18S rRNA for other genes) and presented as a relative fold change to controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney *U* test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), $*p < 0.5$, $**p < 0.01$, and $***p < 0.001$.

and 1,135-fold change ($p = 0.0002$) for GI.2 (Figure 5G). However, a change in *Bach1* expression in the spleen was noted only in infection with GI.2 and was reduced by 2.3-fold (56% reduction, $p = 0.02$ vs. control; Figure 5H).

An extensively upregulated miR-132 expression was noted in the liver (3.9-fold, $p = 0.0003$ for GI.1 and 3.4-fold, $p = 0.002$ for GI.2; Figure 6A) and spleen (3.6-fold, $p = 0.002$ and 3.9-fold, $p < 0.0001$; Figure 6G) during viral infection compared to the control group. In the liver, where expression of miR-132 was increased, the expression of *Nrf-2*, a critical factor in antioxidant defense, was inhibited in comparison to controls in both infected groups, with a 1.7-fold reduction (41% reduction, $p = 0.02$) for GI.1 and a 2.4-fold reduction (58% reduction, $p = 0.007$) for GI.2 (Figure 6B). In the lung in both infected groups, no change was demonstrated in levels of miR-132 and *Nrf-2* (Figures 6C,D). The same observations as for the lungs were made for kidney tissue in the GI.2 group (Figures 6E,F). In the kidneys, an increase in miR-132 expression only occurred during GI.1 infection (1.6-fold change, $p = 0.02$; Figure 6E). Whereas in the kidney in the GI.1 group, the expression of *Nrf-2* was inhibited 2-fold (50% reduction, $p = 0.025$ vs. control; Figure 6F). In the spleen, an increase in miR-132 expression was accompanied by an increase in *Nrf-2* expression for both *L. europaeus* genotypes (2.4-fold change, $p = 0.02$ for GI.1 and 1.8-fold change, $p = 0.008$ for GI.2) compared to healthy rabbits (Figure 6H).

## 3.5 *HO-I* as a biomarker of the response to oxidative stress during *Lagovirus europaeus* GI.1 and GI.2 genotype infection in rabbits

To assess the antioxidative response of *Bach1* and *Nrf-2* during *L. europaeus* GI.1 and GI.2 genotype infection in rabbits, we estimated their effect on the downstream target gene *HO-I*. We found that *HO-I* levels were 3.1-fold (67% reduction, $p = 0.005$ vs. control) lower in livers from rabbits infected with *L. europaeus* GI.1 and 2.8-fold (64% reduction, $p = 0.026$ vs. control) lower in livers from rabbits infected with GI.2 (Figure 7A). In the kidney, inhibition of *HO-I* was observed only during *L. europaeus* GI.1 infection (2.4-fold reduction, 58% reduction, $p = 0.02$; Figure 7C). Moreover, *HO-I* mRNA expression levels were 2.1-fold lower (52% reduction, $p = 0.02$) for the GI.1 group and 3.2-fold lower (69% reduction, $p = 0.045$) for the GI.2 group than

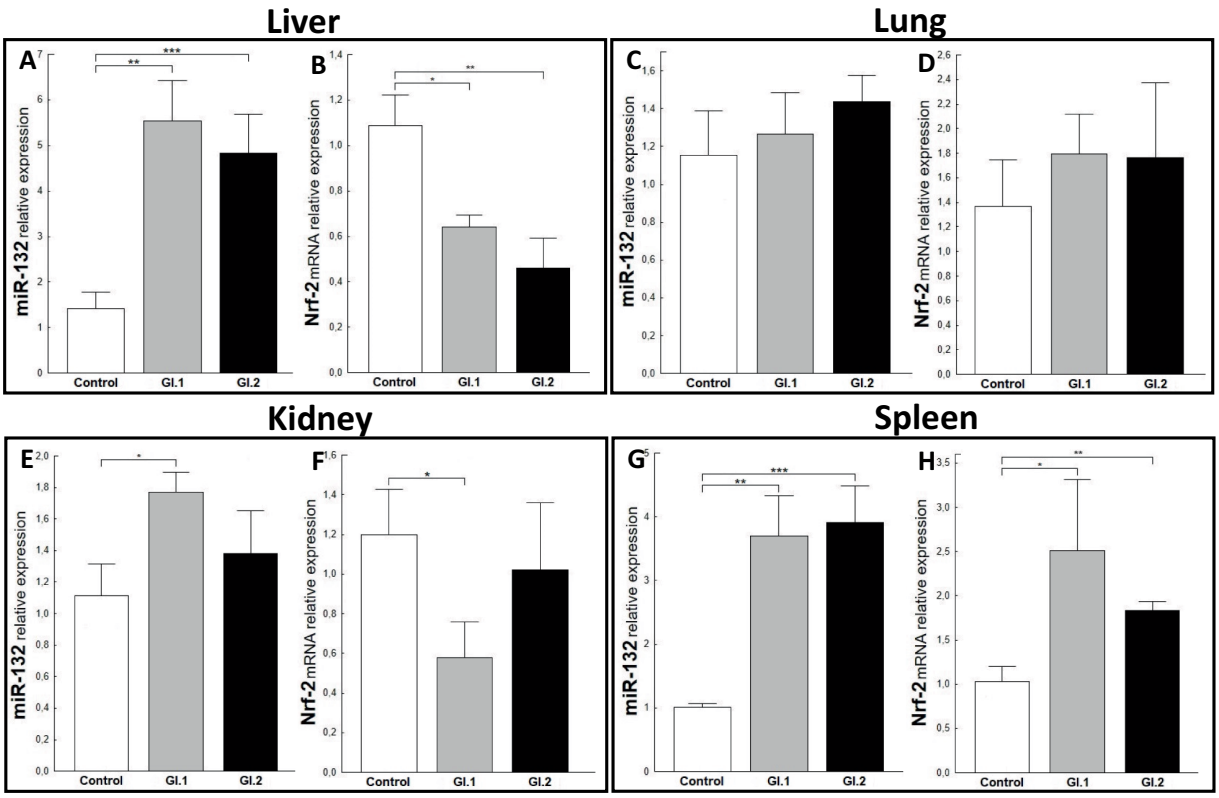


FIGURE 6
Expression of miR-132 and *Nrf-2* mRNA in four tissues during infection with *L. europaeus*—two genotypes (GI.1 and GI.2). Relative expression levels of miR-132 **(A,C,E,G)** and *Nrf-2* **(B,D,F,H)** in the liver **(A,B)**, lung **(C,D)**, kidney **(E,F)**, and spleen **(G,H)** of controls ($n = 10$), GI.1 ($n = 10$), and GI.2 ($n = 10$). The expression of all genes is normalized to an endogenous reference (miR-103a for miR-132 and 18S rRNA for other genes) and presented as a relative fold change to controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney *U* test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), $*p < 0.5$, $**p < 0.01$, and $***p < 0.001$.

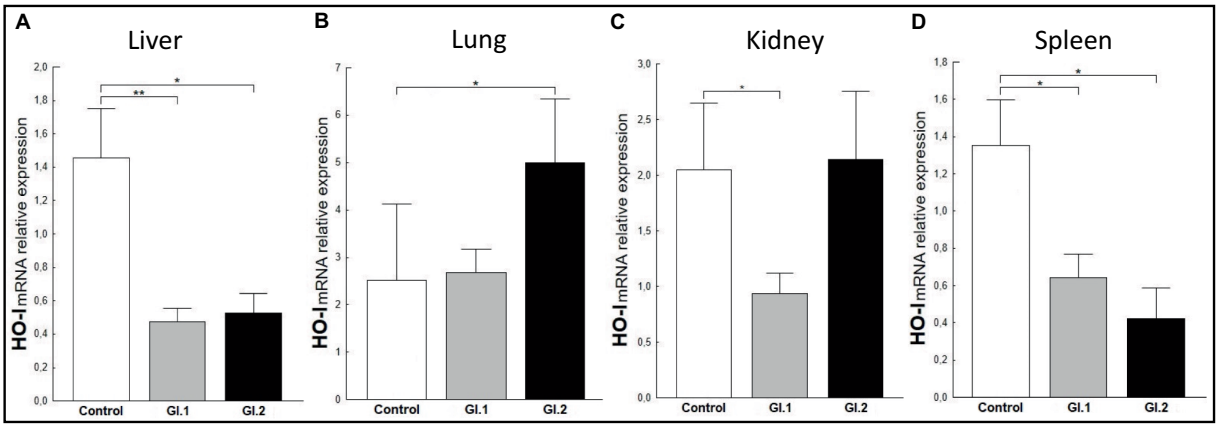


FIGURE 7
Expression of biomarker *HO-1* mRNA in four tissues during infection with *Lagovirus europaeus*—two genotypes (GI.1 and GI.2). Relative expression levels of *HO-1* **(A–D)** in the liver **(A)**, lung **(B)**, kidney **(C)**, and spleen **(D)** of controls ($n = 10$), GI.1 ($n = 10$), and GI.2 ($n = 10$). The expression of the gene is normalized to an endogenous reference 18S rRNA and presented as a relative fold change to controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The mRNA levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney *U* test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), $*p < 0.5$, $**p < 0.01$, and $***p < 0.001$.

in controls in the spleen (Figure 7D). However, in the lung in group GI.2, we observed an upregulation as a 2-fold change ($p = 0.02$ vs. control; Figure 7B) of *HO-I*.

## 4 Discussion

Little is known about the molecular signatures of regulatory interactions between specific miRs, apoptosis, and oxidative stress in viral diseases. We were the first to examine the expression of three miRs with proapoptotic (miR-16b, miR-34a) and antiapoptotic (miR-21) effects, which regulate both the intrinsic and extrinsic apoptosis pathways, to determine their regulatory effects on target genes and their potential impact on the pathways in infection with *L. europaeus*. In the case of oxidative stress, we studied miR-122 and miR-132 and their target genes. To investigate apoptosis and oxidative stress as a response to the viral stimulus, we examined apoptosis biomarkers (*Bax, Bax/Bcl-2 ratio, Caspase-3, PARP*) and oxidative stress (*HO-I*).

Bcl-2 family proteins are important regulatory molecules of apoptosis. Members of this family can be divided into the antiapoptotic family, which includes Bcl-2, and the apoptotic family, which includes Bax. The ratio of antiapoptotic Bcl-2 to proapoptotic Bax contributes to the susceptibility of a given cell to apoptosis (San-Miguel et al., 2006). After infection of rabbits with *L. europaeus* GI.1 and GI.2 genotypes, there is an increase in the expression of the *Bax* gene, which promotes programmed cell death (PCD). The *Bax* biomarker increases as much as 12.3-fold in the liver of rabbits during *L. europaeus* GI.2 infection, which indicates rapid (32 h p.i.) and extensive apoptosis of hepatocytes in this organ in response to the viral stimulus. Moreover, Chen et al. (2018) reported increased mRNA *Bax* expression during RHDV infection in cells transfected with NSP6 at 24 and 36 h p.i. compared to the control. The same result regarding the upregulation of Bcl-2 family proteins (determined at the level of *Bax* and *Bcl-2* mRNA) in the liver tissue and in the spleen and kidneys after infection of rabbits with the *L. europaeus* GI.2 genotype was obtained by Bebnowska et al. (2023, 2024). In our lung and spleen results, the *Bax* mRNA gene expression level indicates a lower degree of apoptosis (statistically insignificant). It is difficult to interpret the lack of changes in *Bax* mRNA expression in the kidneys, and it may result from negative regulation by *p53*. Because *p53* promotes the apoptotic capacity of *Bax* as the main transcription factor, and since we have a reduction (22% reduction) of *p53* in the kidneys during *L. europaeus* GI.1 infection and no changes during GI.2 infection, such a scenario is possible. Vallejo et al. (2014) stated that after infection with *L. europaeus* GI.1 at 30 and 36 h p.i., there is a significant inhibition of the expression of Bcl-2 and Bcl-xL, two antiapoptotic proteins involved in the intrinsic apoptosis pathway.

During apoptosis, Bax protein bound to Bcl-2 protein activates a cascade of reactions by releasing cytochrome c from mitochondria, which helps in the successive activation of Caspases and ultimately leads to cell death (Pepper et al., 1997). It has been proposed (Pepper et al., 1997) that the association and ratio of Bax to Bcl-2 determine cell survival or death after an apoptotic stimulus. High Bax levels and/or a decrease in Bcl-2 and a high Bax/Bcl-2 ratio promote apoptosis (Pepper et al., 1997). We demonstrated a decrease in antiapoptotic *Bcl-2* and an increase in proapoptotic *Bax* during *L. europaeus* GI.1 and GI.2 genotype infection in four tissues of rabbits. We showed that the *Bax/Bcl-2* ratio promotes apoptosis during infection with *L. europaeus*—two genotypes—and was more strongly expressed (2–3 times) during GI.2 infection. This process included, in order, the liver (535-fold and 181-fold, respectively, GI.2 and GI.1), spleen (28.3-fold and 15.8-fold, GI.2 and GI.1), and lungs (7.9-fold GI.2). In the kidney, a different effect was observed (11.2-fold and 35.6-fold, GI.2 and GI.1). Our studies are consistent with previous observations by other authors, which were carried out after infection with *L. europaeus* GI.1 genotype and GI.2 genotype and concerned mainly the liver (San-Miguel et al., 2006; Chen et al., 2018), as well as the kidney and spleen; however, no changes were noted in the lungs and spleen (Bebnowska et al., 2023).

Caspase-3 is the main effector of Caspase in the external and internal apoptosis pathways, acting on enzymes involved in DNA fragmentation and chromatin condensation (Duprez et al., 2009). In our studies, the recorded increase in the *Caspase-3* gene at a similar level in the lungs (2.1-fold change and 2.3-fold change, respectively, GI.1 and GI.2) and in the spleen (2.1-fold change and 1.4-fold change, GI.1 and GI.2) indicates the activation of apoptosis in these organs. Garcia-Lastra et al. (2010) have shown a marked increase in Caspase-3 activity in the liver at 36 h (12.5-fold) and 48 h (12.6-fold) with RHDV-infected animals. Chen et al. (2018) found that non-structural protein 6 (NSP6)-induced Caspase-3 activity in *Oryctolagus cuniculus* kidney cells (RK13). However, other studies showed a significant increase in *Caspase-3* mRNA in rabbit liver (Bebnowska et al., 2024) and cleaved protein *Caspase-3* in lung, heart, kidney, and spleen during infection with the *L. europaeus* GI.2 genotype (Bebnowska et al., 2023). This methodological approach is better for measuring apoptosis levels/activity biomarkers (Caspase-3, PARP, and Bax/Bcl-2 proteins). On the other hand, in our own studies after infection of rabbits with *L. europaeus* GI.2, a reduction in the relative mRNA level of *Caspase-3* was observed in the liver (2.2-fold reduction, 54% reduction) and in the kidneys during *L. europaeus* GI.1 infection (2.9-fold reduction, 66% reduction). These results are consistent with the observations of Vallejo et al. (2014), who showed that apoptosis in RHD is induced by *L. europaeus* GI.1 genotype in the liver occurred without significantly decreasing Caspase-3 activity at 12 h, 18 h, and 24 h p.i. In the same research (Vallejo et al., 2014), infection of rabbits with RHDV resulted in a marked increase of Caspase-3 activity only at 30 and 36 h p.i. in the liver.

PARP is widely known as an enzyme that plays a role in DNA damage detection and repair. Activation of PARP in the event of DNA damage enables poly(ADP-ribosylation) of appropriate proteins, influencing repair systems, which helps maintain genome stability (Chaitanya et al., 2010). Inhibition of the PARP protein leads to the accumulation of DNA damage, which contributes to cell death (Chaitanya et al., 2010). Caspase-3 is primarily responsible for cleaving the 116 kDa PARP-1 protein into an 85 kDa fragment, whose presence indicates that cells are undergoing apoptosis (San-Miguel et al., 2006). Our results indicate that during *L. europaeus* infection, there is a reduction of mRNA *PARP* of 76%–95% in the liver, kidneys, and spleen (no significance was found in the lungs), which may indicate the accumulation of DNA damage and the process of apoptosis in cells. In Western blot

analysis, San-Miguel et al. (2006) and Vallejo et al. (2014) demonstrated that in later periods of RHDV infection (36 h and 38 h p.i.), there was marked proteolysis of PARP-1, a nuclear enzyme whose cleavage into an 85 kDA fragment by Caspase-3 confirms that cells are undergoing apoptosis. Using the same technique, other authors (Bebnowska et al., 2023) showed elevated levels of the cleaved form of PARP in the heart, kidney, and spleen during *L. europaeus* GI.2 genotype infection in rabbits. No significant changes were observed in the lungs. The above result indicates that in the infection of rabbits with *L. europaeus*—two genotypes (GI.1 and GI.2)—cell death is activated by apoptosis in four tissues—liver, lungs, kidneys, and spleen—and it was more strongly expressed after *L. europaeus* GI.2 infection. Apoptosis was accompanied by increased expression or reduction of apoptosis biomarkers *Bax, Bax/Bcl-2 ratio, Caspase-3, and PARP*.

Our study demonstrated that miR-21, involved in the regulation of the external apoptosis pathway (Su et al., 2015), has antiapoptotic effects in all tissues tested after *L. europaeus* infection and has an inhibitory effect on the target genes *PTEN* and *PDCD4* and an effect on *Caspase-3* which is equivalent to reduced apoptosis. The exception is the lungs, where no inhibitory mechanism is observed during infection with *L. europaeus*. For this event, it can be suggested that there are other mechanisms of *PTEN* induction (one of the most important genes of the apoptosis pathway) and *PDCD4* induction that require further investigation. A similar regulation of miR-21 was observed in studies on the HBV, Chandipura virus, and hepatic stellate cells (Qiu et al., 2013; Wei et al., 2013; Fu et al., 2017; Pandey et al., 2021).

Our studies show that miR-16b, involved in regulating the intrinsic apoptosis pathway (Su et al., 2015), has a proapoptotic effect in most of the examined tissues, inhibiting *Bcl-2*, which increases apoptosis and increases *Bax* expression. This result is confirmed by previous research by Hukowska-Szematowicz et al. (2020) during *L. europaeus* GI.1 (GI.1a variant, strain Erfurt) infection in rabbits (2.5-fold change) and other studies (Su et al., 2015). Our current research excludes the lungs, where we do not observe changes in miR-16b and *Bcl-2* expression during *L. europaeus* GI.1 and GI.2 genotype infection. Moreover, we have shown that miR-16b affects the increase in the chemoattractant *CXCL10* in the liver, spleen, and kidneys, which may result in increased apoptosis and tissue damage. The exception is the lungs, where no change in miR-16b expression was observed, but an increase in *CXCL10* expression was observed in both *L. europaeus* genotypes. The increase in *CXCL10* expression differs from the literature data because no inhibitory mechanism via miR-16b is observed (Gao and Zhao, 2020). Perhaps in the case of infection with *L. europaeus*, there is an activating mechanism through miR-16b. According to one theory, miR-16b binds to the gene promoter and influences its increased transcription, which may have been reflected in our research on *CXCL10*. Our results differ from those recorded after HBV infection, where a downregulation of miR-16 in HepG2 cells was noted during transfection of HBV X protein. However, the results may differ due to the research model (*in vitro* vs. *in vivo* study) and the occurrence of acute and chronic forms of RHD during *L. europaeus* infection. A rapid course of infection may lead to faster damage to the organ and an increase in the level of miR-16 associated with apoptosis (Wu et al., 2011).

MiR-34a regulates the extrinsic apoptosis pathway, and its targets are *SIRT1* and *p53* (Yamakuchi et al., 2008; Hermeking, 2010; Su et al., 2015; Sharma et al., 2018; Jiang et al., 2019; Hao et al., 2021; Zhu et al., 2021). The literature describes a feedback mechanism for miR-34a in which miR-34a inhibits *SIRT1*, thereby increasing the expression of *p53*, which is known as a transcriptional activator of miR-34a (Yamakuchi et al., 2008; Jiang et al., 2019; Hao et al., 2021; Zhu et al., 2021). Our study demonstrated that miR-34a has a proapoptotic effect after infection of rabbits with *L. europaeus* GI.1 in the liver and after infection with *L. europaeus* GI.1 and GI.2 (in the spleen) and does not affect *SIRT1* expression but induces *p53* in these organs (GI.1 and GI.2). This induction of *p53* leads to an increase in apoptosis. This increase in apoptosis may occur because miR-34 is a transcriptional target of *p53*, suggesting a positive feedback loop between *p53* and miR-34a. A noticeable regulatory effect of miR-34a was observed in the lungs, the decrease of which induced an increase in *SIRT1* and did not alter the regulation of *p53*. Our observations are partly consistent with those of other researchers. Jiang et al. (2019) report that the coxsackie B3 virus increases the expression of miR-34a during infection, which induces cardiomyocyte apoptosis by activating the *SIRT1-p53* pathway. Studies (Sharma et al., 2018) also show that during T-lymphotropic virus-1 infection, there is an increase in miR-34a, which increases the level of *p53*. Additionally, researchers (Hao et al., 2021) have shown that treating infected cell lines with the *p53* activator nutlin-3a leads to a further increase in miR-34a, creating a feedback loop similar to that observed during *L. europaeus* infection in the liver and spleen with *L. europaeus* GI.1 or GI.2 genotypes, respectively. Furthermore, other factors that influence organ damage increase the expression of miR-34a and *p53*, contributing to apoptosis and increasing the *Bax/Bcl-2* ratio, similar to our studies (Sharma et al., 2018; Jiang et al., 2019; Hao et al., 2021).

Based on our previous literature review (Ostrycharz and Hukowska-Szematowicz, 2022), we indicated that miR-122 is involved in oxidative stress and regulates *Bach1*, thereby influencing the level of *HO-I* (the regulatory effect of miR-122 on the *Bach1*/*HO-I* axis;Shan et al., 2007; Qiu et al., 2010; Ostrycharz and Hukowska-Szematowicz, 2022; Wang et al., 2022). Many studies have shown that miR-122 is essential for HCV replication (Jopling et al., 2005; Jopling, 2008; Jopling et al., 2008; Amador-Canizares et al., 2018). On the other hand, literature data indicate that in the course of HCV and HBV infection in people (Ostrycharz and Hukowska-Szematowicz, 2022), there is a decrease in the expression of miR-122. It, in turn, inhibits the increase in the expression of *Bach1*, which in turn affects the increase in the expression of *HO-I*, and exerts a protective effect in this tissue microenvironment (Shan et al., 2007; Qiu et al., 2010; Ostrycharz and Hukowska-Szematowicz, 2022; Wang et al., 2022). No such effect was observed in our studies. Therefore, we propose an alternative mechanism for the action of miR-122 in the examined tissues during *L. europaeus* infection. Our studies indicate that miR-122 may participate in cell damage, and its increase in expression in almost all examined tissues (except for the lungs) decreases *Bach1*. The upregulation of miR-122, in turn, impacts the inhibition of *HO-I* activity, which can lead to increased tissue damage. Our results indicate that *HO-I* in *L. europaeus* infection has no protective effect. The association of the proposed pathway with clinical documentation indicates that the protective role of *HO-I* can only be considered in the lungs during *L. europaeus* GI.2 infection. In the case of miR-122, the highest expression of these molecules was recorded

in the spleen, kidney, lung, and liver during *L. europaeus* GI.1 infection and the same tissues except the liver with GI.2 infection. The latter result is consistent with previous observations by Hukowska-Szematowicz et al. (2020) during *L. europaeus* GI.1 (GI.1a variant, strain Erfurt) infection in rabbits, which did not show miR-122 expression in the liver but in the serum.

The regulatory effect of miR-132 in the oxidative stress pathway in viral infection proposed in our study has yet to be studied. The involvement of miR-132 in oxidative stress has been confirmed in other diseases (Wasik et al., 2017; Zhou et al., 2020; Xu et al., 2021). Our studies suggested that miR-132 can be involved in tissue damage during *L. europaeus* infection. Overexpression of miR-132 during *L. europaeus* GI.1 and GI.2 infection may be an element of the pathogenesis of RHD. The conducted research showed increased miR-132 in the liver and spleen during *L. europaeus* GI.1 and GI.2 infection. Whereas in the kidney, it was noted that miR-132 expression increased only during infection with *L. europaeus* GI.1. The mechanism of *Nrf-2* inhibition via miR-132 is observed only in the liver and kidneys. This mechanism correlates with a simultaneous decrease in *HO-I* expression, which may increase liver and kidney damage. Interestingly, in the spleen, despite the increase in miR-132, an increased level of *Nrf-2* is observed with a simultaneous decrease in the level of *HO-I*. Further research is required to investigate this mechanism. Data on Nrf-2 signaling (a critical factor in oxidative defense) in viral infections are limited (Herengt et al., 2021). Evidence suggests that activation of Nfr-2 in host cells is protective during viral infections. Protection may be through either antiviral activity, inhibition of cell death to protect against excessive tissue damage, or both (Herengt et al., 2021). There was no change in miR-132 expression in the lungs after infection with *L. europaeus* GI.1 and GI.2. In the lungs, after the *L. europaeus* GI.1 infection, no *HO-I* changes were noted. It can be assumed that the result of these reactions is less oxidative damage in the lungs and more significant damage in the liver, spleen, and kidneys. Our results regarding the expression of *Nrf-2* in response to *L. europaeus* infection confirm other studies (San-Miguel et al., 2006; Hu et al., 2020). Hu et al. (2020) showed that both the mRNA and protein levels of Nrf-2 were significantly reduced after RHDV infection, which shows that RHDV infection inhibits Nrf-2 activity and the antioxidant response. San-Miguel et al. (2006) showed that oxidative stress is a primary pathway for apoptosis in RHDV.

A detailed analysis of the first *HO-I* deficiency in a human showed that *HO-I* protects many tissues and organs against oxidative stress and excessive inflammatory responses by releasing many molecules with stress and antioxidant properties (Yachie, 2021). In addition, it protects against programmed cell death, and this cytoprotective effect is based on its ability to catabolize free heme and prevent cells from sensitizing to apoptosis. *HO-I* production is induced *in vivo* in selected cell types, including renal tubular epithelium, liver Kupffer cells, vascular endothelium, and monocytes/macrophages, suggesting that *HO-I* plays a crucial role in these cells. Data from reported cases of *HO-I* deficiency in humans and numerous studies in animal models suggest that *HO-I* plays a crucial role in various clinical conditions involving oxidative stress (Yachie, 2021). The magnitude of HO-I induction after oxidative stress and the wide distribution of this enzyme in systemic tissues, combined with the biological activity of the catalytic byproducts carbon monoxide, iron, and bilirubin, make HO-I a very attractive and interesting biomarker of oxidative stress and may play an essential role in mediating protection against liver, lung, spleen, and kidney damage (Choi and Alam, 1996). Because of the above facts, our results indicate that the reduction of *HO-I* mRNA at the level of 52%–69% in rabbit organs (liver, kidneys, spleen) after infection with *L. europaeus* GI.1 and GI.2 genotypes does not protect cells against oxidative damage but, on the contrary, may intensify, which is consistent with the results of experimental studies on various animal models (Choi and Alam, 1996; Yachie, 2021). The increase in *HO-I* mRNA expression (2-fold change, $p = 0.02$ vs. control) in the lungs during *L. europaeus* GI.2 infection, observed in our studies, indicates a role in protection against lung damage. Accumulating evidence suggests that oxidative stress plays a central role in the pathogenesis of many pulmonary diseases, including adult respiratory distress syndrome, emphysema, asthma, bronchopulmonary dysplasia, and interstitial pulmonary fibrosis (Choi and Alam, 1996).

### 4.1 Proposed miRs, target genes, and pathways apoptosis and oxidative stress during *Lagovirus europaeus* GI.1 and GI.2 infection

Our research proposes five pathways in *L. europaeus* infection, three of which are involved in apoptosis pathways and two in oxidative stress (Figures 8, 9). Additionally, Spearman's rank correlations for examined miRs, mRNA, and biomarkers of processes in four tissues of rabbits during *L. europaeus* GI.1 and GI.2 infection were described. Correlations are provided for statistically significant results (Figures 8, 9).

## 5 Conclusion

Our report is the first to present the regulatory effects of miRs on apoptosis and oxidative stress genes in rabbit infection with *L. europaeus*—two genotypes (GI.1 and GI.2) in four tissues (liver, lungs, kidneys, and spleen). Our research provides new data that are critical for understanding the pathogenesis of Rabbit Hemorrhagic Disease caused by *L. europaeus*—two genotypes (GI.1 and GI.2), regarding the molecular regulation of apoptosis and oxidative stress by miRs (as two essential biological processes in viral infections). The regulatory effect of miRs indicates that, on the one hand, miRs can intensify apoptosis (miR-16b, miR-34a) in the examined organs in response to a viral stimulus and, on the other hand, inhibit (miR-21), which in both cases may be a determinant of the pathogenesis of RHD and tissue damage. Biomarkers of the *Bax and Bax/Bcl-2* ratio promote more intense apoptosis after infection with the *L. europaeus* GI.2 genotype. Our findings demonstrate that miR-122 and miR-132 regulate oxidative stress in the pathogenesis of RHD, which is associated with tissue damage. The *HO-I* biomarker in the course of rabbit hemorrhagic disease indicates oxidative tissue damage. Our findings show that miR-21, miR-16b, and miR-34a regulate three apoptosis pathways. Meanwhile, miR-122 and miR-132 are involved in two oxidative stress pathways. The results of our research also have diagnostic (searching for potential disease biomarkers) and

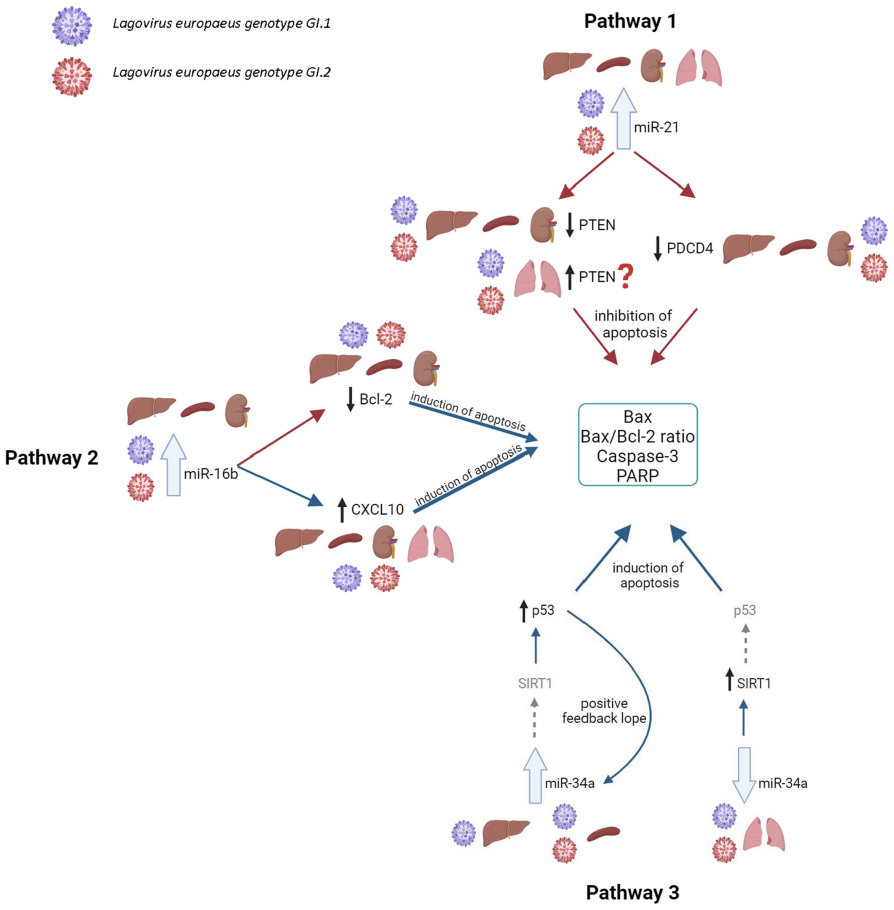


FIGURE 8
Contribution of miRs/target genes involved in the apoptosis pathway in *Lagovirus europaeus* infection. **Pathway 1**—increase miR-21 expression during *L. europaeus*—GI.1 and GI.2 genotypes inhibit *PTEN* and *PDCD4* in the liver (*PTEN*—Spearman's rank correlation Rho: −0.74, $p = 0.013$, *PDCD4*—Spearman's rank correlation Rho: −0.73, $p = 0.015$ for GI.1 and *PTEN*—Spearman's rank correlation Rho: −0.68, $p = 0.029$, *PDCD4*—Spearman's rank correlation Rho: −0.78, $p = 0.007$ for GI.2), kidney (*PTEN*—Spearman's rank correlation Rho: −0.84, $p = 0.002$, PDCD4—Spearman's rank correlation Rho: −0.83, $p = 0.003$ for GI.1 and *PTEN*—Spearman's rank correlation Rho: −0.66, $p = 0.038$, *PDCD4*—Spearman's rank correlation Rho: −0.72, $p = 0.019$ for GI.2), and spleen (*PTEN*—Spearman's rank correlation Rho: −0.64, $p = 0.048$, *PDCD4*—Spearman's rank correlation Rho: −0.71, $p = 0.022$ for GI.1 and *PTEN*—Spearman's rank correlation Rho: −0.78, $p = 0.008$, *PDCD4*—Spearman's rank correlation Rho: −0.94, $p < 0.001$ for GI.2) and effect on *Caspase-3* (in kidney Spearman's rank correlation Rho: 0.7, $p = 0.025$ for GI.1, in spleen Spearman's rank correlation Rho: 0.84, $p = 0.002$ for GI.1 and Spearman's rank correlation Rho: 0.89, $p = 0.0005$ for GI.2), *Bax* (Spearman's rank correlation Rho: 0.66, $p = 0.04$) and *Bax/Bcl-2* ratio (Spearman's rank correlation Rho: 0.68, $p = 0.029$) in the liver for GI.1. The exception is the lungs, where no inhibitory mechanism is observed during infection with *L. europaeus* in both genotypes. **Pathway 2**—increase miR-16b expression during *L. europaeus* GI.1 and GI.2 in the liver (Spearman's rank correlation Rho: −0.64, $p = 0.043$ for GI.1 and Spearman's rank correlation Rho: −0.84, $p = 0.002$ for GI.2), spleen (Spearman's rank correlation Rho: −0.85, $p = 0.002$ for GI.1 and Spearman's rank correlation Rho: −0.74, $p = 0.013$ for GI.2), and kidney (Spearman's rank correlation Rho: −0.77, $p = 0.009$ for GI.1 and Spearman's rank correlation Rho: −0.64, $p = 0.043$ for GI.2) inhibit the *Bcl-2* target gene, which is an antiapoptotic gene. In this case, the exception is the lungs, where after infection with both *L. europaeus* genotypes, we do not observe a change in the expression of miR-16b and *Bcl-2* but an increase in the expression of the proapoptotic *Bax*. Moreover, miR-16b affects the growth of the chemoattractant *CXCL10* in the liver (Spearman's rank correlation Rho: 0.77, $p = 0.009$ for GI.1 and Spearman's rank correlation Rho: 0.79, $p = 0.006$ for GI.2), spleen (Spearman's rank correlation Rho: 0.73, $p = 0.016$ for GI.1 and Spearman's rank correlation Rho: 0.75, $p = 0.013$ for GI.2), and kidney (Spearman's rank correlation Rho: 0.63, $p = 0.048$ for GI.1 and Spearman's rank correlation Rho: 0.76, $p = 0.011$ for GI.2). The exception is the lungs, where no change in miR-16b expression was observed, but an increase in *CXCL10* expression was observed in both genotypes. Additionally, in the liver, miR-16 correlates with the *Bax/Bcl-2* ratio (Spearman's rank correlation Rho: 0.63, $p = 0.047$ for GI.1) and with *Caspase-3* (Spearman's rank correlation Rho: 0.83, $p = 0.003$ for GI.2). In the kidney, the analysis showed a positive correlation of miR-16 with the *Bax/Bcl-2* ratio (Spearman's rank correlation Rho: 0.85, $p = 0.002$ for GI.1 and Spearman's rank correlation Rho: 0.89, $p = 0.0005$ for GI.2). Whereas correlation miR-16 with *Caspase-3* has been demonstrated only during infection of GI.2 in the kidney (Spearman's rank correlation Rho: 0.67, $p = 0.03$) and in the spleen (Spearman's rank correlation Rho: 0.71, $p = 0.02$). **Pathway 3**—increased miR-34a expression during *L. europaeus* GI.1 infection in the liver and during GI.1 and GI.2 in the spleen do not affect *SIRT1* expression but induce *p53* in the liver (Spearman's rank correlation Rho: 0.68, $p = 0.028$ for GI.1) and spleen (Spearman's rank correlation Rho: 0.73, $p = 0.016$ for GI.1 and Spearman's rank correlation Rho: 0.77, $p = 0.009$ for GI.2; GI.1 and GI.2), which leads to increased apoptosis. Despite the lack of statistical significance in the increase in miR-34a in the liver during GI.2 infection, it correlates with a decrease (also statistically insignificant) in *SIRT1* mRNA level (Spearman's rank correlation Rho: −0.75, $p = 0.011$). Additionally, *p53* positively correlates with biomarkers of apoptosis. In the liver, *p53* correlates with *Caspase-3* (Spearman's rank correlation Rho: 0.65, $p = 0.042$ for GI.1 and Spearman's rank correlation Rho: 0.63, $p = 0.047$ for GI.2) and with the *Bax/Bcl-2* ratio (Spearman's rank correlation Rho: 0.75, $p = 0.01$ for GI.1 and Spearman's rank correlation Rho: 0.77, $p = 0.009$ for GI.2). Whereas *p53* in the liver correlates with *Bax* only during GI.1 infection (Spearman's rank correlation Rho: 0.8, $p = 0.004$). In the spleen, *p53* correlates with *Bax/Bcl-2* ration in both genotypes (Spearman's rank correlation Rho: 0.78, $p = 0.008$ for GI.1 and Spearman's rank correlation Rho: 0.81, $p = 0.004$ for GI.2) and with *Caspase-3* only in GI.2 (Spearman's rank correlation Rho: 0.75, $p = 0.01$). A noticeable regulatory effect of miR-34a was observed in the lungs, which correlated with an increase in *SIRT1* (Spearman's rank correlation Rho:−0.64, $p = 0.042$ for GI.1 and Spearman's rank correlation Rho:−0.77, $p = 0.009$ for GI.2) did not affect the regulation of p53. ?—further research is necessary; the gray arrow indicates the lack of regulatory influence in our research.

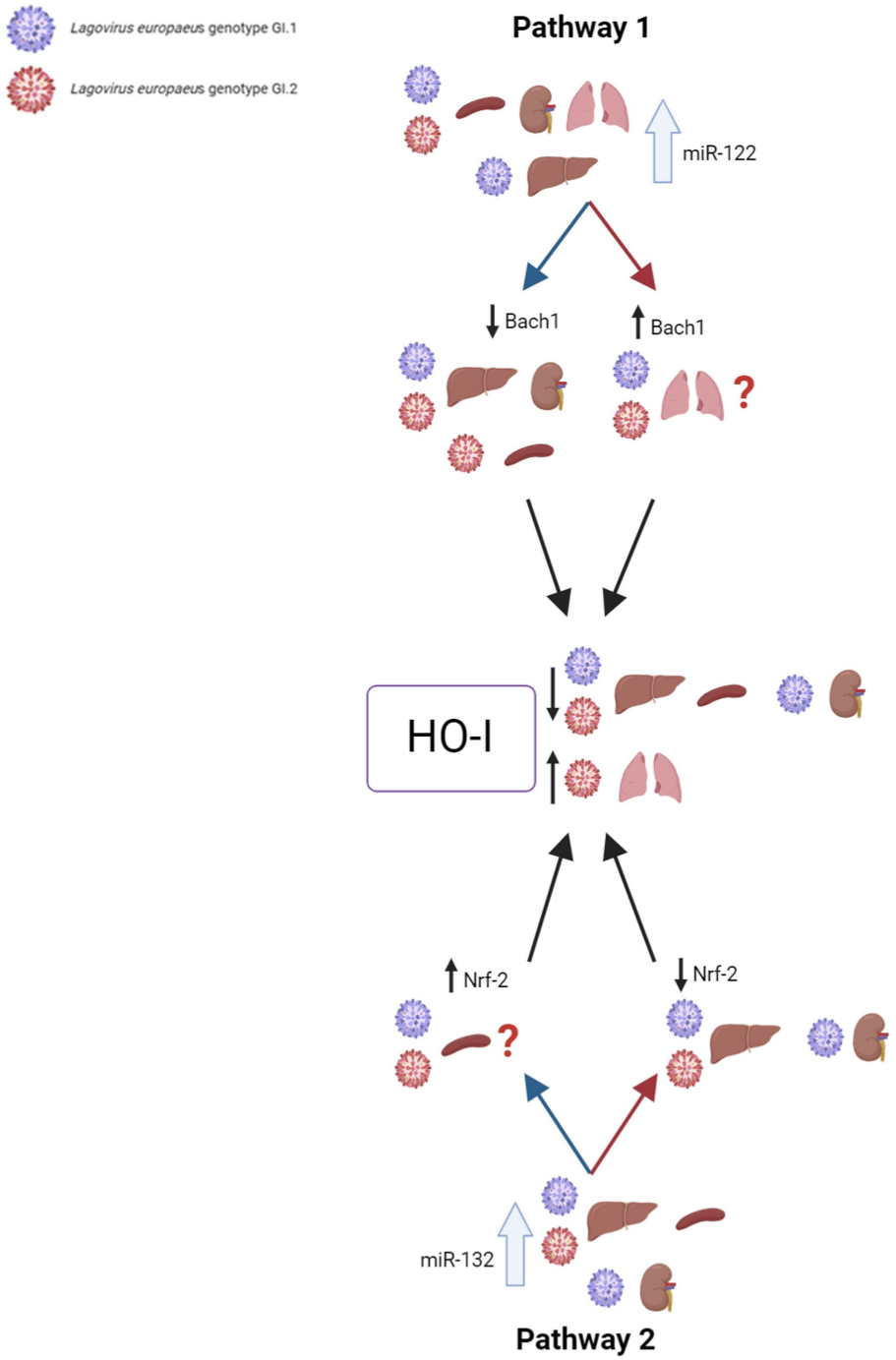


FIGURE 9
Contribution of miRs/target genes involved in the oxidative stress pathway in *Lagovirus europaeus* infection. **Pathway 1**—increase miR-122 expression during *L. europaeus* GI.1 and GI.2 genotype infection leads to a decrease in *Bach1* in all organs except in the lungs. miR-122 correlates with *Bach1* in the liver (Spearman's rank correlation Rho: −0.71, $p = 0.021$ for GI.1 and Spearman's rank correlation Rho: −0.66, $p = 0.037$ for GI.2), kidneys (Spearman's rank correlation Rho: −0.76, $p = 0.011$ for GI.1 and Spearman's rank correlation Rho: −0.8, $p = 0.005$ for GI.2), and spleen (Spearman's rank correlation Rho: −0.85, $p = 0.002$ for GI.2). The upregulation of miR-122 leads to a decrease in *HO-1* mRNA levels in the liver (Spearman's rank correlation Rho: −0.95, $p < 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.7, $p = 0.025$ for GI.2), spleen (Spearman's rank correlation Rho: −0.81, $p = 0.004$ for GI.1 and Spearman's rank correlation Rho: −0.92, $p = 0.0001$ for GI.2), and kidney (Spearman's rank correlation Rho: −0.91, $p = 0.0002$ for GI.1 and Spearman's rank correlation Rho: −0.79, $p = 0.006$ for GI.2), which leads to increased tissue damage. Therefore, *HO-1* has no protective effect on *L. europaeus* infection (except for the lungs). **Pathway 2**—it has not been previously described in viral infections, so it is a novelty. miR-132 expression during *L. europaeus* GI.1 and GI.2 in the liver, spleen, and kidney (only GI.1) inhibits *Nrf-2* in the liver (Spearman's rank correlation Rho: −0.83, p = 0.003 for GI.1 and Spearman's rank correlation Rho: −0.7, $p = 0.025$ for GI.2) and kidneys (Spearman's rank correlation Rho: −0.87, $p = 0.001$ for GI.1). Further research is needed to identify the factor influencing the increase in *Nrf-2* expression in the spleen during infection with *L. europaeus*. The correlation analyses performed indicate miR-122 as the main inhibitor of *HO-1* levels (not *Bach1* or *Nrf-2*) during *L. europaeus* infection, which can lead to increased tissue damage. ?—further research is necessary.

therapeutic (modulating miR-dependent pathways) potential during acute liver failure (ALF) and multi-organ failure (MOF) of viral etiology, which we encounter during *L. europaeus* infection.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Ethics statement

The animal study was approved by the Local Ethical Committee for Animal Experiments in Poznań, Poland (no. 51/2022). The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

EO: conceptualization, data curation, investigation, methodology, software, validation, visualization, writing—original draft, and writing—review and editing. AF: investigation, resources, and writing—original draft. AK: investigation, resources, and writing—original draft. AS: investigation, resources, and writing—original draft. BHS: conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, writing—original draft, writing—review and editing, funding acquisition, and project administration.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## References

Abolfathi, H., Arabi, M., and Sheikhpour, M. (2023). A literature review of microRNA and gene signaling pathways involved in the apoptosis pathway of lung cancer. *Respir. Res.* 24:55. doi: 10.1186/s12931-023-02366-w

Abrantes, J., van der Loo, W., Le Pendu, J., and Esteves, P. J. (2012). Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. *Vet. Res.* 43:12. doi: 10.1186/1297-9716-43-12

Agarwal, V., Bell, G. W., Nam, J. W., and Bartel, D. P. (2015). Predicting effective microRNA target sites in mammalian mRNAs. *eLife* 4:e05005. doi: 10.7554/eLife.05005

Alonso, C., Oviedo, J. M., Martin-Alonso, J. M., Diaz, E., Boga, J. A., and Parra, F. (1998). Programmed cell death in the pathogenesis of rabbit hemorrhagic disease. *Arch. Virol.* 143, 321–332. doi: 10.1007/s007050050289

Amador-Canizares, Y., Panigrahi, M., Huys, A., Kunden, R. D., Adams, H. M., Schinold, M. J., et al. (2018). miR-122, small RNA annealing and sequence mutations alter the predicted structure of the hepatitis C virus 5' UTR RNA to stabilize and promote viral RNA accumulation. *Nucleic Acids Res.* 46, 9776–9792. doi: 10.1093/nar/gky662

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., et al. (2000). Gene ontology: tool for the unification of biology. The gene ontology consortium. *Nat. Genet.* 25, 25–29. doi: 10.1038/75556

Asim, M., Amin, F., and El-Menyar, A. (2020). Multiple organ dysfunction syndrome: contemporary insights on the clinicopathological spectrum. *Qatar Med J* 2020:22. doi: 10.5339/qmj.2020.22

Banerjee, J., Khanna, S., and Bhattacharya, A. (2017). MicroRNA regulation of oxidative stress. *Oxid. Med. Cell. Longev.* 2017, 2872156–2872153. doi: 10.1155/2017/2872156

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297. doi: 10.1016/s0092-8674(04)00045-5

Beacon Designer. (2024). Computationaly tools for online. Available at: http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1.

Bebnowska, D., Hrynkiewicz, R., Rzeszotek, S., Freus, M., Poniewierska-Baran, A., and Niedzwiedzka-Rystwej, P. (2024). Apoptotic cell death in an animal model of virus-induced acute liver failure-observations during *Lagovirus europaeus*/GI.2 infection. *Int. J. Mol. Sci.* 25:798. doi: 10.3390/ijms25020798

Bebnowska, D., Hrynkiewicz, R., Wisniewska, K., Zabinska, M., Rintz, E., Pierzynowska, K., et al. (2023). Apoptosis activation during *Lagovirus europaeus*/GI.2 infection in rabbits. *Front. Microbiol.* 14:1308018. doi: 10.3389/fmicb.2023.1308018

Buscaglia, L. E., and Li, Y. (2011). Apoptosis and the target genes of microRNA-21. *Chin. J. Cancer* 30, 371–380. doi: 10.5732/cjc.011.10132

Chaitanya, G. V., Steven, A. J., and Babu, P. P. (2010). PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. *Cell Commun. Signal* 8:31. doi: 10.1186/1478-811X-8-31

Chen, J. (2015). MicroRNAs, signaling pathways and diseases. *Ann Transl Med* 3:329. doi: 10.3978/j.issn.2305-5839.2015.12.16

Chen, M., Liu, X., Hu, B., Fan, Z., Song, Y., Wei, H., et al. (2018). Rabbit hemorrhagic disease virus non-structural protein 6 induces apoptosis in rabbit kidney cells. *Front. Microbiol.* 9:3308. doi: 10.3389/fmicb.2018.03308

Chen, Y., and Wang, X. (2020). miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Res.* 48, D127–D131. doi: 10.1093/nar/gkz757

Choi, A. M., and Alam, J. (1996). Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am. J. Respir. Cell Mol. Biol.* 15, 9–19. doi: 10.1165/ajrcmb.15.1.8679227

Chou, C. H., Shrestha, S., Yang, C. D., Chang, N. W., Lin, Y. L., Liao, K. W., et al. (2018). miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res.* 46, D296–D302. doi: 10.1093/nar/gkx1067

Cimmino, A., Calin, G. A., Fabbri, M., Iorio, M. V., Ferracin, M., Shimizu, M., et al. (2005). miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc. Natl. Acad. Sci. U. S. A.* 102, 13944–13949. doi: 10.1073/pnas.0506654102

Duprez, L., Wirawan, E., Vanden Berghe, T., and Vandenabeele, P. (2009). Major cell death pathways at a glance. *Microbes Infect.* 11, 1050–1062. doi: 10.1016/j.micinf.2009.08.013

Esteves, P. J., Abrantes, J., Baldauf, H. M., BenMohamed, L., Chen, Y., Christensen, N., et al. (2018). The wide utility of rabbits as models of human diseases. *Exp. Mol. Med.* 50, 1–10. doi: 10.1038/s12276-018-0094-1

Fioravanti, A., Giordano, A., Dotta, F., and Pirtoli, L. (2022). Crosstalk between MicroRNA and oxidative stress in physiology and pathology 2.0. *Int. J. Mol. Sci.* 23:6831. doi: 10.3390/ijms23126831

Fitzner, A., Kesy, A., Bulenger, K., and Niedbalski, W. (2021). Evidence of independent introductions of RHDV2 strains in Poland based on the genome analysis of viral isolates from 2016-2018. *Acta Biochim. Pol.* 68, 255–263. doi: 10.18388/abp.2020_5547

Fu, R. Q., Hu, D. P., Hu, Y. B., Hong, L., Sun, Q. F., and Ding, J. G. (2017). miR-21 promotes alpha-SMA and collagen I expression in hepatic stellate cells via the Smad7 signaling pathway. *Mol. Med. Rep.* 16, 4327–4333. doi: 10.3892/mmr.2017.7054

Gain, C., Song, S., Angtuaco, T., Satta, S., and Kelesidis, T. (2022). The role of oxidative stress in the pathogenesis of infections with coronaviruses. *Front. Microbiol.* 13:1111930. doi: 10.3389/fmicb.2022.1111930

Gao, X., and Zhao, S. (2020). miRNA-16-5p inhibits the apoptosis of high glucose-induced pancreatic beta cells via targeting of CXCL10: potential biomarkers in type 1 diabetes mellitus. *Endokrynol. Pol.* 71, 404–410. doi: 10.5603/EP.a2020.0046

Garcia-Lastra, R., San-Miguel, B., Crespo, I., Jorquera, F., Alvarez, M., Gonzalez-Gallego, J., et al. (2010). Signaling pathways involved in liver injury and regeneration in rabbit hemorrhagic disease, an animal model of virally-induced fulminant hepatic failure. *Vet. Res.* 41:02. doi: 10.1051/vetres/2009050

Ghafouri-Fard, S., Abak, A., Shoorei, H., Mohaqiq, M., Majidpoor, J., Sayad, A., et al. (2021). Regulatory role of microRNAs on PTEN signaling. *Biomed. Pharmacother.* 133:110986. doi: 10.1016/j.biopha.2020.110986

Hao, R., Song, X., Sun-Waterhouse, D., Tan, X., Li, F., and Li, D. (2021). MiR-34a/Sirt1/p53 signaling pathway contributes to cadmium-induced nephrotoxicity: a preclinical study in mice. *Environ. Pollut.* 282:117029. doi: 10.1016/j.envpol.2021.117029

He, G., Ding, J., Zhang, Y., Cai, M., Yang, J., Cho, W. C., et al. (2021). microRNA-21: a key modulator in oncogenic viral infections. *RNA Biol.* 18, 809–817. doi: 10.1080/15476286.2021.1880756

Herengt, A., Thyrsted, J., and Holm, C. K. (2021). NRF2 in viral infection. *Antioxidants* 10:1491. doi: 10.3390/antiox10091491

Hermeking, H. (2010). The miR-34 family in cancer and apoptosis. *Cell Death Differ.* 17, 193–199. doi: 10.1038/cdd.2009.56

Hu, B., Wei, H., Song, Y., Chen, M., Fan, Z., Qiu, R., et al. (2020). NF-kappaB and Keap1 interaction represses Nrf2-mediated antioxidant response in rabbit hemorrhagic disease virus infection. *J. Virol.* 94, e00016–e00020. doi: 10.1128/JVI.00016-20

Hukowska-Szematowicz, B. (2013). Genetic and immunological characteristic of European strains of RHD (rabbit haemorrhagic disease) virus. *Pol. J. Environ. Stud.* 2, 1–114.

Hukowska-Szematowicz, B., Maciejak-Jastrzebska, A., Blatkiewicz, M., Maciak, K., Gora, M., Janiszewska, J., et al. (2020). Changes in MicroRNA expression during rabbit hemorrhagic disease virus (RHDV) infection. *Viruses* 12:965. doi: 10.3390/v12090965

Hukowska-Szematowicz, B., Ostrycharz, E., Dudzinska, W., Roszkowska, P., Siennicka, A., and Wojciechowska-Koszko, I. (2023). Digital PCR (dPCR) quantification of miR-155-5p as a potential candidate for a tissue biomarker of inflammation in rabbits infected with *Lagovirus europaeus*/rabbit hemorrhagic disease virus (RHDV). *Viruses* 15:1578. doi: 10.3390/v15071578

Jiang, D., Li, M., Yu, Y., Shi, H., and Chen, R. (2019). microRNA-34a aggravates coxsackievirus B3-induced apoptosis of cardiomyocytes through the SIRT1-p53 pathway. *J. Med. Virol.* 91, 1643–1651. doi: 10.1002/jmv.25482

Jopling, C. L. (2008). Regulation of hepatitis C virus by microRNA-122. *Biochem. Soc. Trans.* 36, 1220–1223. doi: 10.1042/BST0361220

Jopling, C. L., Schutz, S., and Sarnow, P. (2008). Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* 4, 77–85. doi: 10.1016/j.chom.2008.05.013

Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M., and Sarnow, P. (2005). Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 309, 1577–1581. doi: 10.1126/science.1113329

Jung, J. Y., Lee, B. J., Tai, J. H., Park, J. H., and Lee, Y. S. (2000). Apoptosis in rabbit haemorrhagic disease. *J. Comp. Pathol.* 123, 135–140. doi: 10.1053/jcpa.2000.0403

Klieser, E., Mayr, C., Kiesslich, T., Wissniowski, T., Fazio, P. D., Neureiter, D., et al. (2019). The crosstalk of miRNA and oxidative stress in the liver: from physiology to pathology and clinical implications. *Int. J. Mol. Sci.* 20:5266. doi: 10.3390/ijms20215266

Lai, X., Dreyer, F. S., Cantone, M., Eberhardt, M., Gerer, K. F., Jaitly, T., et al. (2021). Network- and systems-based re-engineering of dendritic cells with non-coding RNAs for cancer immunotherapy. *Theranostics* 11, 1412–1428. doi: 10.7150/thno.53092

Le Pendu, J., Abrantes, J., Bertagnoli, S., Guitton, J. S., Le Gall-Recule, G., Lopes, A. M., et al. (2017). Proposal for a unified classification system and nomenclature of lagoviruses. *J. Gen. Virol.* 98, 1658–1666. doi: 10.1099/jgv.0.000840

Liu, S. J. X. H. P., Pu, B. Q., and Qian, N. H. (1984). A new viral disease in rabbits. *Anim. Husb. Vet. Med.* 16, 253–255.

Mao, M., Yang, L., Hu, J., Liu, B., Zhang, X., Liu, Y., et al. (2022). Oncogenic E3 ubiquitin ligase NEDD4 binds to KLF8 and regulates the microRNA-132/NRF2 axis in bladder cancer. *Exp. Mol. Med.* 54, 47–60. doi: 10.1038/s12276-021-00663-2

Marques, R. M., Costa, E. S. A., Aguas, A. P., Teixeira, L., and Ferreira, P. G. (2010). Early acute depletion of lymphocytes in calicivirus-infected adult rabbits. *Vet. Res. Commun.* 34, 659–668. doi: 10.1007/s11259-010-9437-7

Ni, Z., Wei, W., Liu, G. Q., Chen, L., Yu, B., Yun, T., et al. (2009). Studies on the apoptosis of RK13 cells induced by rabbit hemorrhagic disease virus. *Bing Du Xue Bao* 25, 316–317.

Niedzwiedzka-Rystwej, P., and Deptula, W. (2012). Apoptosis of peripheral blood leukocytes from rabbits infected with non-haemagglutinating strains of rabbit haemorrhagic disease virus (RHDV). *Vet. Immunol. Immunopathol.* 149, 54–57. doi: 10.1016/j.vetimm.2012.06.012

Niedzwiedzka-Rystwej, P., Hukowska-Szematowicz, B., Tokarz-Deptula, B., Trzeciak-Ryczek, A., Dzialo, J., and Deptula, W. (2013a). Apoptosis of peripheral blood leucocytes in rabbits infected with different strains of rabbit haemorrhagic disease virus. *Acta Biochim. Pol.* 60, 65–69. doi: 10.18388/abp.2013_1952

Niedzwiedzka-Rystwej, P., Tokarz-Deptula, B., and Deptula, W. (2013b). Apoptosis of granulocytes and lymphocytes in peripheral blood in rabbits infected with haemagglutinating and non-haemagglutinating antigenic variants of the RHD (rabbit haemorrhagic disease) virus. *Pol. J. Vet. Sci.* 16, 223–229. doi: 10.2478/pjvs-2013-0032

Ostrycharz, E., Blatkiewicz, M., and Hukowska-Szematowicz, B. (2021). *Młodzi naukowcy 2.0. Tom 1. Expression of the master anti-inflammatory regulator IL-10 during Lagovirus europaeus GI.1/RHDV (rabbit hemorrhagic disease virus) infection*. Fundacji Centrum Badań Socjologicznych (Centre of Sociological Research); Szczecin, Poland: pp. 59–72.

Ostrycharz, E., and Hukowska-Szematowicz, B. (2022). Micro-players of great significance-host microRNA signature in viral infections in humans and animals. *Int. J. Mol. Sci.* 23:10536. doi: 10.3390/ijms231810536

O'Toole, A. D., Mohamed, F. M., Zhang, J., and Brown, C. C. (2022). Early pathogenesis in rabbit hemorrhagic disease virus 2. *Microb. Pathog.* 173:105814. doi: 10.1016/j.micpath.2022.105814

Pandey, N., Rastogi, M., and Singh, S. K. (2021). Chandipura virus dysregulates the expression of hsa-miR-21-5p to activate NF-kappaB in human microglial cells. *J. Biomed. Sci.* 28:52. doi: 10.1186/s12929-021-00748-0

Park, J. H., Lee, Y. S., and Itakura, C. (1995). Pathogenesis of acute necrotic hepatitis in rabbit hemorrhagic disease. *Lab. Anim. Sci.* 45, 445–449.

Pepper, C., Hoy, T., and Bentley, D. P. (1997). Bcl-2/Bax ratios in chronic lymphocytic leukaemia and their correlation with in vitro apoptosis and clinical resistance. *Br. J. Cancer* 76, 935–938. doi: 10.1038/bjc.1997.487

Primer-BLAST. (2024). Computational tools for online. Available at: https://www.ncbi.nlm.nih.gov/tools/primer-blast/.

Qiu, X., Dong, S., Qiao, F., Lu, S., Song, Y., Lao, Y., et al. (2013). HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene* 32, 3296–3305. doi: 10.1038/onc.2013.150

Qiu, L., Fan, H., Jin, W., Zhao, B., Wang, Y., Ju, Y., et al. (2010). miR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. *Biochem. Biophys. Res. Commun.* 398, 771–777. doi: 10.1016/j.bbrc.2010.07.021

Rocchi, M., and Dagleish, M. (2018). Diagnosis and prevention of RHVD2 infection. *Vet. Rec.* 182, 604–605. doi: 10.1136/vr.k2313

Sadri Nahand, J., Shojaie, L., Akhlagh, S. A., Ebrahimi, M. S., Mirzaei, H. R., Bannazadeh Baghi, H., et al. (2021). Cell death pathways and viruses: role of microRNAs. *Mol Ther Nucleic Acids* 24, 487–511. doi: 10.1016/j.omtn.2021.03.011

San-Miguel, B., Alvarez, M., Culebras, J. M., Gonzalez-Gallego, J., and Tunon, M. J. (2006). N-acetyl-cysteine protects liver from apoptotic death in an animal model of fulminant hepatic failure. *Apoptosis* 11, 1945–1957. doi: 10.1007/s10495-006-0090-0

Santus, P., Danzo, F., Zuffi, A., Pini, S., Saad, M., Visconti, A., et al. (2022). Oxidative stress and viral infections: rationale, experiences, and perspectives on N-acetylcysteine. *Eur. Rev. Med. Pharmacol. Sci.* 26, 8582–8590. doi: 10.26355/eurrev_202211_30395

Semerjyan, A. B., Sargsyan, M. A., Arzumanyan, H. H., Hakobyan, L. H., Abroyan, L. O., Semerjyan, Z. B., et al. (2019). Immune cell pathology in rabbit hemorrhagic disease. *Vet World* 12, 1332–1340. doi: 10.14202/vetworld.2019.1332-1340

Shan, Y., Zheng, J., Lambrecht, R. W., and Bonkovsky, H. L. (2007). Reciprocal effects of micro-RNA-122 on expression of heme oxygenase-1 and hepatitis C virus genes in human hepatocytes. *Gastroenterology* 133, 1166–1174. doi: 10.1053/j.gastro.2007.08.002

Sharma, V. K., Raimondi, V., Ruggero, K., Pise-Masison, C. A., Cavallari, I., Silic-Benussi, M., et al. (2018). Expression of miR-34a in T-cells infected by human T-Lymphotropic virus 1. *Front. Microbiol.* 9:832. doi: 10.3389/fmicb.2018.00832

Su, Z., Yang, Z., Xu, Y., Chen, Y., and Yu, Q. (2015). MicroRNAs in apoptosis, autophagy and necroptosis. *Oncotarget* 6, 8474–8490. doi: 10.18632/oncotarget.3523

Sun, W., Liu, S., Yan, Y., Wang, Q., Fan, Y., and Okyere, S. K. (2023). Pseudorabies virus causes splenic injury via inducing oxidative stress and apoptosis related factors in mice. *Sci. Rep.* 13:23011. doi: 10.1038/s41598-023-50431-7

Teixeira, L., Marques, R. M., Aguas, A. P., and Ferreira, P. G. (2012). Regulatory T cells are decreased in acute RHDV lethal infection of adult rabbits. *Vet. Immunol. Immunopathol.* 148, 343–347. doi: 10.1016/j.vetimm.2012.05.006

Thomson, B. J. (2001). Viruses and apoptosis. *Int. J. Exp. Pathol.* 82, 65–76. doi: 10.1111/j.1365-2613.2001.iep0082-0065-x

Trzeciak-Ryczek, A., Tokarz-Deptula, B., and Deptula, W. (2015). The importance of liver lesions and changes to biochemical and coagulation factors in the pathogenesis of RHD. *Acta Biochim. Pol.* 62, 169–171. doi: 10.18388/abp.2014_943

Trzeciak-Ryczek, A., Tokarz-Deptula, B., and Deptula, W. (2016). Expression of IL-1beta, IL-2, IL-10, TNF-beta and GM-CSF in peripheral blood leukocytes of rabbits experimentally infected with rabbit haemorrhagic disease virus. *Vet. Microbiol.* 186, 71–81. doi: 10.1016/j.vetmic.2016.02.021

Trzeciak-Ryczek, A., Tokarz-Deptula, B., and Deptula, W. (2017). Expression of IL-1Ra, IL-6, IL-8, IL-18, TNF-alpha and IFN-gamma genes in peripheral blood leukocytes of rabbits infected with RHDV (rabbit Haemorrhagic disease virus). *Dev. Comp. Immunol.* 76, 310–315. doi: 10.1016/j.dci.2017.07.005

Tunon, M. J., San Miguel, B., Crespo, I., Jorquera, F., Santamaria, E., Alvarez, M., et al. (2011). Melatonin attenuates apoptotic liver damage in fulminant hepatic failure induced by the rabbit hemorrhagic disease virus. *J. Pineal Res.* 50, 38–45. doi: 10.1111/j.1600-079X.2010.00807.x

Tunon, M. J., Sanchez-Campos, S., Garcia-Ferreras, J., Alvarez, M., Jorquera, F., and Gonzalez-Gallego, J. (2003). Rabbit hemorrhagic viral disease: characterization of a new animal model of fulminant liver failure. *J. Lab. Clin. Med.* 141, 272–278. doi: 10.1067/mlc.2003.30

Vallejo, D., Crespo, I., San-Miguel, B., Alvarez, M., Prieto, J., Tunon, M. J., et al. (2014). Autophagic response in the rabbit hemorrhagic disease, an animal model of virally-induced fulminant hepatic failure. *Vet. Res.* 45:15. doi: 10.1186/1297-9716-45-15

Valyi-Nagy, T., and Dermody, T. S. (2005). Role of oxidative damage in the pathogenesis of viral infections of the nervous system. *Histol. Histopathol.* 20, 957–967. doi: 10.14670/HH-20.957

Verma, A. K., Ghosh, S., and Basu, A. (2018). Chandipura virus induced neuronal apoptosis via calcium signaling mediated oxidative stress. *Front. Microbiol.* 9:1489. doi: 10.3389/fmicb.2018.01489

Wang, H., Zhang, C., Zhang, C., Wang, Y., Zhai, K., and Tong, Z. (2022). MicroRNA-122-5p regulates coagulation and inflammation through MASP1 and HO-1 genes. *Infect. Genet. Evol.* 100:105268. doi: 10.1016/j.meegid.2022.105268

Wasik, U., Milkiewicz, M., Kempinska-Podhorodecka, A., and Milkiewicz, P. (2017). Protection against oxidative stress mediated by the Nrf2/Keap1 axis is impaired in primary biliary cholangitis. *Sci. Rep.* 7:44769. doi: 10.1038/srep44769

Wei, J., Feng, L., Li, Z., Xu, G., and Fan, X. (2013). MicroRNA-21 activates hepatic stellate cells via PTEN/Akt signaling. *Biomed. Pharmacother.* 67, 387–392. doi: 10.1016/j.biopha.2013.03.014

Wu, G., Yu, F., Xiao, Z., Xu, K., Xu, J., Tang, W., et al. (2011). Hepatitis B virus X protein downregulates expression of the miR-16 family in malignant hepatocytes in vitro. *Br. J. Cancer* 105, 146–153. doi: 10.1038/bjc.2011.190

Xu, K., Chen, C., Wu, Y., Wu, M., and Lin, L. (2021). Advances in miR-132-based biomarker and therapeutic potential in the cardiovascular system. *Front. Pharmacol.* 12:751487. doi: 10.3389/fphar.2021.751487

Yachie, A. (2021). Heme Oxygenase-1 deficiency and oxidative stress: a review of 9 independent human cases and animal models. *Int. J. Mol. Sci.* 22:1514. doi: 10.3390/ijms22041514

Yamakuchi, M., Ferlito, M., and Lowenstein, C. J. (2008). miR-34a repression of SIRT1 regulates apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* 105, 13421–13426. doi: 10.1073/pnas.0801613105

Yamakuchi, M., and Lowenstein, C. J. (2009). MiR-34, SIRT1 and p53: the feedback loop. *Cell Cycle* 8, 712–715. doi: 10.4161/cc.8.5.7753

Yang, X., Liu, X., Nie, Y., Zhan, F., and Zhu, B. (2023). Oxidative stress and ROS-mediated cellular events in RSV infection: potential protective roles of antioxidants. *Virol. J.* 20:224. doi: 10.1186/s12985-023-02194-w

Zhou, Y., Li, K. S., Liu, L., and Li, S. L. (2020). MicroRNA-132 promotes oxidative stress-induced pyroptosis by targeting sirtuin 1 in myocardial ischaemia-reperfusion injury. *Int. J. Mol. Med.* 45, 1942–1950. doi: 10.3892/ijmm.2020.4557

Zhu, H., Lin, Y., and Liu, Y. (2021). miR-34a increases inflammation and oxidative stress levels in patients with necrotizing enterocolitis by downregulating SIRT1 expression. *Mol. Med. Rep.* 24:664. doi: 10.3892/mmr.2021.12303

# Publikacja 3 [P-3]

Ostrycharz-Jasek Ewa, Fitzner Andrzej, Siennicka Aldona, Budkowska Marta, Hukowska-Szematowicz Beata

MicroRNAs regulate the expression of genes related to the innate immune and inflammatory response in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 Genotypes

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# MicroRNAs Regulate the Expression of Genes Related to the Innate Immune and Inflammatory Response in Rabbits Infected with *Lagovirus europaeus* GI.1 and GI.2 Genotypes

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**Abstract:** MicroRNAs (miR) are a group of small, non-coding RNAs of 17–25 nucleotides that regulate gene expression at the post-transcriptional level. Dysregulation of miRNA expression or function may contribute to abnormal gene expression and signaling pathways, leading to disease pathology. *Lagovirus europaeus* (*L. europaeus*) causes severe disease in rabbits called rabbit hemorrhagic disease (RHD). The symptoms of liver, lung, kidney, and spleen degeneration observed during RHD are similar to those of acute liver failure (ALF) and multi-organ failure (MOF) in humans. In this study, we assessed the expression of miRs and their target genes involved in the innate immune and inflammatory response. Also, we assessed their potential impact on pathways in *L. europaeus* infection—two genotypes (GI.1 and GI.2)—in the liver, lungs, kidneys, and spleen. The expression of miRs and target genes was determined using quantitative real-time PCR (qPCR). We assessed the expression of miR-155 (*MyD88*, *TAB2*, *p65*, *NLRP3*), miR-146a (*IRAK1*, *TRAF6*), miR-223 (*TLR4*, *IKKα*, *NLRP3*), and miR-125b (*MyD88*). We also examined biomarkers of inflammation: *IL-1β*, *IL-6*, *TNF-α*, and *IL-18* in four tissues at the mRNA level. Our study shows that the main regulators of the innate immune and inflammatory response in *L. europaeus*/GI.1 and GI.2 infection, as well as RHD, are miR-155, miR-223, and miR-146a. During infection with *L. europaeus*/RHD, miR-155 has both pro- and anti-inflammatory effects in the liver and anti-inflammatory effects in the kidneys and spleen; miR-146a has anti-inflammatory effects in the liver, lungs and kidneys; miR-223 has anti-inflammatory effects in all tissues; however, miR-125b has anti-inflammatory effects only in the liver. In each case, such an effect may be a determinant of the pathogenesis of RHD. Our research shows that miRs may regulate three innate immune and inflammatory response pathways in *L. europaeus* infection. However, the result of this regulation may be influenced by the tissue microenvironment. Our research shows that infection of rabbits with *L. europaeus*/GI.1 and GI.2 genotypes causes an overexpression of two critical acute phase cytokines: *IL-6* in all examined tissues and *TNF-α* (in the liver, lungs, and spleen). *IL-1β* was highly expressed only in the lungs after *L. europaeus* infection. These facts indicate a strong and rapid involvement of the local innate immune and inflammatory response in *L. europaeus* infection—two genotypes (GI.1 and GI.2)—and in the pathogenesis of RHD. Profile of biomarkers of inflammation in rabbits infected with *L. europaeus*/GI.1 and GI.2 genotypes are similar regarding the nature of changes but are different for individual tissues. Therefore, we propose three inflammation profiles for *L. europaeus* infection for both GI.1 and GI.2 genotypes (pulmonary, renal, liver, and spleen).

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**Keywords:** microRNA; *Lagovirus europaeus*/GI.1; GI.2; rabbit hemorrhagic disease (RHD); RHDV; innate immune; inflammation; biomarker; cytokine; rabbits

## 1. Introduction

Rabbit hemorrhagic disease (RHD) is caused by *L. europaeus* [1,2]. There are four genotypes within the *Lagovirus* genus (GI.1, GI.2, GI.3 and GI.4) [1]. The *L. europaeus*/GI.1 genotype includes four variants (GI.1a, GI.1b, GI.1c, and GI.1d), with the GI.1a variant causing an acute, inflammatory, highly contagious, and fatal disease with a mortality rate of 90–100% [3]. However, the *L. europaeus*/GI.2 genotype in infected rabbits causes a disease with variable mortality (from 50% to 80%, depending on the strain) and clinical presentation from sudden death in the hyperacute form to subacute or chronic disease [4,5]. The main target tissues of *L. europaeus* are the liver, lungs, spleen, and kidneys. However, where the most significant pathological changes are observed, the liver replicates the virus [3]. During infection, the liver becomes enlarged with a distinct lobular pattern. Moreover, increased virus-induced hepatocyte loss is observed in the liver [3,4]. The main changes in RHD are acute liver, spleen, lung, and kidney inflammation. Other pathological changes in RHD include spleen and kidney enlargement and pulmonary edema [3]. Inflammatory foci rich in neutrophils, T and B lymphocytes are found in the liver, lungs, spleen, and kidneys, and the activation of Kupffer cells occurs in the liver and alveolar macrophages in the lungs [3,4]. However, coagulation disorders, microthrombi formation, and massive disseminated intravascular coagulation (DIC) influence the occurrence of hemorrhages [3,5,6]. Studies have shown that necrosis is another critical factor in the pathogenesis of RHD [7]. As well as increased apoptosis, not only in the liver but in all infected tissues [8–18] and apoptosis of T and B lymphocytes in the liver and spleen and granulocytes and lymphocytes in peripheral blood [13,19–21], there is also increased oxidative stress [10,16,22].

In our recent studies [16,23–25], we indicated that another element in the pathogenesis of RHD are microRNAs (miRs), representing a subclass of small non-coding, single-stranded RNAs that, by binding to mRNA, post-transcriptionally regulate gene expression [26]. Our reports [16,24] are the first to present the regulatory effects of miRs on apoptosis, oxidative stress, and inflammation genes in *L. europaeus* infection—two genotypes (GI.1 and GI.2)—in four rabbit tissues (liver, lung, kidneys, and spleen). Our studies provide new data that are critical for understanding the pathogenesis of RHD caused by *L. europaeus*—two genotypes (GI.1 and GI.2)—concerning the molecular regulation of apoptosis and oxidative stress by miRs (as two essential biological processes in infectious viruses). Increased expression of miR-132 and miR-122 through the regulation of *Nrf-2*, *Bach1*, and *HO-1* genes regulates oxidative stress in the pathogenesis of RHD and affects tissue damage in *L. europaeus*-infected animals. Additionally, changes in the expression levels of proapoptotic (miR-16b and miR-34a) and antiapoptotic (miR-21) miRs and their impact on apoptosis-related target genes (*Bcl-2*, *PTEN*, *SIRT1/p53*) may intensify apoptosis, contributing to more severe disease and the death of animals [16]. Our findings show that miR-21, miR-16b, and miR-34a regulate three apoptosis pathways. Meanwhile, miR-122 and miR-132 are involved in two oxidative stress pathways [16].

The innate and adaptive immune response, including peripheral blood leukocytes and systemic inflammation, also plays an essential role in the pathogenesis of RHD.

The innate immune response after infection of rabbits with over thirty different strains of *L. europaeus* was mainly manifested by variable phagocytosis activity by neutrophils and the killing capacity of leukocytes through the activity of enzymes with killing/antiviral properties—myeloperoxidase (MPO) and lysozyme (LZM) [20,27–33]. The participation and intensity of these processes depended on the *L. europaeus* variant/strain. Moreover, in the infected organs (mainly the liver, lungs, kidneys, and spleen), infiltrates rich in leukocytes (neutrophils, T and B cells) and an increase in the inflammatory biomarker miR-155 are observed [23,34,35]. It has also been shown that during *L. europaeus* infection,

the levels of both pro- and anti-inflammatory cytokines increase in tissues (liver and spleen) and peripheral blood leukocytes. These cytokines include IL-1, IL-6, IL-8, IL-10, TNF-α, TNF-β, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [36–41]. Additionally, O'Toole et al. [42] indicate that *L. europaues* infection/GI.2 genotype drives the pathogenesis of RHD through a cytokine storm. Hepatocytes excessively produce TNF-α, IL-1β and IL-6, leading to hypercoagulability. In 2024, Yu et al. [43] showed that pro-inflammatory cytokines (IL-1α, IL-6, IL-8, IL-22) and chemokines (CCL2, CXCL9), involved in inflammation, are significantly increased in the spleen in the late stages of *L. europaeus* infection/GI.2 genotype. These data suggests that *L. europaeus*/GI.2 genotype (RHDV2) infection may induce dysregulation of the cytokine network and weaken the spleen's resistance to viral infection, leading to inflammatory disorders.

However, the adaptive immune response was characterized by variable activity of lymphocytes with different phenotypes. These phenotypes included T (CD5+), Th (CD4+), Tc (as CTLs) (CD8+),Tregs (CD25+), and B lymphocytes (CD19+). This variability indicates an impaired immune response, sometimes resulting in the complete loss of effector cells in a short time and changes in the total amount of immunoglobulins [20,28,29,44,45].

MiRs are also involved in the host's immune and inflammatory response to an inflammatory stimulus [46]. The role of miRs in the innate immune and inflammatory response context may be twofold [46–48]. On the one hand, the expression of miRs may be directly regulated by innate immune responses (since intracellular levels of different miRs are initially regulated at the transcriptional level by transcription factors dependent on the cell, tissue type, and environmental stimuli) [46]. On the other hand, miRs can regulate critical genes of innate immunity, among others: miR-155 regulated *MyD88* (Myeloid differentiation primary response protein MyD88), *TAB2* (GF-beta-activated kinase 1 and MAP3K7-binding protein 2), the *p65* subunit of NF-κβ (Transcription factor p65), and the *NLRP3* inflammasome (NACHT, LRR and PYD domains-containing protein 3); miR-146a regulated *IRAK1* (Interleukin-1 receptor-associated kinase 1), and *TRAF6* (TNF receptor-associated factor 6); miR-223 regulated *TLR4* (Toll-like receptor 4), *IKKα* (Inhibitor of nuclear factor kappa-B kinase subunit alpha), and the *NLRP3* inflammasome (NACHT, LRR and PYD domains-containing protein 3); and miR-125b regulated *MyD88*, thereby regulating innate immune and inflammatory responses (becoming pro-inflammatory or anti-inflammatory/or even both) [46–49]. Researchers have identified several dozen miRs involved in regulating the innate immune and inflammatory response, with the well-described being miR-155, miR-146a, miR-223, and miR-125b [46–52].

So far, apart from our studies [16,23–25,53], there is a lack of information on the molecular signatures of regulatory interactions between miRs and biological processes occurring in *L. europaeus* infection/RHD pathogenesis. There is a lack of studies on the molecular regulatory interactions between miRs and the innate immune and inflammatory response in *L. europaeus* infection. Therefore, this issue is the aim of this study. The study assessed the expression of miRs and their target genes involved in the regulation of the innate immune and inflammatory response, as well as presenting their potential impact on the pathways in *Lagovirus europaeus* infection—two genotypes (GI.1 and GI.2)—of different virulence in four tissues (liver, lungs, kidneys, and spleen). Based on in-silico analysis and previous literature data [24,25,46], we selected known miRs and target genes involved in the regulation of the innate immune and inflammatory response (essential in the TLR4-MyD88, NF-κB, NLRP3 inflammasome signaling pathway): miR-155 (*MyD88*, *TAB2*, *p65*, *NLRP3*), miR-146a (*IRAK1*, *TRAF6*), miR-223 (*TLR4*, *IKKα*, *NLRP3*), and miR-125b (*MyD88*). Also, we examined biomarkers of inflammation at the mRNA level: *IL-1β*, *IL-6*, *TNF-α*, and *IL-18* in the examined tissue.

Understanding the above molecular interactions has diagnostic potential (search for potential molecular biomarkers of inflammation/disease) and therapeutic potential (modulation of miR-dependent pathways, e.g., NF-κB and NLRP3 inflammasome) in the course of acute liver failure (ALF) and organ dysfunction in multi-organ failure (MOF) of a viral etiology that we encounter during *Lagovirus europaeus* infection.

## 2. Results

### 2.1. MiRs Expression Levels and Its Downstream Targets Involved in Innate Immune and Inflammatory Responses in Four Tissues in Rabbits during Lagovirus europaeus/GI.1 and GI.2 Genotype Infection

We analyzed the expression of miRs (miR-155, miR-146a, miR-223, and miR-125b) and its downstream targets involved in the innate immune and inflammatory response in four tissues (liver, lung, spleen, and kidney) in rabbits infected with *L. europaeus*/GI.1 and GI.2 genotypes.

#### 2.1.1. Liver

In the liver after infection with *L. europaeus*, a very similar increased miR-155 expression was observed (13.6-fold change vs. control, $p < 0.001$ and 13.7-fold change vs. control, $p < 0.001$ for GI.1 and GI.2, respectively; Figure 1A). In the case of infection with *L. europaeus*/GI.1 and GI.2 genotypes, upregulation of miR-155 was accompanied by decreased levels of *MyD88* (17.5-fold reduction vs. control, $p = 0.01$ and 32-fold reduction vs. control, $p < 0.001$, respectively; Figure 1B) and *p65* (11.5-fold reduction vs. control, $p < 0.001$ and 5.7-fold reduction vs. control, $p < 0.001$, respectively; Figure 1D). However, in the case of *TAB2*, the change in expression was noted only during infection with the GI.1 genotype and was upregulated by a 2.4-fold change ($p < 0.001$) compared to the control (Figure 1C). However, compared to the GI.2 group, in the GI.1 group, the expression was a higher 1.5-fold change ($p = 0.03$; Figure 1C). Our research demonstrated that in the liver, an increase in miR-155 was accompanied by overexpression of the NLRP3 inflammasome by a 53.6-fold change for the GI.1 group compared to the control ($p < 0.001$) and a 37.7-fold change for the GI.2 group compared to the control (Figure 1E). Additionally, the expression of *NLRP3* showed a 1.4-fold change higher in the case of GI.1 infection than in the GI.2 infected group ($p = 0.03$; Figure 1E).

The expression of miR-146a, like miR-155, was enhanced in both infected groups. Compared to the control group during *L. europaeus*/GI.1 infection, the increase in expression of miR-146a was a 10.5-fold change ($p < 0.001$); however, during infection with genotype GI.2, the expression increase was a 9.9-fold change ($p < 0.001$) (Figure 1F). Upregulation of miR-146a was associated with a significant downregulation of *TRAF6* (9-fold change, $p = 0.003$ for GI.1 vs. control and 30.6-fold change, $p < 0.001$ for GI.2 vs. control; Figure 1H). *IRAK1* overexpression was noted during infection of GI.1 and GI.2 (2.1-fold change, $p = 0.01$ vs. control and 2.6-fold change, $p = 0.001$ vs. control; Figure 1G).

The highest increase in expression among miRs in the liver was observed in the case of miR-223. Compared to the control upregulation of miR-223, it was a 816-fold change in the GI.1 group ($p < 0.001$) and 826-fold change in the GI.2 group ($p < 0.001$) (Figure 1I). No statistically significant change was observed in the expression level of *TLR4*, a target gene of miR-223, in the GI.1 and GI.2 groups (Figure 1J). Upregulation of miR-223 was associated with decreased *IKKα* levels in both infected groups compared to the control (3-fold change, $p = 0.003$ for GI.1 and 6.7-fold change, $p = 0.002$ for GI.2; Figure 1K). However, during infection with the *L. europaeus*/GI.1 genotype, the *IKKα* expression level was a 2.3-fold change higher ($p = 0.02$) than the GI.2 genotype (Figure 1K). In the case of another miR-223 target, the *NLRP3* inflammasome, an increase was observed (53.6-fold change for GI.1 vs. control, $p < 0.001$ and 37.7-fold change for GI.2 vs. control, $p < 0.001$; Figure 1L). Additionally, the expression of *NLRP3* was a 1.4-fold change higher in GI.1 compared to the GI.2 genotype ($p = 0.03$) (Figure 1L).

In the liver also, miR-125b was significantly higher during infection of both L. europaeus genotypes compared to the control (2.7-fold change, $p < 0.001$ for GI.1 and 3-fold change, $p < 0.001$ for GI.2; Figure 1M). This fact was accompanied by decreased *MyD88* expression levels (17.5-fold reduction vs. control, $p = 0.01$ and 32-fold reduction vs. control, $p < 0.001$; Figure 1N).

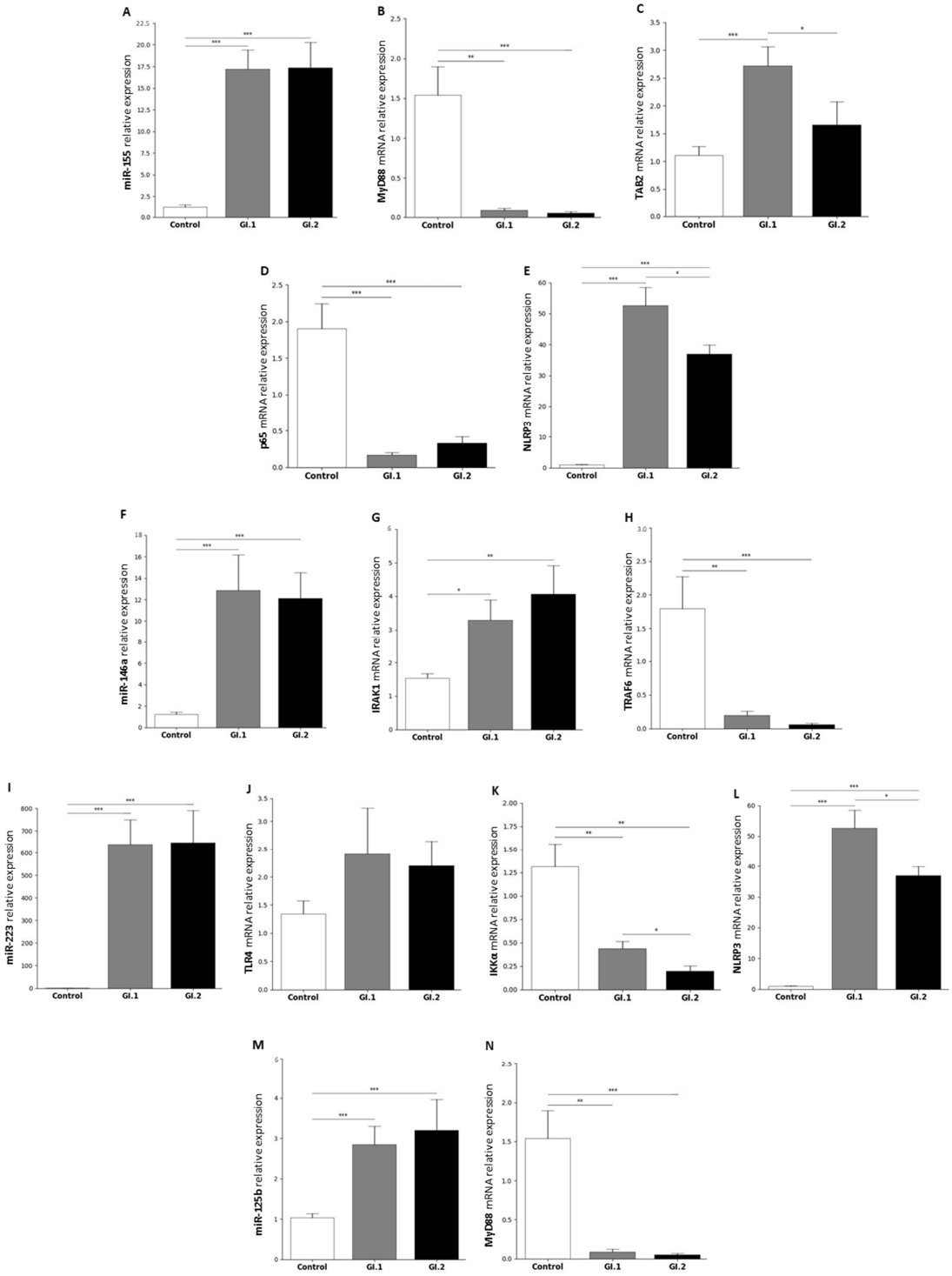


**Figure 1.** Relative expression of miRs and downstream targets: miR-155 (**A**) (*MyD88* (**B**), *TAB2* (**C**), *p65* (**D**), *NLRP3* (**E**)), miR-146a (**F**) (*IRAK1* (**G**), *TRAF6* (**H**)), miR-223 (**I**) (*TLR4* (**J**), *IKKα* (**K**), *NLRP3* (**L**)), miR-125b (**M**) (*MyD88* (**N**)) in the liver during rabbits infection with *Lagovirus europaeus*—two genotypes (GI.1 ($n$ = 10) and GI.2 ($n$ = 10)) and controls ($n$ = 10). The expression of all genes is normalized to an endogenous reference (miR-103a for all tested miRs and 18S rRNA for other genes) and presented as a relative fold change to controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA or the ANOVA Kruskal–Wallis test. The $t$-test, or Mann–Whitney U test, assessed the differences in parameter concentrations. $p$-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), * $p < 0.5$, ** $p < 0.01$, and *** $p < 0.001$.

### 2.1.2. Lung

In the lung, changes in miR levels were observed only in two of four studies miRs. They were miR-146a and miR-223. No change was detected for the miR-155 (Figure 2A) and its target genes *TAB2* and *p65* (Figure 2C,D), except *MyD88* and *NLRP3* (Figure 2B,E). Our research showed an increase in the mRNA level for *MyD88* (3-fold change in the GI.1 group ($p = 0.007$) and 5-fold change in the GI.2 group ($p = 0.003$); Figure 2B). The highest increase in expression compared to the control was recorded in the *NLRP3* gene (5-fold ($p < 0.001$) for the GI.1 and the GI.2 −5.8-fold ($p < 0.001$) (Figure 2E).

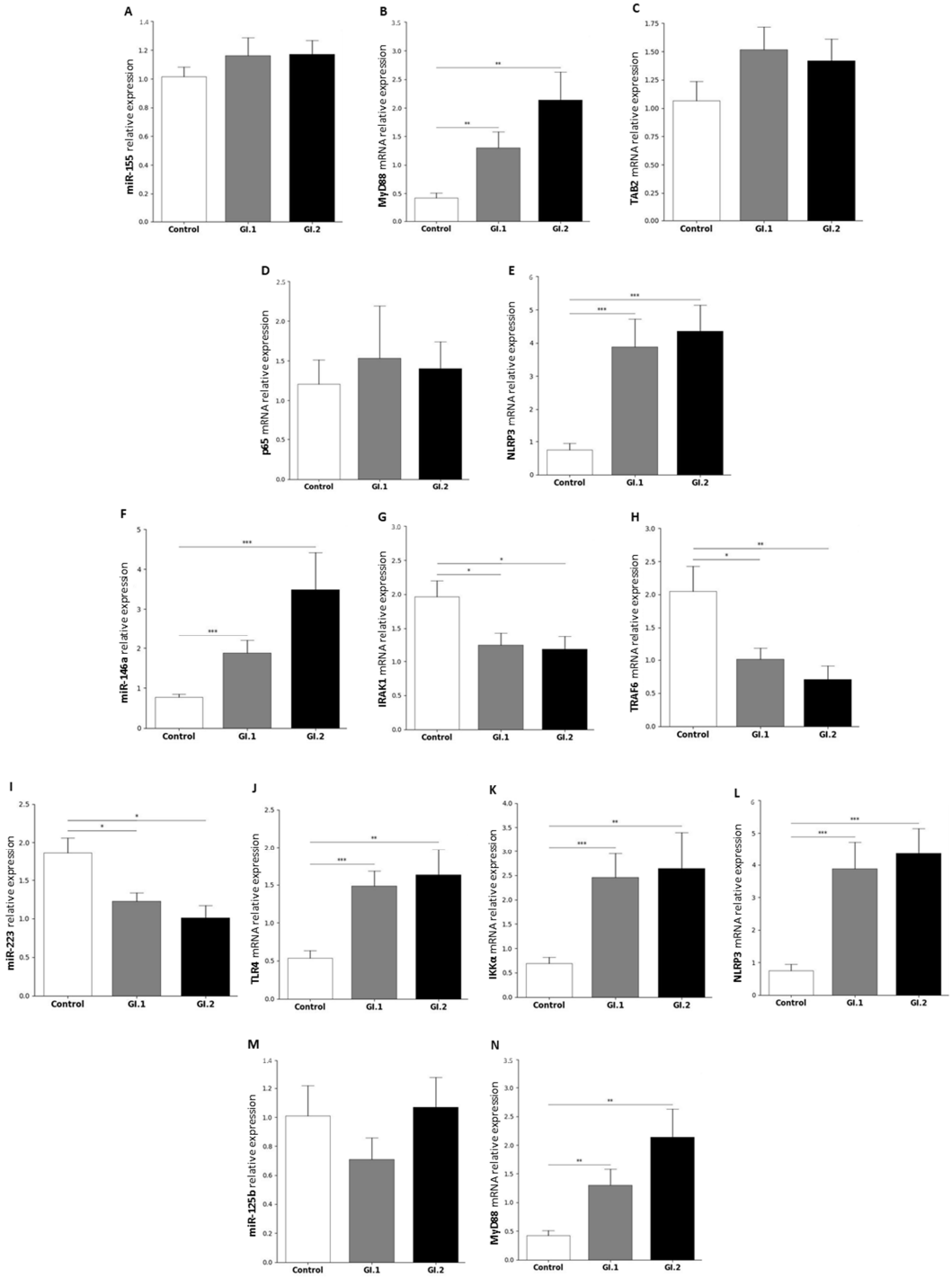


**Figure 2.** Relative expression of miRs and downstream targets: miR-155 (**A**) (*MyD88* (**B**), *TAB2* (**C**), *p65* (**D**), *NLRP3* (**E**)), miR-146a (**F**) (*IRAK1* (**G**), *TRAF6* (**H**)), miR-223 (**I**) (*TLR4* (**J**), *IKKα* (**K**), *NLRP3* (**L**)),

and miR-125b (**M**) (*MyD88* (**N**)) in the lungs during rabbits infection with *Lagovirus europaeus*—two genotypes (GI.1 ($n$ = 10) and GI.2 ($n$ = 10))—and controls ($n$ = 10). The expression of all the genes is normalized to an endogenous reference (miR-103a for all tested miRs and 18S rRNA for other genes) and presented as a relative fold change to the controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The $t$-test, or Mann–Whitney U test, was performed to assess the differences in parameter concentrations. $p$-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), * $p < 0.5$, ** $p < 0.01$, and *** $p < 0.001$.

During *L. europaeus* infection of both genotypes, our studies showed an increase in expression of miR-146a (2.4-fold change for GI.1 vs. control, $p < 0.001$ and 4.5-fold change for GI.2 vs. control, $p < 0.001$; Figure 2F). The increase in miR-146a was associated with a decrease in *IRAK1* and *TRAF6* gene expression. The decrease in the expression level of *IRAK* was similar in both infected groups (GI.1 and GI.2) and amounted to a 1.6-fold reduction for GI.1 group ($p = 0.03$) and a 1.7-fold reduction for GI.2 ($p = 0.02$) compared to the control (Figure 2G). However, during *L. europaeus* GI.1 infection we noted a downregulation of *TRAF6* was a 2-fold reduction ($p = 0.02$) compared to the control, while during *L. europaeus* GI.2 genotype infection a downregulation of *TRAF6* was 2.9-fold reduction ($p = 0.005$ vs. control) (Figure 2H).

Different expressions of miR-223 were observed in the lungs compared to the liver. The expression level of miR-223 was significantly lower in the lungs in both infected groups of rabbits (1.5-fold reduction ($p = 0.017$) for GI.1 and 1.9-fold reduction ($p = 0.014$) for GI.2 (Figure 2I). The downregulation of miR-223 was accompanied by increased expression of all target genes. The expression of *TLR4* for GI.1 and GI.2 group increased 2.7-fold, ($p < 0.001$ vs. control) and 3-fold, ($p < 0.001$ vs. control), respectively (Figure 2J). In the case of the *IKKα* gene, we observed an upregulation by a 3.5-fold change in the GI.1 infected group ($p < 0.001$ vs. control) and a 3.7-fold change in the GI.2 group ($p = 0.004$ vs. control) (Figure 2K). However, the highest increase in expression compared to the control was recorded in the *NLRP3* inflammasome gene (5-fold ($p < 0.001$) for the GI.1 and 5.8-fold ($p < 0.001$) for the GI.2) (Figure 2L). Similarly to miR-155, our studies showed no change in the expression level of miR-125b (Figure 2M), but with a clearly marked increase in *MyD88* expression (3-fold change in GI.1 group ($p = 0.007$) and 5-fold change in GI.2 ($p = 0.003$); Figure 2N).

#### 2.1.3. Kidney

In the kidneys, our studies showed an increase in the expression of almost all tested miRs during infection with both L. europaeus genotypes except miR-125b.

The expression of miR-155 was significantly different in the infected rabbits compared to the control rabbits during infection with *L. europaeus* (1.6-fold change, $p = 0.03$ for GI.1 and 2.5-fold change, $p = 0.004$ for GI.2) (Figure 3A).

Overexpression of miR-155 was associated with decreased expression levels of *MyD88* (7.7-fold reduction for GI.1, $p < 0.001$ vs. control and 2.7-fold reduction for GI.2, $p = 0.001$ vs. control; Figure 3B) and *p65* (8.9-fold reduction for GI.1, $p < 0.001$ vs. control and 2.4-fold reduction for GI.2, $p = 0.009$ vs. control; Figure 3D) in both infected groups. Whereas no statistically significant change was observed in the expression level of the *TAB2* gene (Figure 3C). The studies showed only a decrease in the *TAB2* gene (1.4-fold reduction, $p = 0.038$) between the GI.1 and GI.2 infected groups (Figure 3C). In the case of the *NLRP3* inflammasome gene, a decreasing expression was observed (2.6-fold reduction during *L. europaeus*/GI.1 ($p = 0.02$) and 1.7-fold reduction during GI.2 ($p = 0.03$) infection compared to the control; Figure 3E).

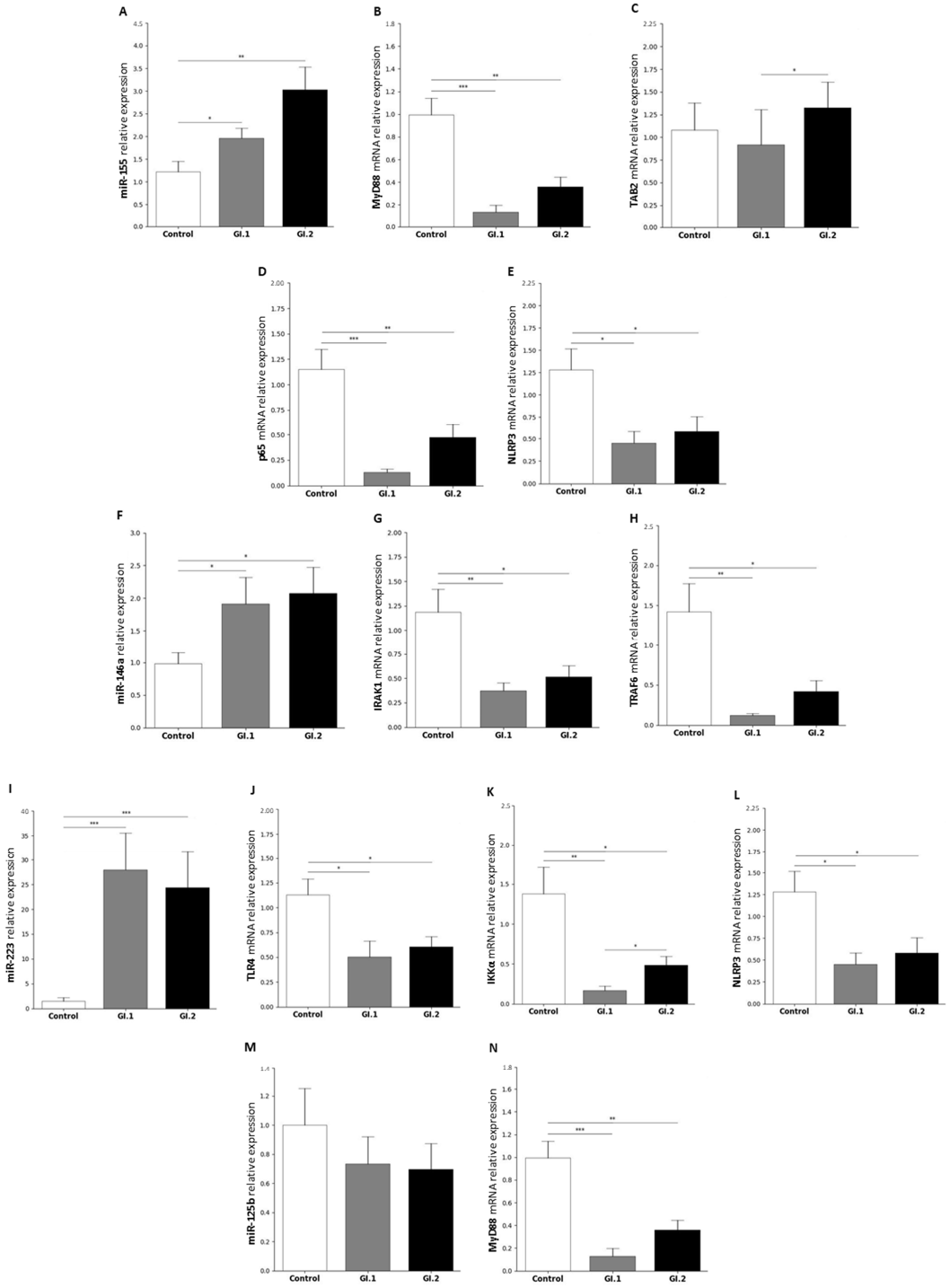


**Figure 3.** Relative expression of miRs and downstream targets: miR-155 (**A**) (*MyD88* (**B**), *TAB2* (**C**), *p65* (**D**), *NLRP3* (**E**)), miR-146a (**F**) (*IRAK1* (**G**), *TRAF6* (**H**)), miR-223 (**I**) (*TLR4* (**J**), *IKKα* (**K**), *NLRP3* (**L**)), and miR-125b (**M**) (*MyD88* (**N**)) in the kidney during rabbits infection with *Lagovirus europaeus*—two genotypes (GI.1 ($n$ = 10) and GI.2 ($n$ = 10))—and controls ($n$ = 10). The expression of all genes is normalized to an endogenous reference (miR-103a for all tested miRs and 18S rRNA for other genes) and presented as a relative fold change to the controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney U test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), * $p < 0.5$, ** $p < 0.01$, and *** $p < 0.001$.

Upregulation of miR-146a (1.9-fold change for GI.1, $p = 0.047$ vs. control and 2-fold change for GI.2, $p = 0.02$ vs. control; Figure 3F) in the kidney was associated with downregulation of *IRAK1* and *TRAF6*. In the GI.1 group, the reduction of *IRAK1* was a 3.2-fold reduction, while in GI.2 it was a 2.3-fold reduction (Figure 3G). Compared to the control, the level of *TRAF6* expression decreased during infection of GI.1 (11.5-fold reduction ($p = 0.002$)), while during GI.2, it was only a 3.4-fold reduction ($p = 0.017$) (Figure 3H). *TRAF6* gene expression was also decreased between groups (3.4-fold lower in the GI.1 than in the GI.2 ($p = 0.045$); Figure 3H).

Similarly to the liver, the highest increase in expression among miRs was observed in the case of miR-223. The greatest increase occurred during infection with the *L. europaeus*/GI.1 genotype (18.6-fold change compared to the control ($p < 0.001$)). Followed by GI.2 (16.3-fold change vs. control ($p < 0.001$)) (Figure 3I).

All target genes for MiR-223 were downregulated. The expression of *TLR4* for the GI.1 and GI.2 group decreased by a 2.8-fold reduction, ($p < 0.017$ vs. control) and 1.8-fold reduction, ($p < 0.011$ vs. control), respectively (Figure 3J). In the case of the *IKKα* gene, we observed an 8.4-fold reduction in the GI.1 group ($p = 0.001$ vs. control) and a 2.8-fold reduction in GI.2 ($p = 0.02$ vs. control) (Figure 3K). Additionally, the expression of *IKKα* was a 2.9-fold reduction in the case of GI.1 infection compared to the GI.2 infected group ($p = 0.03$; Figure 3K). Furthermore, the expression of *NLRP3* was downregulated (2.6-fold reduction, $p = 0.021$ and 1.7-fold reduction, $p = 0.028$, during GI.1 and GI.2 infection) compared to healthy rabbits (Figure 3L). Our study showed no change in the expression level of miR-125b in both infected groups (Figure 3M). However, the expression level of the *MyD88* gene decreased in both groups (7.7-fold reduction during GI.1 ($p = 0.02$) and 2.7-fold reduction during GI.2 ($p = 0.03$) infection compared to the control) (Figure 3N).

#### 2.1.4. Spleen

Our studies show that only miR-155 is upregulated in the spleen during infection with both *L. europaeus* genotypes. MiR-155 was upregulated by a 3.3-fold change ($p < 0.001$) for the GI.1 group, while for GI.2, there was a 4.7-fold change ($p < 0.001$) compared to the control (Figure 4A). As in the kidney, the increase in miR-155 was associated with a decrease in its target genes, *MyD88* and *p65*. The expression levels of *MyD88* for the GI.1 and GI.2 groups decreased by a 3-fold reduction ($p = 0.026$ vs. control) and 5.6-fold reduction ($p < 0.001$ vs. control), respectively (Figure 4B). Expression of the *p65* gene was a 3-fold reduction ($p = 0.038$) in the case of infection with the *L. europaeus*/GI.1 genotype and 4.8-fold lower ($p = 0.01$) in GI.2 (Figure 4D). In the spleen, the change in *TAB2* gene expression was observed only during infection with the GI.1 genotype (1.8-fold change ($p = 0.04$) compared to the control) (Figure 4C).

The remaining miRs were expressed only during infection with the GI.1 genotype, and all of them showed reduced expression. The expression level of miR-146a was downregulated by a 1.8-fold reduction ($p = 0.017$; Figure 4F). However, no change in the expression level of the target gene *IRAK1* was observed in the GI.1 group compared to the control group (Figure 4G). Moreover, in the second group, GI.2, *IRAK1* also showed no altered expression (Figure 4G). However, interestingly, our studies showed a decrease in *TRAF6* expression in both infected groups (2.5-fold reduction for GI.1, $p = 0.0046$ and 3.7-fold reduction for GI.2, $p < 0.01$) compared to the healthy rabbits (Figure 4H).

The expression level of miR-223 was significantly lower for the GI.1 genotype (2.5-fold reduction compared to the control; ($p = 0.005$); Figure 4I). No changes in *TLR4* gene expression were observed in both infected groups (Figure 4J). Expression of *IKKα* was reduced in both *L. europaeus* infected groups, with a 2.8-fold reduction for GI.1 ($p < 0.001$ vs. control) and a 3.9-fold reduction for GI.2 ($p < 0.001$ vs. control) (Figure 4K). At the same time, the reduction miR-223 was associated with a 3-fold change in *NLRP3* gene expression level ($p = 0.006$; Figure 4L). Additionally, our studies showed a statistically significant difference in *NLPR3* expression between the infected groups (2.3-fold higher in the GI.1 group compared to the GI.2 group ($p = 0.02$); Figure 4L). MiR-125b expression was a 2-fold reduction ($p = 0.13$) only in the group GI.1 compared to the control, and 3-fold lower for the GI.1 group compared to GI.2 ($p = 0.02$;

Figure 4M). Additionally, the expression level of the *MyD88* gene decreased in both groups (3-fold reduction for GI.1 ($p = 0.02$) and 5.6-fold reduction for GI.2 ($p = 0.03$) compared to the control) (Figure 4N).

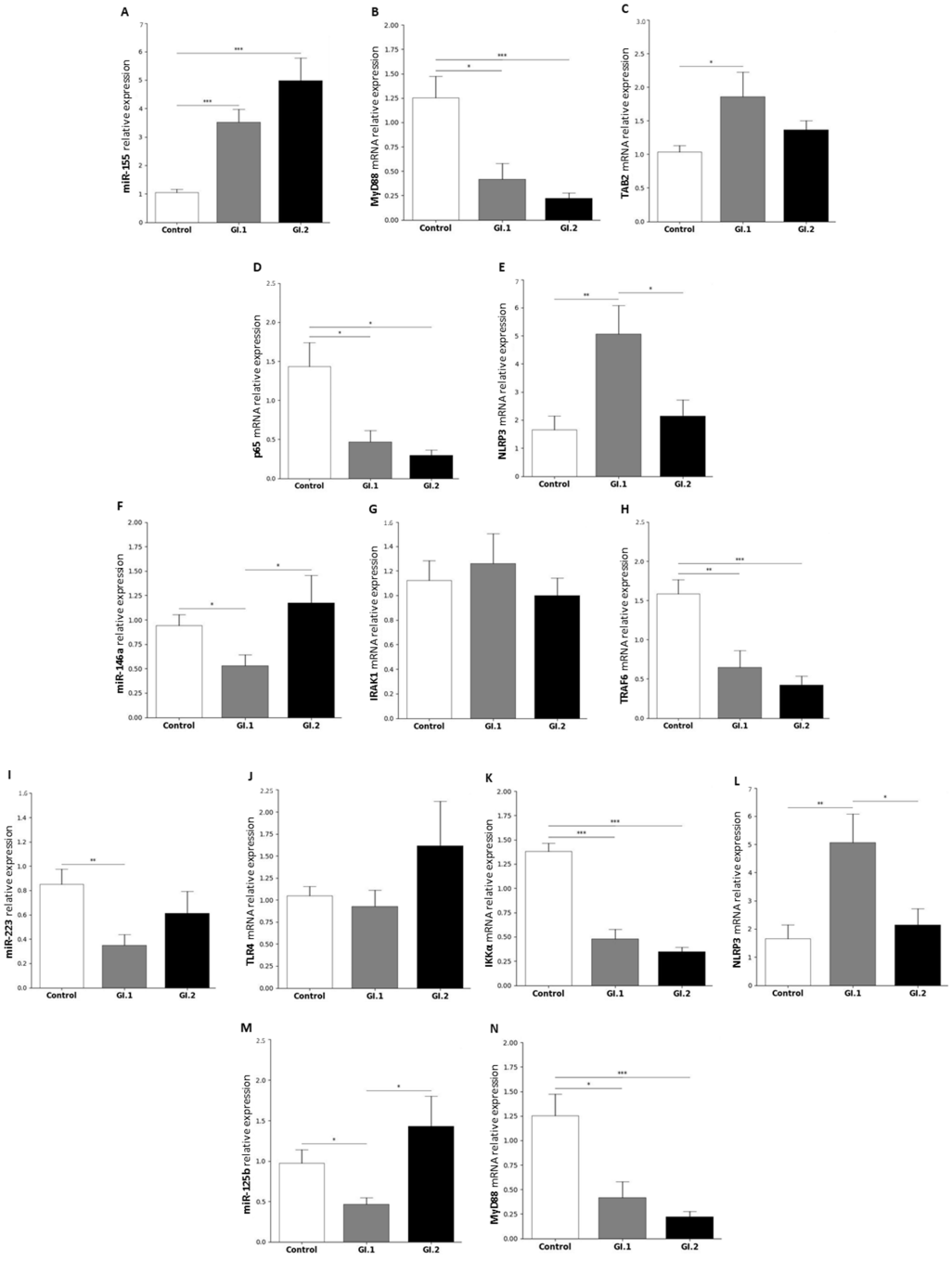


**Figure 4.** Relative expression of miRs and downstream targets: miR-155 (**A**) (*MyD88* (**B**), *TAB2* (**C**), *p65* (**D**), *NLRP3* (**E**)), miR-146a (**F**) (*IRAK1* (**G**), *TRAF6* (**H**)), miR-223 (**I**) (*TLR4* (**J**), *IKKα* (**K**), *NLRP3* (**L**)), and miR-125b (**M**) (*MyD88* (**N**)) in the spleen during rabbits infection with *Lagovirus europaeus*—two genotypes (GI.1 ($n = 10$) and GI.2 ($n = 10$))—and controls ($n = 10$). The expressions of all genes are normalized to an endogenous reference (miR-103a for all tested miRs and 18S rRNA for other genes) and presented as a relative fold change to the controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The miR and target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney U test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), * $p < 0.5$, ** $p < 0.01$, and *** $p < 0.001$.

### *2.2. Biomarkers of Inflammation in the Liver, Lung, Kidney, and Spleen during Lagovirus europaeus/GI.1 and GI.2 Genotype Infection in Rabbits*

The study aimed to determine the relative level of mRNA expression of selected biomarkers of inflammation, *IL-1β*, *IL-6*, *TNF-α*, and *IL-18* (proinflammatory cytokines), involved in the acute phase response (rapid, non-specific immune and inflammatory response, including systemic metabolic-physiological changes, affecting tissues and organs) (Figures 5 and 6, Table 1).

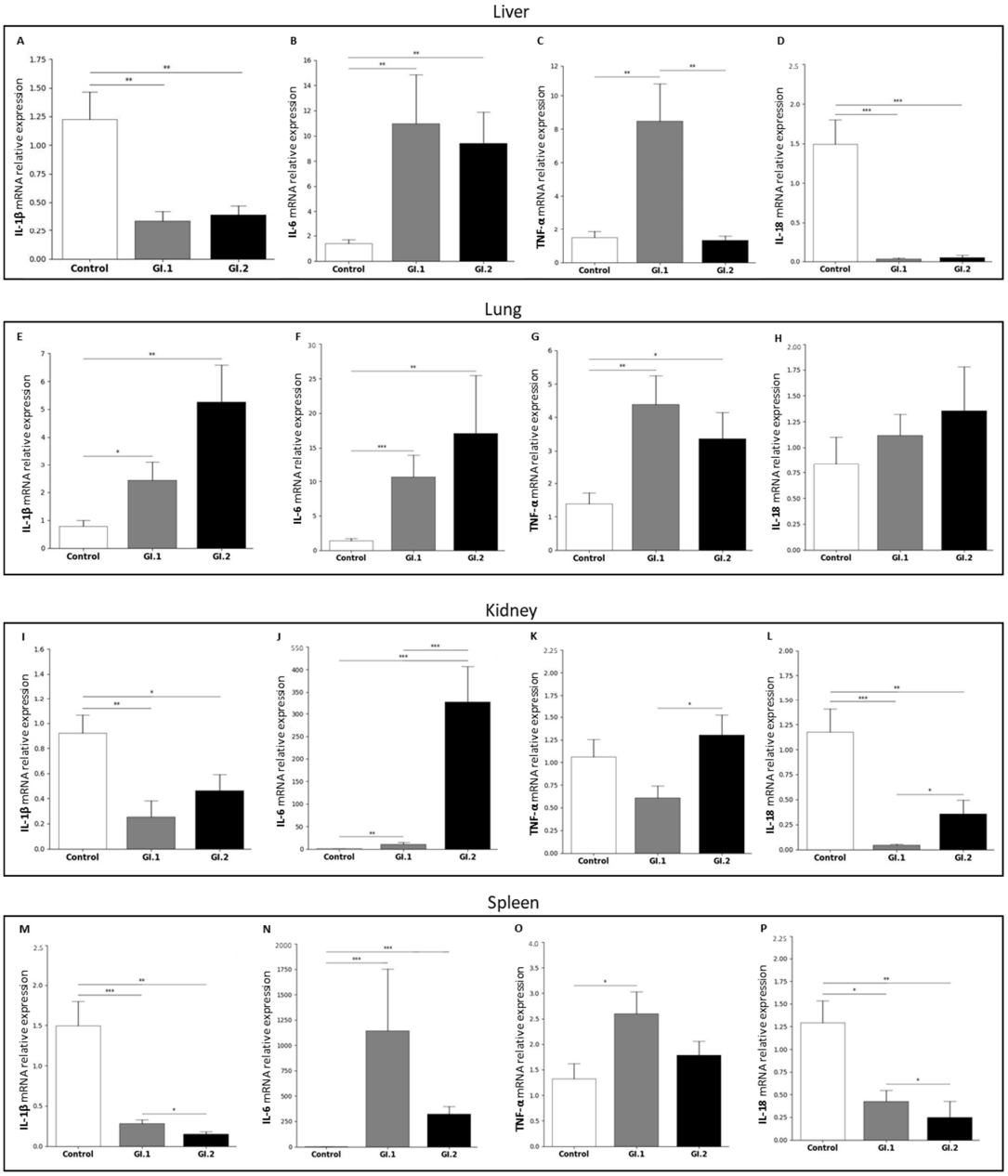


**Figure 5.** Expression biomarkers of inflammation: IL-1β (**A**,**E**,**I**,**M**), IL-6 (**B**,**F**,**J**,**N**), TNF-α (**C**,**G**,**K**,**O**) and IL-18 (**D**,**H**,**L**,**P**) in the liver (**A**–**D**), lung (**E**–**H**), kidney (**I**–**L**) and spleen (**M**–**P**) of controls rabbits ($n$ = 10), and *L. europaeus*/GI.1 ($n$ = 10) and GI.2 ($n$ = 10) genotype infections. The expressions of all genes are normalized to an endogenous reference (18S rRNA) and presented as a relative fold change to the controls according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The target gene levels were evaluated using real-time PCR. Data were compared with the one-way ANOVA test or the ANOVA Kruskal–Wallis test. The *t*-test, or Mann–Whitney U test, was performed to assess the differences in parameter concentrations. *p*-values below 0.05 were considered statistically significant. Bars indicate the mean ± standard error of the mean (SEM), * $p < 0.5$, ** $p < 0.01$, and *** $p < 0.001$.

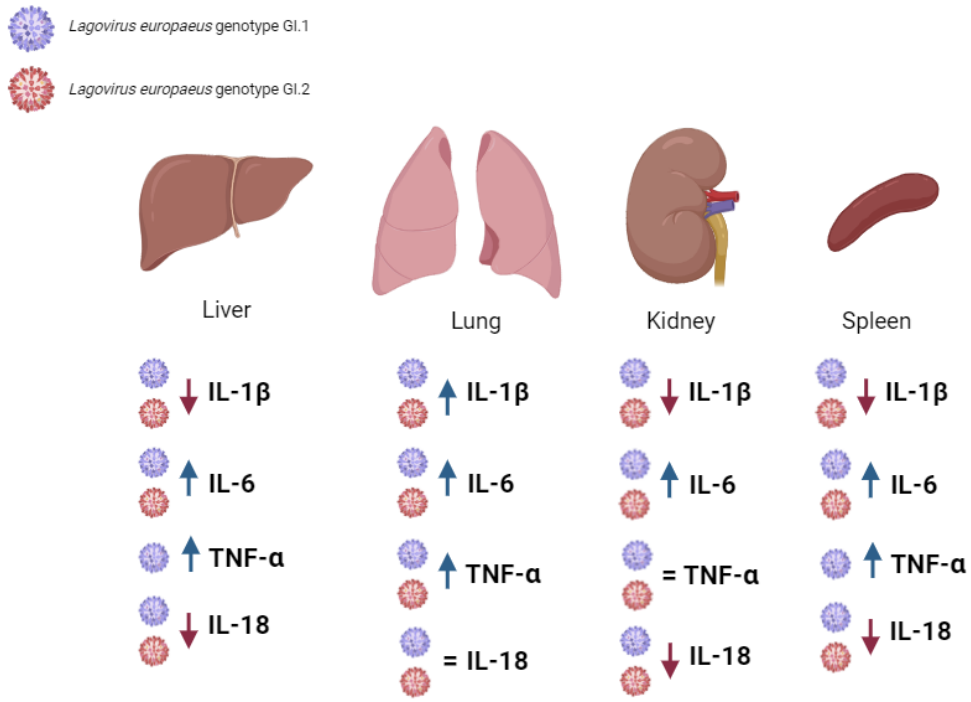


**Figure 6.** Biomarkers of inflammation during *Lagovirus europaeus*/GI.1 and GI.2 genotype infection in rabbits.

**Table 1.** Comparison of expression biomarkers of inflammation in four tissues of rabbits infected with *Lagovirus europaeus*/GI.1 and GI.2 genotypes.

| Tissues | Proinflammatory Cytokines Determined at the mRNA Level | | | |
|---|---|---|---|---|
| **Gene** | ***IL-1β*** | ***IL-6*** | ***TNF-α*** | ***IL-18*** |
| **Liver** | | | | |
| Control vs. GI.1 | ↓ ×3.6 | ↑ ×8.0 | ↑ ×5.7 | ↓ ×46.8 |
| Control vs. GI.2 | ↓ ×3.1 | ↑ ×6.8 | = | ↓ ×31.0 |
| GI.1 vs. GI.2 | = | = | ↑ ×6.4 | = |
| **Lung** | | | | |
| Control vs. GI.1 | ↑ ×3.1 | ↑ ×7.7 | ↑ ×3.1 | = |
| Control vs. GI.2 | ↑ ×6.7 | ↑ ×12.3 | ↑ ×2.4 | = |
| GI.1 vs. GI.2 | = | = | = | = |
| **Kidney** | | | | |
| Control vs. GI.1 | ↓ ×3.6 | ↑ ×8.5 | = | ↓ ×27.5 |
| Control vs. GI.2 | ↓ ×2.0 | ↑ ×261.0 | = | ↓ ×3.3 |
| GI.1 vs. GI.2 | = | ↓ ×30.0 | ↓ ×2.1 | ↓ ×8.3 |
| **Spleen** | | | | |
| Control vs. GI.1 | ↓ ×5.5 | ↑ ×997.0 | ↑ ×1.9 | ↓ ×3.0 |
| Control vs. GI.2 | ↓ ×1.9 | ↑ ×282.0 | = | ↓ ×5.1 |
| GI.1 vs. GI.2 | ↑ ×5.2 | = | = | ↑ ×1.7 |

Explanations: ↑—increase in expression; ↓—decrease in expression; and = no statistical significance.

In the liver the relative level of mRNA expression of *IL-1β* was decreased during infection with *L. europaeus* of both genotypes (3.6-fold reduction for genotype GI.1 ($p = 0.002$) and 3.1-fold reduction for GI.2 ($p = 0.004$)) compared to the control group (Figure 5A). In the case of *IL-6* (the most important mediator of the inflammation, stimulating the synthesis of acute phase protein), we observed its increase after infection with *L. europaeus* of both genotypes (8-fold change, $p = 0.003$ for GI.1 vs. control and 6.8-fold change, $p = 0.004$ for GI.2 vs. control; Figure 5B). It should be emphasized that this cytokine was more strongly expressed in the group of rabbits infected with the *L. europaeus*/GI.1 genotype. In

our studies, we also observed an increased level of the main mediator of tissue damage, *TNF-α*, during infection, but only in the GI.1 group (5.7-fold change, $p = 0.006$ vs. control; Figure 5C). Additionally, we observed 6.4-fold higher *TNF-α* expression during infection with the GI.1 genotype compared to the GI.2 infected group ($p = 0.04$; Figure 5C). The expression level of the *IL-18* gene was the lowest in the liver, from all examined tissues, where downregulation was a 46.8-fold reduction ($p < 0.001$) and 31-fold reduction ($p < 0.001$) for GI.1 and GI.2 (Figure 5D).

In the lungs, our studies showed an increase in almost all tested cytokines except *IL-18*, whose expression was unchanged (Figure 5H). The greatest changes in expression levels in the lung were observed in the case of *IL-6*. Compared to the control, the *IL-6* mRNA relative expression was 7.7-fold enhanced for GI.1 ($p < 0.001$) and 12.3-fold enhanced for GI.2 ($p = 0.002$) (Figure 5F). During infection with the *L. europaeus*/GI.1 genotype, we noted a similar increase in the expression level of *IL-1β* and *TNF-α*, which was a 3.1-fold change ($p = 0.045$ for *IL-1β* (Figure 5E) and $p = 0.004$ for *TNF-α* (Figure 5G)). Whereas during the *L. europaeus*/GI.2 infection, the level of *IL-1β* was enhanced by a 6.7-fold change ($p = 0.045$ vs. control; Figure 5E) and a 2.4-fold change for *TNF-α* ($p = 0.003$ vs. control; Figure 5F).

Similar to the liver, we observed a significant decrease in *IL-1β* and *IL-18* levels but also increased levels of *IL-6* in the kidney and the spleen. In the kidney, expression of *IL-1β* was decreased by a 3.6-fold reduction ($p = 0.003$ vs. control; Figure 5I) in the GI.1 group and a 2-fold reduction for *IL-1β* in GI.2 ($p = 0.04$; Figure 5I). The expression of *IL-6* was significantly increased in the rabbits infected with the *L. europaeus*/GI.1 and GI.2 genotypes, with an 8.5-fold change ($p = 0.004$) and 261-fold change ($p < 0.001$), respectively (Figure 5J). We also noted a difference in *IL-6* expression levels between infected groups. During *L. europaeus* GI.1 infection, the level of *IL-6* expression was 30-fold lower compared to the GI.2 group ($p < 0.001$; Figure 5J). Our study showed no change in *TNF-α* expression in the kidneys in both infected groups compared to the control group (Figure 5K). However, we observed a statistically significant change in the *TNF-α* expression level in both infected groups (2.1-fold lower in the GI.1 group ($p = 0.003$) vs. GI.2; Figure 5K). In the kidney In the GI.1 group, expression of *IL-18* was decreased by a 27.5-fold reduction ($p = 0.01$ vs. control; Figure 5L). However, for the GI.2 genotype, we noted a 3.3-fold reduction in *IL-18* ($p = 0.004$; Figure 5L) compared to the control. Our studies showed a difference in expression levels of *IL-18* between rabbits infected with *L. europaeus* for both genotypes.Comparing GI.1 to the GI.2 group, the level of *IL-18* was downregulated (8.3-fold reduction ($p = 0.03$); Figure 5L).

In the spleen, the relative expression of *IL-1β* mRNA was a 5.5-fold reduction for GI.1 ($p = 0.04$) and a 1.9-fold reduction for GI.2 ($p = 0.004$) compared to the healthy tissues (Figure 5M). Additionally, we observed 5.2-fold higher *IL-1β* expression during infection with the GI.1 genotype compared to the GI.2 infected group ($p = 0.04$; Figure 5M). Our studies have shown that during infection with both *L. europaeus* genotypes, the level of *IL-6* increases most in the spleen. During infection with the GI.1 genotype, the expression level increased by a 997-fold change ($p < 0.001$) compared to the control group, while for the GI.2 genotype, the increase was a 282-fold change ($p < 0.001$) (Figure 5N). In the case of *TNF-α*, our studies show an increase in the relative expression of mRNA only during infection with the GI.1 genotype (1.9-fold change, $p = 0.02$ vs. control; Figure 5O). Similarly to other tissue, in the spleen, we observed a decrease in expression levels of *IL-18* during *L. europaeus* infection with both genotypes. Compared to the control, in the GI.1 group, the decrease was a 3-fold reduction ($p = 0.045$), while in the GI.2 group, it was a 5.1-fold reduction ($p = 0.004$) (Figure 5P). Similar to *IL-1β*, our studies showed a change in *IL-18* expression levels between infected groups (1.7-fold change for GI.1, $p = 0.038$ vs. GI.2; Figure 5P).

### 2.3. Clinical Signs of Disease and Post-Mortem Analysis

Animals infected with both *L. europaeus* genotypes—GI.1 and GI.2—showed clinical signs consistent with RHD (apathy, dyspnea, body temperature > 41 °C, anorexia, and neurological symptoms). Post-mortem findings of infected rabbits showed characteristic

anatomopathological organ changes during RHD. Two rabbits after the *L. europaeus*/GI.2 infection died asymptomatically. Mortality after infection with *L. europaeus* in both genotypes was 90% at 60 hpi. The *L. europaeus*/GI.2 genotype was more virulent, causing 90% mortality in rabbits within 32 hpi and a fulminant course of the disease. The disease ranged from per-acute to acute in animals infected with this *L. europaeus* genotype. Whereas, after *L. europaeus*/GI.1 infection, the mortality rate was −10% at 32 hpi, 40% at 36 hpi, and 40% between 56 and 60 hpi.

## 3. Discussion

So far, apart from our studies [16,23–25,53], there is a lack of information on the molecular signatures of regulatory interactions between miRs and biological processes involved in *L. europaeus* infection/RHD pathogenesis. We examined miRs and target genes involved in regulating the innate immune and inflammatory response. To investigate the immune and inflammatory response to the viral stimulus in the examined tissues, we examined biomarkers of the inflammation *IL-1β*, *IL-6*, *TNF-α*, and *IL-18* involved in acute phase response [54–58].

The immune and inflammatory response is the coordinated activation of many cells, substances, and signaling pathways that regulate the levels of inflammatory mediators in tissue-resident cells and inflammatory cells recruited from the blood. Although inflammatory response processes depend on the exact nature of the initial stimulus and its location in the body, they all share a common mechanism: cell surface receptors recognize harmful stimuli; inflammatory pathways are activated; inflammatory biomarkers are released; and inflammatory cells are recruited [59].

For the first time, we examined inflammatory biomarkers (in liver, lungs, kidneys, and spleen) in rabbits infected with *L. europaeus*—two genotypes: GI.1 and GI.2. We examined the expression level of cytokines *IL-1β*, *IL-6*, *TNF-α*, and *IL-18* in each post-mortem organ. Our research shows that the expression profile of biomarkers of inflammation in rabbits infected with *L. europaeus* is similar for GI.1 and GI.2 genotypes in terms of the nature of changes (increase/decrease/no changes in expression) but is different for individual tissues.

The inflammatory biomarker profile in the liver of infected rabbits was characterized by a decrease in *IL-1β* expression at very similar levels after infection with both genotypes (3.6-fold reduction and 3.1-fold reduction for GI.1 and GI.2, respectively). An increase in *IL-6* mRNA expression for both genotypes was slightly higher in the case of the GI.1 (8-fold change) genotype. The rise in *TNF-α* expression after *L. europaeus*/GI.1 infection was a 5.7-fold change and 6.4-fold higher *TNF-α* expression in GI.1 compared to the GI.2 infection group. This fact suggests the development of an inflammatory response and liver damage. We also recorded a drastic reduction in the level of *IL-18* mRNA expression after *L. europaeus* infection (both genotypes), with a stronger reduction in the GI.1 infection group (46.8-fold reduction).

In turn, the inflammatory biomarker profile in the lungs of infected rabbits was characterized by an increase in the expression of three acute-phase cytokines (*IL-1β*, *IL-6*, and *TNF-α*). After infection with the *L. europaeus*/GI.2 genotype, *IL-1β* expression in the lungs increased by 6.7-fold, and 3.1-fold changes for GI.1. We recorded a similar situation for *IL-6* (12.3-fold change, GI.2; 7.7-fold change, GI.1). Moreover, an increase in the expression of *TNF-α* after infection with the GI.1 and GI.2 genotypes (3.1-fold change, and 2.4, for GI.1 and GI.2) at a similar level indicates a similar degree of lung damage (slightly more significant for GI.1), perhaps due to the more extended survival period of the animals. In the lung, *IL-18* expression remained unchanged in response to *L. europaeus* infection (both genotypes).

The profile of Inflammatory biomarkers In the kidneys Is characterized by a decrease in *IL-1β* mRNA expression with both genotypes. There was a critical increase in the level of *IL-6* expression in response to infection with *L. europaeus*, genotype GI.2 (261-fold change), and much smaller in the GI.1 group (8.5-fold change). In the kidney, unlike all other tissues

examined, *TNF-α* expression remained unchanged in response to infection. Similarly to the liver, infection with *L. europaeus*/GI.1 and GI.2 drastically reduced the expression level of *IL-18* in the kidneys by a 27.5-fold and 3.3-fold reduction.

The profile of Inflammatory biomarkers In the spleen regarding the nature of changes (increase/decrease expression) was very similar to the liver. There was a 5.5-fold reduction in *IL-1β* for GI.1 and a 1.9-fold reduction for GI.2. The highest increase in *IL-6* mRNA expression (997-fold change) was observed in rabbits infected with the GI.1 and with GI.2 genotype (282-fold change). Increased *TNF-α* expression was observed after *L. europaeus*/GI.1 infection at the level of a 1.9-fold change. We also recorded a reduction in the level of *IL-18* mRNA expression after *L. europaeus* infection (both genotypes), with a stronger expression in the GI.2 infection group (5.1-fold reduction) and GI.1 (3-fold reduction).

Our research shows that infection of rabbits with *L. europaeus*/GI.1 and GI.2 genotypes causes an increase in the expression of two critical acute phase cytokines—*IL-6* in all examined tissues (liver, lungs, kidneys, and spleen) and *TNF-α* (in the liver, lungs, and spleen). Cytokine *IL-1β* was highly expressed only in the lungs after *L. europaeus* infection—both genotypes. *IL-6* production following *L. europaeus* infection/GI.1 and GI.2 was most dramatic in the spleen (for GI.1 and GI.2, respectively), kidney (GI.2 and GI.1), lung (GI.2 and GI.1) and liver (GI.1 and GI.2). Increases in *TNF-α* expression were most significant in the liver (for GI.1 only), lung (GI.1 and GI.2), and spleen (GI.1 only). These facts indicate a strong and rapid involvement of the local innate immune and inflammatory response in *L. europaeus* infection for both genotypes (GI.1 and GI.2) and in the pathogenesis of RHD. Dramatic levels of cytokines in tissues may drive the pathogenesis of RHD, which may lead to hypercoagulability, formation of microthrombi (as observed post-mortem), acute liver failure, multi-organ failure, coagulopathy with hemorrhage and ultimately, death of animals. The marked upregulation and overexpression of cytokines-*IL-6* and *TNF-α* in the examined organs and *IL-1β* (only in the lungs) is probably for the resulting per-acute/acute (in the case of *L. europaeus*/GI.2 infection) and acute (in the case of *L. europaeus*/GI.1 infection) form of the disease and mortality of animals.

IL-6 is a promising biomarker of the early phase of inflammation because it has a longer half-life than other cytokines (e.g., IL-1β), and its concentration in blood and tissues may increase several thousand times within the first 2–3 h after the initiation of inflammatory processes, reaching critical values in fatal states [60], which we also recorded in our research. Monocytes, macrophages, and neutrophils produce it. Its primary role is in the regulation of anti-infective immune response and inflammation. Moderate and acute increases in IL-6 levels contribute to hepatocyte regeneration. However, persistently elevated levels of IL-6 lead to apoptosis of hepatocytes, other cells, damage to the vascular endothelium, and activation of coagulation [61].

In turn, TNF-α is one of the central cytokines of the inflammatory response with proinflammatory and anti-infective effects [62–64]. The biological effects of TNF-α depend on the intensity of secretion of this cytokine. Rapid secretion of large amounts of TNF-α leads to shock, acute respiratory failure/lung damage, hepatocyte apoptosis, adrenal hemorrhage, and DIC [64]. In turn, chronic secretion of small amounts of this cytokine causes liver and spleen enlargement. TNF-α blockade in an animal model with induced ALF led to attenuated hepatocyte damage and increased regeneration [64]. Changes in the level of TNF-α can predict COVID-19 progression, lung damage, and disease severity. Using TNF-α inhibitors or blockers to treat COVID-129 can prevent mortality in severe COVID-19 patients [63]. O'Toole et al. [42] described for the first time the profile of acute phase cytokines (IL-6, IL-1β, TNF-α) after administration to rabbits per os inoculum (in the form of liver homogenate containing the *L. europaeus*/GI.2 genotype). Similarly, they found increased expression of *IL-6* in the liver and spleen (at 36 and 48 hpi) and increased expression of *TNF-α* in the liver and spleen (from 12 h to 144 hpi). Similar to our study, Yu et al. [43] showed that after infection of rabbits with the *L. europaeus*/GI.2 genotype, there is an increase in *IL-6* expression in the spleen. Other researchers [37,39,41] noticed increased expression of *IL-6* and *TNF-α* during *L. europaeus* infection, GI.1 genotype, in

serum and peripheral blood leukocytes. Marques et al. [41] observed an increase in the expression of *IL-6* (from 24 h) and *TNF-α* (48 h) in the serum, and similar to Semeryjan et al. [39], an increase in *TNF-α* (1 and 2 dpi). Trzeciak-Ryczek et al. [37] showed increased *IL-6* and *TNF-α* (36 and 48 hpi) expression in peripheral blood leukocytes.

Our study only found increased *IL-1β* mRNA expression in the lungs (GI.2 and GI.1, respectively). IL-1β is secreted mainly by monocytes and macrophages from various tissues by microbial products that activate PRR receptors and other cytokines. IL-1, systemically and at the site of inflammation, causes biological effects that facilitate the development of the inflammatory reaction, among others, and induces increased production of neutrophils and monocytes. It plays a crucial role in the acute phase of inflammation by inducing IL-6 [55]. The short half-life of IL-1β may limit its use in diagnosing the inflammatory process, which may have been the case in our studies [60]. Our studies' results differ from those obtained by O'Toole et al. [42] and Yu [43] after the infection of rabbits with the *L. europaeus*/GI.2 genotype. The first team [42] showed an increase in *IL-1β* expression in the liver (36 hpi and 48 hpi) and spleen (36, 48, 144 hpi). Yu et al. [43] showed increased *IL-1β* expression in the spleen. The same results after infection with *L. europaeus*—genotype GI.1—was reported by Marques et al. [41], showing an increase in *IL-1* (up to 18 hpi) and Trzeciak-Ryczek et al. [38], showing a decrease in *IL-1β* (8 hpi, and 24 and 28 hpi) in peripheral blood leukocytes.

Our study also found dramatically reduced levels of *IL-18* mRNA expression in the liver, kidney, and spleen. In the lungs, *IL-18* expression remained unchanged. The literature data indicate that IL-18 strategically generates the immune response against viral infections and is usually proinflammatory [56,57]. Macrophages produce IL-18 at the very early stages of viral infection and induce the production of IL-6 and IFN-γ. As a rule [58], an increase in the level of IL-18 in plasma is observed during viral infections. Infections with EBV, HIV, chronic HB and HC infections, and acute dengue infections have been evident of this fact. Moreover, circulating IL-18 and ferritin levels may strongly correlate with dengue disease severity and can be considered a tool for predicting disease progression [58]. Our results are difficult to interpret unambiguously at this research stage because, as Slaats et al. [58] point out, distinct inflammatory programs in *IL-18*-mediated viral infection require investigation. Our study results are consistent with Trzeciak-Ryczek et al. [37], who recorded decreased *IL-18* expression (8 hpi and between 24 and 36 hpi) in peripheral blood leukocytes during *L. europaeus*/GI.1 infection. Moreover, the study by these authors [37] showed a negative correlation between reduced *IL-18* expression and the survival time of infected rabbits (36 h).

Our studies confirm that in *L. europaeus* infection/GI.1 and GI.2 genotypes, miRs are crucial in regulating innate immune and inflammatory responses. MiRs, which regulate inflammation, may have pro- and anti-inflammatory effects [46,47,49,50]. MiRs regulate complex gene networks, leading to the observation of opposite phenotypes for the same miR. The levels of miRs (miR-155, miR-146a, miR-223, miR-125b) induced after a viral stimulus can be different and usually lead to placing the cell in a specific state. Induced miRs will be targeted to different pro- or anti-inflammatory functions during the inflammatory response, depending on their target genes [46,47,49,50].

Our studies indicate that in the liver, miR-155 has both pro-inflammatory and anti-inflammatory effects, while miR-146a, miR-223, and miR-125b have anti-inflammatory effects by regulating its target genes that are critical in the TLR4-MyD88, NF-κβ, and NLRP3 inflammasome pathway [46]. The dramatic increase in liver miR expression was miR-223 after infection with *L. europaeus*/GI.1 and GI.2. A similar regulation of miR-223 has been observed in studies on EBV [65], SARS-CoV-1 [66,67], HB virus [68], HIV [68], in liver diseases [69,70], and lung diseases [71]. After *L. europaeus* infection, miR-223 significantly increased and dramatically decreased the expression of the *IKKα* gene at various levels, which was also observed in the kidney. The correlation confirmed the inhibitory effect of miR-223 on the *IKKα* gene in both genotypes. The literature reports [46] that miR-223 responds to *TLR4* by binding its promoter to NF-κB. This induction of miR-223 acts as a negative feedback loop because miR-223 targets the *IKKα* gene, thereby attenuating

the TLR/NF-κB signaling pathway. Additionally, miR-223 also targets the *NLRP3* gene, reducing caspase-1 activation and subsequent IL-1β processing [46,47]. Our study indicates that miR-155, a well-described regulator of inflammation [46,47], has anti-inflammatory and pro-inflammatory effects in the liver in *L. europaeus*/RHD infection. On the one hand, miR-155 dramatically inhibited *MyD88* (an innate immunity gene necessary for the activation of immune cells via TLRs) and the *p65* subunit of the main transcription factor NF-κβ, on which the further fate of the immune and inflammatory response depends. On the other hand, miR-155 exhibited pro-inflammatory effects by upregulating the *NLRP3* inflammasome, a critical component of the innate immune response mediating the severity of inflammation through the secretion of pro-inflammatory cytokines, IL-1β and IL-18, in response to viral infection and cell damage [72,73]. Moreover, the increase in miR-125b expression in the liver inhibited the expression of the *MyD88* gene, which indicates its anti-inflammatory potential. According to one theory, miR-125b confers its anti-inflammatory potential probably by targeting TRAF6-mediated NF-κB signaling, thereby regulating inflammatory gene expression [46]. It is important to emphasize that researchers observed such regulation of miR-125 in the liver and spleen. In turn, overexpression of miR-146a in the liver was manifested by a regulatory effect on the critical immune gene *TRAF6* (a gene in the NF-κβ pathway that activates IKK in response to inflammatory cytokines) [74], causing its decrease. We also recorded increased expression of the *IRAK1* gene (which plays a crucial role in the innate immune response to viruses by inducing acute inflammation) [74]. Further research on these miRs focuses on explaining why, despite the identical overexpression of miR-146a in both GI.1 and GI.2 groups, the decrease in *TRAF6* in the GI.2 group is so significant compared to the GI.1 group.

Our study showed that in the lung as opposed to the liver, altered miRs expression is observed only in the case of miR-146a and miR-223. Our studies indicate that miR-146a and miR-223 have anti-inflammatory effects in the lung. The expression of miR-146a increased in the leach after viral infection, while miR-223 decreased. Limited data on the regulation of miR-223 in different tissue microenvironments indicate that miR-223 may, with varying biological effects, regulate immune target genes and inhibit the synthesis of inflammatory mediators or impede inflammatory signaling pathways, thereby protecting the body from inflammatory damage, which may also take place in the lungs [71]. In the lungs, we showed a decrease in miR-223 expression (similar in both groups), accompanied by an increase in *TLR4*, *IKKα*, and *NLRP3* expression. Researchers have not previously observed such regulation in any of the examined tissues. The correlations performed show a robust negative correlation between miR-223 and its target genes, allowing us to conclude the proposed anti-inflammatory effect of miR-223 in the lungs. However, the observed decrease in miR-223 expression may increase the desired state due to the lack of a mechanism that inhibits target genes involved in the NF-kB and NLRP3 pathways. The limited data on miR-223, NF-κβ (p65 subunits), and NLRP3 signaling are inconsistent. In acute lung injury, miR-223 may regulate the inflammatory response during ALI/ARDS [71]. Moreover, in vitro experiments have shown that reduced miR-223 expression reduces the NLRP3 inflammasome and inhibits the TLR4/NF-κB signaling pathway, leading to exacerbation of lung injury [71]. Other studies have shown that during SARS-CoV-2 pneumonia [66], inhibition of miRNA-223-3p increases mRNA levels of pro-inflammatory cytokines and the NLRP3 inflammasome, suggesting that during lung infection, miRNA-223 may contribute to limiting excessive inflammatory response. We propose this explanation for miR-223 lung regulation in *L. europaeus* infection, GI.1 and GI.2 genotypes. In the lungs, miR-125b and miR-155 expression did not change, but we noted an increase in *MyD88* gene expression in the GI.1 and GI.2 groups. However, this increase may be due to increased *TLR4* expression, which may increase transcriptional initiation rates, thereby increasing *MyD88* expression [75]. Our study showed that miR-146a suppresses the expression of critical immune genes *IRAK1* and *TRAF6*, supporting its anti-inflammatory effects. The correlation confirms the regulatory impact of miR-146a on target genes in our research model analyses.

Our studies indicate that miR-155, miR-146a, and miR-223 have anti-inflammatory effects in the kidney by downregulating their target genes, which are critical in the NF-κB

pathway and may influence its silencing. The expression of the miRs mentioned above increased in response to *L. europaeus* infection with both genotypes. However, no statistically significant changes in miR-125b were observed. The most significant increase in expression in this tissue was for miR-223 (for GI.1 and GI.2). The rise in miR-223 inhibited *TLR4* gene expression at a very similar level after *L. europaeus* infection with both genotypes. MiR-223 also had an inhibitory effect on the *IKKα* gene in case of infection with two genotypes. Additionally, researchers manifested the excessive expression of miR-223 (indicating its anti-inflammatory role in infection with this virus) by inhibiting the expression of the *NLRP3* inflammasome gene. In turn, miR-155, as the primary regulator of inflammation, influenced the inhibition of the *MyD88* gene and caused a decrease in the expression of the *p65* subunit of the main transcription factor NF-κβ. In the latter case, the literature data indicate down-regulation of the p65-NF-κβ-subunit by miR, which may consequently suppress pro-inflammatory pathways [76]. Researchers have shown that the *TAB* gene, necessary for activating the NF-kB pathway, is a target gene of miR-155. Overexpression of miR-155 significantly decreases the *TAB2* transcript [77]. Our studies did not demonstrate the regulatory effect of miR-155 on the *TAB2* gene, which once again—similarly in the lungs—highlights the different behavior of this gene than previously described [77,78]. This event may suggest that other mechanisms of *TAB2* induction require further investigation. However, miR-146a affected the critical immune genes *IRAK1* and *TRAF6*, causing their decline after *L. europaeus* infection. This effect was also observed in the lungs.

In the spleen, changes in the expression levels of the tested MiRs were most diverse. Our result can be explained by the recent research by Yu et al. [43] regarding transcriptional profiles revealing inflammatory disorders conferred by GI.2 genotype/RHDV2 infection. Infection of rabbits with GI.2 in the spleen elicits a significant upregulation in the expression of numerous genes associated with disease, signal transduction, cellular processes, and cytokine signaling categories. Notably, there was an upregulation in the expression of cytokines and chemokines involved in inflammation. These findings suggested that viral infection could disrupt the cytokine network within the spleen and lead to inflammatory disorders. Our research indicates that miR-155 and miR-223 exert anti-inflammatory effects in the spleen. However, determining the nature of miR-146a and miR-125b remains challenging, and their roles are marked with a question mark in the proposed pathways. Expression of miR-155 increased in response to infection, while miR-146a, miR-223, and miR-125b decreased. Our study showed that miR-155 suppresses the expression of two critical immune genes, *MyD88* and *p65*, both in the GI.1 and GI.2 groups, which supports its anti-inflammatory effect, confirmed by correlation analysis. This fact proves that the action of this part of the pathway in the spleen is very similar to that of the liver. The rise in miR-155 gene expression manifested the increase in *TAB2* expression (only in the GI.1 group). However, correlation analysis did not support this effect. The rise in *TAB2* expression in the spleen is similar to its increase in the liver. Similarly to the liver, it does not correlate with miR-155 levels. Further research is needed to identify the factor that increases *TAB2* expression in the GI.1 and GI.2 genotypes. However, a reduction in miR-125b accompanied the decrease in *MyD88* expression in the spleen (only in the GI.1 group), which is challenging to interpret at this study stage. In the splenic environment, there was no regulatory effect of miR-146a (in both GI.1 and GI.2 groups) on the target genes *IRAK1* and *TRAF6*. The decrease in miR-146a in the spleen is different from other tissues. The reduction in miR-223 expression in the GI.1 group and the decrease in *IKKα* gene expression are difficult to interpret in the splenic microenvironment. Typically, miR-223 negatively correlates with *IKKα* to inhibit inflammation, as confirmed in SARS-CoV-2 infection, hepatitis, and pneumonia [67,69,71]. In turn, the decrease in miR-223 correlated with the increase in the *NLRP3* gene in the GI.1 group, suggesting an anti-inflammatory effect. This effect was also observed in the lungs. We realize that the environment of the spleen, as the central organ of the systemic immune and inflammatory response, is very complex, as confirmed by recent studies by Yu et al. [43]. Given the results of Yu et al. [43], our results seem to confirm the complexity of miRs expression profiles in the spleen

microenvironment and their impact on the regulation of innate and inflammatory immune genes in *L. europaeus* infection. Further research in this area is necessary.

### *3.1. Proposed miRs, Target Genes, and Pathways of Innate Immune and Inflammatory Response during Lagovirus europaeus/GI.1 and GI.2 Infection*

Our research shows that miRs may regulate three innate immune and inflammatory response pathways in *L. europaeus* infection. However, the result of this regulation may be influenced by the tissue microenvironment, as observed in studies. (1) TLR4-MyD88 signaling pathway: Activation of *MyD88* by *TLR4* results in signal transduction, where MyD88 (protein) activates IRAK, TRAF6, and TAB2, activating the transcription factor NF-κB (in our studies, *p65* subunit). Although we mention proteins here, our research focused on examining the effects of miRs on target genes involved in this pathway. This factor binds to target elements of the NF-κB response in the genome to drive the expression of genes encoding cytokines [46]. (2) NF-κB signaling pathway (p65): The phosphorylation of IKK leads to the release of NF-κB dimers. Phosphorylated NF-κB binds to NF-κB DNA response elements and induces the transcription of target genes [46]. (3) NRLP3 inflammasome pathway: NF-κB can activate the NLRP3 inflammasome, which induces IL-1β and IL-18, thereby inducing an inflammatory response [79].

The contribution of miRs/target genes involved in the innate immune and inflammatory response pathway in *L. europaeus* infection is presented separately for each tissue (Figures 7–10). Additionally, Spearman's rank correlations for examined miRs and the mRNA in four tissues of rabbits during *L. europaeus*/GI.1 and GI.2 infection were described. Correlations are provided for statistically significant results (Figures 7–10).

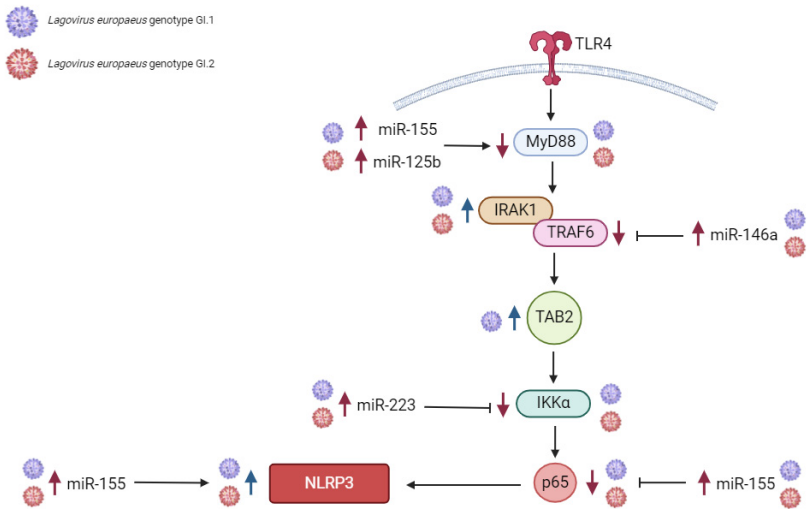


**Figure 7.** Contribution of miRs/target genes involved in the innate immune and inflammatory response pathway in the liver in *Lagovirus europaeus* infection. In the liver, increased miR-155 expression during *L. europaeus*/GI.1 and GI.2 genotypes inhibit *MyD88* and *p65* (*MyD88*—Spearman's rank correlation Rho: −0.75, $p = 0.013$, *p65*—Spearman's rank correlation Rho: −0.93, $p < 0.001$ for GI.1 and *MyD88*—Spearman's rank correlation Rho: −0.93, $p < 0.001$, *p65*—Spearman's rank correlation Rho: −0.7, $p = 0.02$ for GI.2). miR-155 induces *NLRP3* (Spearman's rank correlation Rho: 0.98, $p < 0.001$ for GI.1 and Spearman's rank correlation Rho: 0.8, $p < 0.01$ for GI.2). Increased expression of miR-146a inhibits *TRAF6* (Spearman's rank correlation Rho: −0.91, $p < 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.81, $p < 0.01$ for GI.2). Enhanced expression of miR-223 correlates with reduced expression levels of *IKKα* (Spearman's rank correlation Rho: −0.87, $p = 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.83, $p = 0.003$ for GI.2) and with *TLR4* despite a statistically significant increase in this mRNA (Spearman's rank correlation Rho: −0.9, $p < 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.93, $p < 0.001$ for GI.2). Additionally, an increase miR-125b correlates with a decrease in *MyD88* (Spearman's rank correlation Rho: −0.63, $p = 0.048$ for GI.1 and Spearman's rank correlation Rho: −0.8, $p = 0.004$ for GI.2).

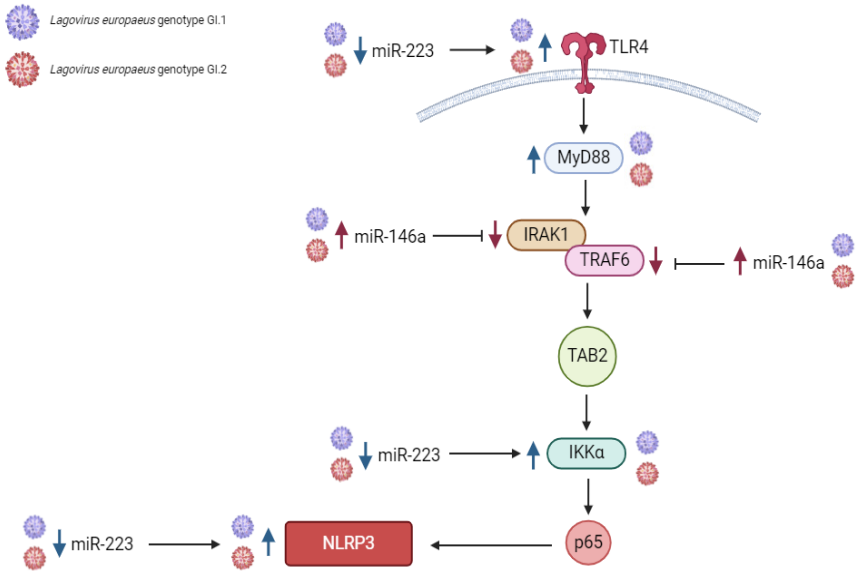


**Figure 8.** Contribution of miRs/target genes involved in the innate immune and inflammatory response pathway in the lungs in *Lagovirus europaeus* infection. In the lungs, reduced miR-223 expression levels results in increased expression of *TLR4* (Spearman's rank correlation Rho: −0.7, $p = 0.025$ for GI.1 and Spearman's rank correlation Rho: −0.95, $p < 0.001$ for GI.2), *IKKα* (Spearman's rank correlation Rho: −0.87, $p < 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.92, $p < 0.001$ for GI.2) and *NLRP3* (Spearman's rank correlation Rho: −0.69, $p = 0.025$ for GI.1 and Spearman's rank correlation Rho: −0.92, $p < 0.001$ for GI.2). Increased miR-146a expression inhibits the *IRAK1* and *TRAF6* target genes (*IRAK1*—Spearman's rank correlation Rho: −0.86, $p = 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.7, $p = 0.02$ for GI.2, *TRAF6*—Spearman's rank correlation Rho: −0.63, $p$ 0.048 for GI.1 and Spearman's rank correlation Rho: −0.96, $p < 0.001$ for GI.2). In the case of the liver and kidney, we did not observe an inhibitory effect of miR-155 on the *TAB2* target gene during *L. europaeus*/GI.1 and GI.2 infection.

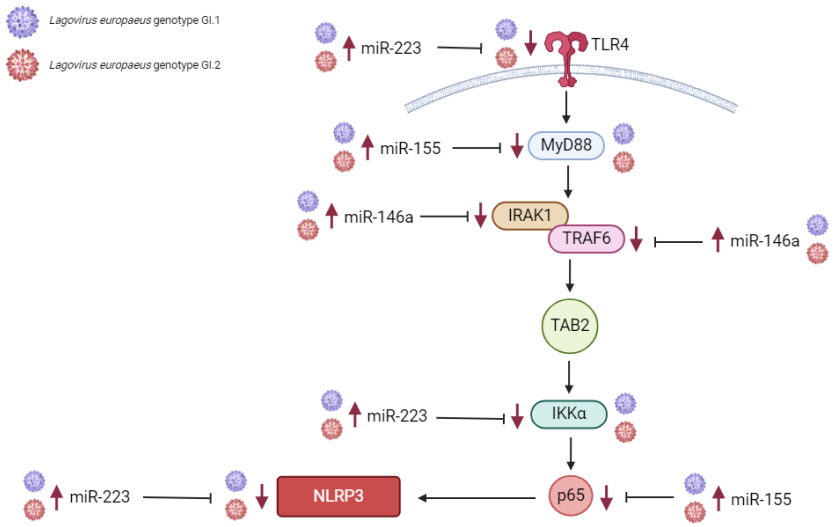


**Figure 9.** Contribution of miRs/target genes involved in the innate immune and inflammatory response pathway in the kidney in *Lagovirus europaeus* infection. In the kidney, an increase in miR-223 during infection of both virus genotypes inhibits *TLR4* (Spearman's rank correlation Rho: −0.84, $p = 0.002$ for GI.1 and Spearman's rank correlation Rho: −0.7, $p = 0.02$ for GI.2), *IKKα* (Spearman's rank correlation Rho: −0.85, $p = 0.0016$ for GI.1 and Spearman's rank correlation Rho: −0.92, $p < 0.001$ for GI.2) and *NLRP3* (Spearman's rank correlation Rho: −0.91, $p < 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.95, $p < 0.001$ for GI.2). Upregulation of miR-155 correlates negatively with

*MyD88* (Spearman's rank correlation Rho: −0.75, $p = 0.01$ for GI.1 and Spearman's rank correlation Rho: −0.92, $p < 0.001$ for GI.2) and *p65* (Spearman's rank correlation Rho: −0.8, $p = 0.005$ for GI.1 and Spearman's rank correlation Rho: −0.98, $p < 0.001$ for GI.2), resulting in inhibition of expression. MiR-146a inhibits the *IRAK1* (Spearman's rank correlation Rho: −0.95, $p < 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.92, $p < 0.001$ for GI.2) and *TRAF6* (Spearman's rank correlation Rho: −0.92, $p < 0.001$ for GI.1 and Spearman's rank correlation Rho: −0.92, $p < 0.001$ for GI.2) target genes.

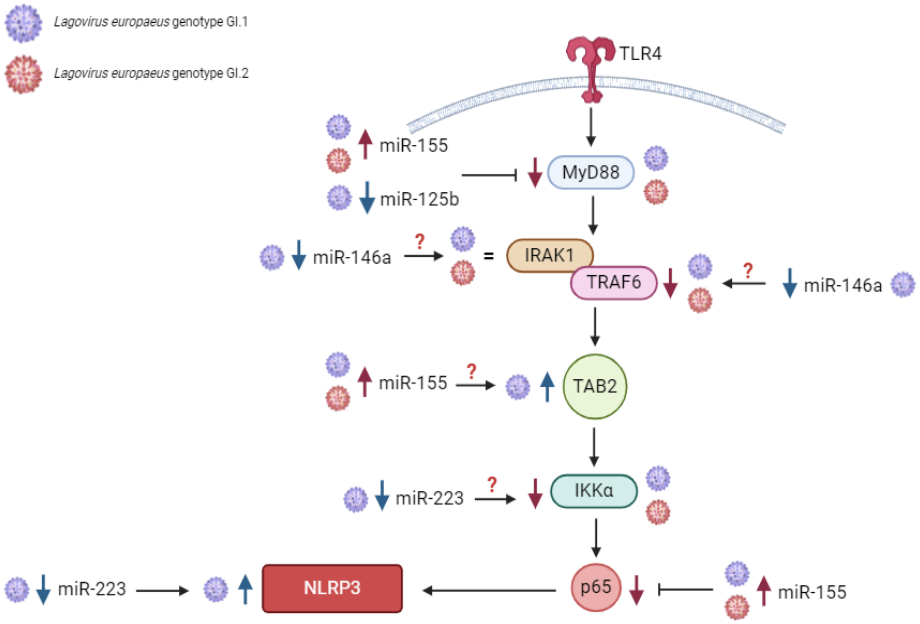


**Figure 10.** Contribution of miRs/target genes involved in the innate immune and inflammatory response pathway in the spleen in *Lagovirus europaeus* infection. In the spleen, the miR-155 expression increases during *L. europaeus*/GI.1 and GI.2 genotypes' infections inhibit *MyD88* and *p65* (*MyD88*—Spearman's rank correlation Rho: −0.81, $p = 0.004$, *p65*—Spearman's rank correlation Rho: −0.98, $p < 0.001$ for GI.1 and *MyD88*—Spearman's rank correlation Rho: −0.81, $p = 0.004$, *p65*—Spearman's rank correlation Rho: −0.91, $p < 0.001$ for GI.2). Decreased expression of miR-223 correlates with upregulated expression level of *NLRP3* (Spearman's rank correlation Rho: −0.95, $p < 0.001$ for GI.1). ?—Requires further research.

### *3.2. Proposed Inflammation Profiles in Response to L. europaeus Infection of GI.1 and GI.2 Genotypes*

Based on the inflammatory biomarkers, we distinguished three inflammatory profiles in the examined tissues (Figure 11):

(1) Pulmonary profile (increase three key acute phase cytokines- IL-1β, IL-6, TNF-α; IL-18-no change);
(2) Renal profile (increase: IL-6, decrease: IL-1β, IL-18; TNF-α -no changes);
(3) Liver and spleen profile (increase: IL-6, TNF-α; decrease: IL-1β, IL-18).

Distinct patterns of tissue cytokines may prove essential to guide the diagnosis and treatment of viral diseases, acute liver failure (ALF), and multi-organ failure (MOF) of viral etiology. In practice, plasma cytokine profiling is routinely used in patients with inflammation to define the pathophysiological phenotype, thereby playing a pivotal role in diagnosing and therapeutic decision making [58].

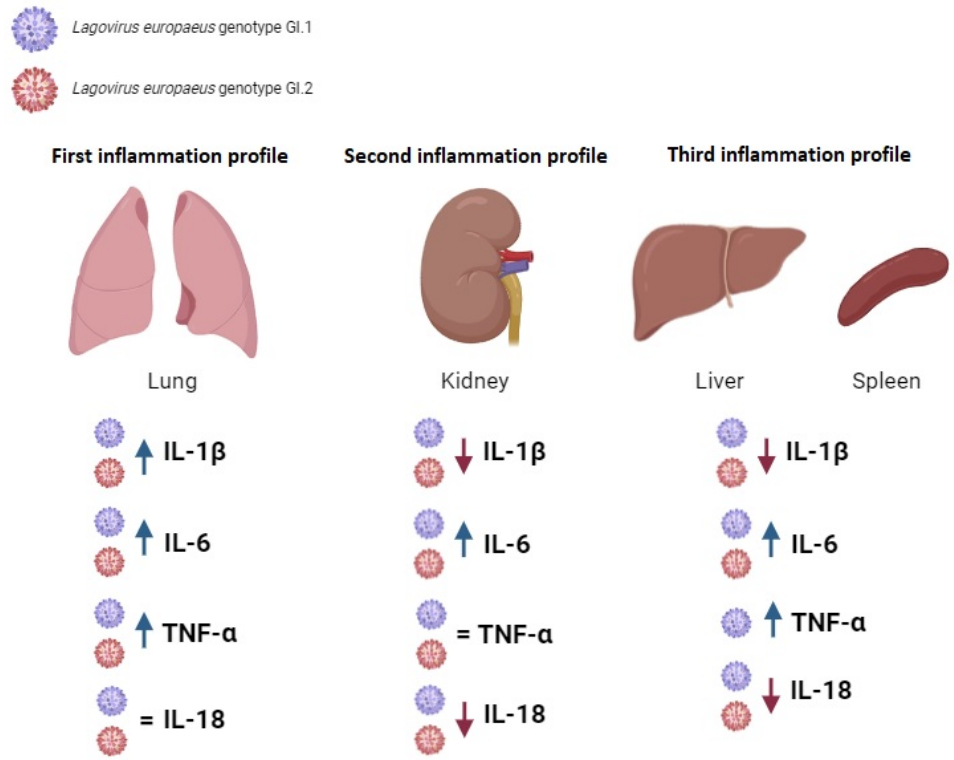


**Figure 11.** Inflammation profiles in response to *L. europaeus* infection of both the GI.1 and GI.2 genotypes.

## 4. Materials and Methods

### *4.1. Ethical Statements*

The experiment was carried out in the experimental animal facility of the Pomeranian Medical University (PUM) in Szczecin based on the consent obtained by the Local Ethical Committee for Animal Experiments in Poznań, Poland (no. 51/2022). Rabbits were maintained according to the European Union and national guidelines for animal experimentation. Additionally, during the experiment, the clinical conditions of the infected and healthy control rabbits were assessed, and clinical signs and mortality were recorded for the infected animals.

### *4.2. Viruses*

The viruses used in the experiment have been prepared at the National Reference Laboratory for Rabbit Hemorrhagic Disease (RHD) and the Department of Foot and Mouth Disease, the National Veterinary Research Institute—State Research Institute in Zduńska Wola, Poland. To provoke infection in rabbits, two viruses were utilized: the *Lagovirus europaeus* genotype GI.1, variant GI.1a, which was designated as BBI (Poland, 2017; GenBank accession no. MG602005), and *Lagovirus europaeus* genotype GI.2 which was labeled as PIN (Poland, 2018; GenBank accession no. MN853660) [80]. Both viruses were titer-determined by the hemagglutination (HA) assay. The infectious titer of the *L. europaeus* GI.1 genotype inoculum (1 mL) was determined to be 0.5 u/mL (1 HA unit corresponds to $10^4$ particles per mL), and for *L. europaeus* GI.2 genotype to be 2.048 u/mL (1 HA unit corresponds to $10^4$ particles per mL).

### *4.3. Experimental Models*

The study was performed on 30 European rabbits. *Oryctolagus cuniculus*—Crl:KBL (NZW)/052 purchased from AnimaLab Limited Liability Company (branch in Poland, Poznań). The rabbits were 6 months old, with body weights of 4.0–4.5 kg, and of both sexes (50:50 ratio). After the animals were delivered to the university's experimental facility, there was a 3-week adaptation period. The experiment was conducted in accordance with the

European Union Directive with regard to temperature and humidity, as well as the lighting and size of cages for animals [81], and ARRIVE guidelines [82,83]. The autonomous air conditioning system maintained a temperature of 22 °C (±1 °C) with humidity levels between 50% and 60%. The system facilitated 15–20 air changes per hour, ensuring a controlled environment. The rooms were equipped with artificial lighting, automatically controlled to provide 12 h of light followed by 12 h of darkness, supplemented with red night lighting. Food and water were available to the animals *ad libitum*. The animals were randomly divided into three groups for the study. The experimental animals were infected by intramuscular injection of the 1 mL selected virus—the *L. europaeus*/GI.1 genotype (named BBI strain, Poland 2017) for group 1, and the *L. europaeus*/GI.2 genotype (PIN strain, Poland 2018) for group 2, while the control group was injected with a form of PBS (phosphate-buffered saline) as a placebo. The onset of severe symptoms of rabbit hemorrhagic disease was considered the terminal moment of the experiment. Animals qualified for euthanasia were anesthetized by intravenous administration of the preparation ketamine 35–50 mg/kg, xylazine 5–10 mg/kg, followed by administration of the cardiac arrest-inducing preparation sodium pentobarbital (at 240 mg/kg).

### 4.4. Tissue Sample Collection

Tissue samples for the study (liver, lung, spleen, and kidney) were obtained from infected rabbits ($n$ = 20) immediately post-mortem and from healthy rabbits ($n$ = 10) after euthanasia. All tissues were washed in cold PBS, placed in liquid nitrogen, and stored at −80 °C until total RNA extraction.

### 4.5. Selection and In-Silico Prediction of miRs Target Genes Involved in the Innate Immune and Inflammatory Response in Oryctolagus cuniculus

In the first stage, miRs involved in innate immune and inflammatory responses were selected [24,25]. At this stage, the miRTarBase database [84] was used to select miRs using various search strategies (by miRs, target gene, pathway/process, validated methods, and disease). The criterion for selecting miRs was the validation method: strong evidence and previous literature data [24,25,46]. *Homo sapiens*' miRs with a described role in the innate immune and inflammatory response (miR-155, miR-146a, miR-223, miR-125b) were selected [24,25,46,84]. In the next stage, the miRTarBase database [84] and miRDB database [85] were used to select target genes (for chosen miRs) previously validated by RT-qPCR, Western blot, or a reporter assay in other species (validation methods: strong evidence). Next, the set of genes was used to conduct a gene ontology (GO) analysis via a GO enrichment analysis powered by protein annotation through evolutionary relationships [86]. The analysis included analysis type: PANTHER overrepresentation test; reference list: all *Homo sapiens'* genes in the database; annotation data set: GO biological process complete; test type: Fisher's exact; and correction: calculate false discovery rate (FDR). From all the processes with FDR $p < 0.05$, those correlated with immune response, innate immune response, inflammatory response, liver diseases, and multi-organ dysfunction in humans and animals were used for further steps. The 3′-UTR sequences of the *Oryctolagus cuniculus* genes involved in the selected processes were assessed to determine if they featured binding sites for miR-155, miR-146a, miR-223, and miR-125b using the TargetScan database [87]. In the next stage, to verify the importance of miR-155, miR-146a, miR-223, and miR-125b in *L. europaeus* infection, an in-silico analysis of putative target genes was conducted. Due to the inability to use one database to demonstrate the miR–mRNA interactions in *Oryctolagus cuniculus*, the following approach was selected: (i) mature sequences of these miRs in *Oryctolagus cuniculus* and *Homo sapiens* were compared, and no differences were found. We decided to use the miRTarBase [84], which lists genes with validated miR–mRNA interactions by RT-qPCR or luciferase assays in *Homo sapiens*. Finally, four groups were created containing 110 for miR-155, 90 for miR-146a, 100 for miR-223, and 50 for miR-125b; (ii) there was an attempt to determine the processes related to RHD that miR-155, miR-146a, miR-223, and miR-125b might regulate. For this purpose, a GO analysis was conducted

on the putative target genes for every miR separately. Thus, 772 processes for miR-155, 250 processes for miR-146a, 300 processes for miR-223, and 50 processes for miR-125b were identified. These groups' processes correlated with RHD pathogenesis, liver diseases, ALF, and MOF and were subsequently chosen for further analysis: 22 for miR-155, 20 for miR-146a, 20 for miR-223, and 18 for miR-125b. At this step, all analyses were performed based on miR–mRNA interactions in *Homo sapiens*. The TargetScan database confirmed whether these regulations might also occur in *Oryctolagus cuniculus*. This tool enabled us to verify if the predicted binding sites were conserved in *Oryctolagus cuniculus*. Genes engaged in RHD, ALF, and MOF processes were selected. Each miR–3′-UTR interaction was checked independently. The TargetScan analysis revealed that 63 out of 159 genes for ocu-miR-155, 25 out of 50 genes for ocu-miR-146a, 20 out of 80 genes for ocu-miR-223, and 11 out of 40 genes for ocu-miR-125b, have binding sites in 3′-UTR in *Oryctolagus cuniculus* genes. Selected miRs/target genes are presented in Table 2.

**Table 2.** Selected studied miRs/target genes involved in innate immune and inflammatory responses.

| miRs | Target Genes | Gene Product | Reference Used to Select Target Gene |
|---|---|---|---|
| miR-155 | *MyD88* | Myeloid differentiation primary response protein MyD88 | [46,88,89] |
| | *TAB2* | TGF-beta-activated kinase 1 and MAP3K7-binding protein 2 | [46,90,91] |
| | *p65* (subunit of NF-κβ) | Transcription factor p65 | [46,92,93] |
| | *NLRP3 inflammasome* | NACHT, LRR, and PYD domains containing protein 3 | [46] |
| miR-146a | *IRAK1* | Interleukin-1 receptor-associated kinase 1 | [25,46,94] |
| | *TRAF6* | TNF receptor-associated factor 6 | [25,46,94] |
| miR-223 | *TLR4* | Toll-like receptor 4 | [46,68,95] |
| | *IKKα* | Inhibitor of nuclear factor kappa-B kinase subunit alpha | [46,67,96] |
| | *NLRP3* | NACHT, LRR, and PYD domains-containing protein 3 | [46,97] |
| miR-125b | *MyD88* | Myeloid differentiation primary response protein MyD88 | [50,98] |

### 4.6. miRs and mRNA Isolation from Tissues

The total RNA, encompassing miRs, was extracted from 50 mg of each tissue sample of the infected and healthy rabbits using the miRNeasy Mini Kit (Qiagen, Hilden, Germany). Isolation of total RNA was performed following the manufacturer's protocol. Samples were mechanically homogenized in 700 µL of QIAzol Lysis Reagent, followed by a 5 min incubation at room temperature. Chloroform (140 µL) was added, and the mixture was shaken. After a 3 min incubation, the samples were centrifuged at 12,000× *g* for 15 min at 4 °C. The upper aqueous phase was carefully transferred to a new tube, and RNA was precipitated by adding 1.5 volumes of 100% ethanol. The mixture was then applied to an RNeasy Mini column, and centrifugation was performed at 8000× *g* for 15 s. This step was repeated for the entire sample. Subsequently, the column was washed with 700 µL of Buffer RWT, followed by two washes with 500 µL of Buffer RPE; the second included a 2 min centrifugation to ensure membrane dryness. Finally, RNA was eluted in 50 µL of RNase-free water after centrifugation at 9000× *g* for 1 min. RNA concentration and quality were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

### 4.7. miRs Polyadenylation and Reverse Transcription Reaction

cDNA synthesis was performed by the reverse transcription (RT) reaction using a miRCURY LNA RT Kit (Qiagen, Hilden, Germany). The total volume of 10 µL of the reaction mixture contained 2 µL of 5× miRCURY RT Reaction Buffer, 1 µL of miRCURY RT Enzyme Mix, 5 µL of RNase-free water and 2 µL of template RNA. Total RNA at a concentration of 5 ng/µL was used for the RT reaction. The reaction and temperature profile were according to the manufacturer's recommendation with the following steps: reverse transcription step by incubation at 42 °C for 60 min, heat inactivation of the reverse transcriptase for 5 min at 95 °C, and immediate cooling to 4 °C. The obtained cDNA samples were stored at −20 °C.

### 4.8. mRNAs Polyadenylation and Reverse Transcription Reaction

The RT reaction to synthesize the cDNA from mRNA was carried out using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). To increase the efficiency of the reaction oligo(dT), random hexamers were used. RT reaction was performed in the mixture with a final volume of 20 µL containing 1 µL of Oligo(dT) Primer and Random Hexamer primer, 4 µL of 5× Reaction Buffer, 1 µL of RiboLock RNase Inhibitor, 1 µL of RevertAid M-MuL V RT (200 U/µL), 4 µL of total RNA template of concentration 200 ng/µL and 8 µL of RNase-free water to replenish the mixture volume. The reaction conditions and temperature profile were determined based on the manufacturer's instructions and were as follows: incubation for 5 min at 25 °C followed by 60 min at 42 °C, then heating at 70 °C for 5 min in order to termination of the reaction, and immediate cooling to 4 °C. The obtained cDNA samples were stored at −20 °C.

### 4.9. Quantification of miRs in Tissue Samples Using Quantitative Real-Time PCR and Data Analysis

The levels of miRs (Table 3) were measured using the quantitative real-time PCR (qPCR) reaction. The expression of miRs (along with miR-103a-3p, which was used as an endogenous control) in tissues samples were measured using the miRCURY LNA miRNA PCR Assay (Qiagen, Hilden, Germany) and the miRCURY LNA SYBR Green PCR Kit, according to the manufacturer's instructions. The reaction mixture consisted of 5 µL of 2× miRCURY SYBR Green Master Mix, 0.05 µL of ROX Reference Dye, 1 µL of specific PCR primer mix, and 1 µL of RNase-free water. Three µL of 60× diluted template cDNA was added to the reaction mixture. The final reaction volume was 10 µL. The thermal cycle conditions were as follows: 95 °C for 2 min for the PCR initial heat activation, 40 cycles of denaturation 95 °C for 10 s and annealing 56 °C for 1 min, and at the end of the reaction, a melting curve analysis was performed in the temperature range of 60–95 °C. Fluorescence data were analyzed using Quant Studio 5 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) and the expression of miRs were calculated using the $2^{-\Delta\Delta Ct}$ method of relative quantification.

**Table 3.** Sequences of the tested microRNAs (miR) of *Oryctolagus cuniculus* (ocu).

| miRs | Sequences |
|---|---|
| **miRs tested** | |
| ocu-miR-155-5p | 5′UUAAUGCUAAUCGUGAUAGGGGUU3′ |
| ocu-miR-146a-5p | 5′UGAGAACUGAAUUCCAUGGGUUG3′ |
| ocu-miR-223-5p | 5′CGUGUAUUUGACAAGCUGAGUUG3′ |
| ocu-miR-125b-5p | 5′UCCCUGAGACCCUAACUUGUGA3′ |
| **reference miRs** | |
| ocu-miR-103a-3p | 5′AGCAGCAUUGUACAGGGCUAUGA3′ |

### 4.10. Quantification of mRNAs in Tissue Samples Using Quantitative Real-Time PCR and Data Analysis

Relative expression mRNA of selected genes (Table 4) was assessed by qPCR using the Quant Studio 5 Real-Time PCR System (Applied Biosystems, United States) and the HOT FIREPol® EvaGreen® qPCR Supermix, 5× (Solis BioDyne, Tartu, Estonia) according to manufacturer's recommendations. The reaction mixture was 20 µL. It consisted of 4 µL of HOT FIREPol EvaGreen qPCR Supermix (5×), 0.4 µL of forward and reverse primers of concentration 10 µM and a variable volume of template cDNA of 10 ng/µL concentration and RNase-free water depending on the primer. The reaction was carried out according to the following temperature–time profile: initial activation by incubation at 95 °C for 12 min, 40 cycles of denaturation at 95 °C for 15 s, annealing at a temperature adapted to the primers (Table 4) for 20–30 s (depending on length product) and extension at 72 °C for 20–30 s. Specific primers for selected target genes for qPCR reaction were designed using the computational tools online Primer-BLAST (2024) [99] and Beacon Designer (2024) [100]. To validate the designed primers, a temperature gradient PCR was performed using the Color OptiTaq PCR Master Mix (2×) kit (Euryx, Gdańsk, Poland), followed by agarose gel electrophoresis. The PCR reaction mixture was 50 µL and consisted of 25 µL Color OptiTaq PCR Master Mix (2×), 0.25 µL of forward and reverse primers of 10 mM concentration, 23.5 µL RNase-free water, and 1 µL of CDNA template of 0.2 µg/µL concentration. The reaction was carried out according to the following temperature–time profile: initial denaturation at 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 15 s, the annealing temperature was carried out in a temperature gradient (temperature range of 52–62 °C) for 30 s, and extension at 72 °C 1 min. Final extension was at 72 °C for 7 min. After the reaction was completed, the samples were cooled to 4 °C. This process enabled determination of the optimal annealing temperature for the primers and to check for the formation of non-specific products. After primer validation, optimization of the real-time PCR reaction was performed. Optimization approach involved sequentially adjusting primer sequences, annealing temperatures, primer concentrations, and a range of cDNA concentrations for each gene tested. The calibration method was used to obtain a standard curve. Using the optimal annealing temperature and primer concentration for each primer pair, serial dilutions of the same cDNA were performed (1:2, 1:4, 1:8, 1:16, and 1:32) from an initial concentration of 10 ng/µL, as recommended by the manufacturer (HOT FIREPol® EvaGreen® qPCR Supermix, 5×; Solis BioDyne, Estonia). It was observed that different primer pairs required different optimal cDNA concentration ranges for each gene to achieve the highest coefficient of determination ($R^2$) and optimal efficiencies (100% ± 5%). Additionally, the specificity of the primers was confirmed by melting curve analysis at the end of the reaction real-time PCR. Fluorescence data were analyzed using a real-time PCR system. The amount of target, normalized to an endogenous reference gene 18S and relative to the expression levels in healthy controls, was determined using the $2^{-\Delta\Delta Ct}$ formula. A melting curve analysis was performed each time.

**Table 4.** qPCR primers used in the study to target genes expression.

| Gene | GenBank Accession No. | Primers | | Ta (°C) | Amplicon Length (bp) | Tm of the Amplification Products (°C) |
|---|---|---|---|---|---|---|
| *MyD88* | XM_002723869.4 | Forward<br>Revers | 5′-CCCCAGCGACATGCAGTTTG-3′<br>5′-TTCTGATGGGCACCTGGAGAG-3′ | 61 | 227 | 90.8 |
| *TAB2* | XM_051836813.1 | Forward<br>Revers | 5′-ACCTCCAGCAGTTCCTCTTC-3′<br>5′-TCATCTCCTGTGGTGGCATT-3′ | 60 | 152 | 83.5 |
| *p65* | XM_051827970.1 | Forward<br>Revers | 5′-CCCTTCCAAGTGCCCATAGA-3′<br>5′-CCTCTTTCTGCACCTTGTCG-3′ | 60 | 250 | 91.5 |
| *NLRP3* | MK829787.1 | Forward<br>Revers | 5′-TGTCTCACGTCCAGCTTTTG-3′<br>5′-AGCCAGAGTCTGCGAATGTT-3′ | 60 | 161 | 87.5 |

**Table 4.** *Cont.*

| Gene | GenBank Accession No. | Primers | | Ta (°C) | Amplicon Length (bp) | Tm of the Amplification Products (°C) |
|---|---|---|---|---|---|---|
| *IRAK1* | XM_051837494.1 | Forward<br>Revers | 5′-GGACTTTGCTGGCTACTGTG-3′<br>5′-CAGGAGGACGTTGGAACTCT-3′ | 60 | 229 | 89.9 |
| *TRAF6* | XM_002709054.4 | Forward<br>Revers | 5′-ACGGGGAACCTTTCTGGCTC-3′<br>5′-TGTGGCCTGCATCCCTTATTG-3′ | 61 | 187 | 86.4 |
| *TLR4* | NM_001082732.2 | Forward<br>Revers | 5′-TTTCACACGGCCACTGCTG-3′<br>5′-ATTGGGAACGACCTCCACAC-3′ | 61 | 142 | 81.4 |
| *IKKα* | XM_002718612.4 | Forward<br>Revers | 5′-GGTAACTCCTCAAGATGGGGAC-3′<br>5′-TGCCCTGTTCCTCATTTGCT-3′ | 60 | 107 | 78.7 |
| *IL-1β* | NM_001082201.1 | Forward<br>Revers | 5′-GGTGTTGTCTGGCACGTATG-3′<br>5′-TTGGGGTCTACACTCTCCAG-3′ | 60 | 210 | 84.0 |
| *IL-6* | NM_001082064.2 | Forward<br>Revers | 5′-GGCGGTGAATAATGAGACCTG-3′<br>5′-ATGAAGTGGATCGTGGTCGT-3′ | 60 | 276 | 87.3 |
| *TNF-α* | NM_001082263.1 | Forward<br>Revers | 5′-CGTAGTAGCAAACCCGCAAG-3′<br>5′-TGATGGCAGAGAGGAGGTTG-3′ | 60 | 245 | 91.3 |
| *IL-18* | NM_001122940.1 | Forward<br>Revers | 5′-TGTATAGAAAATGCACCCCAGAC-3′<br>5′-TCTTTCTGTCCTGCGAGATGT-3′ | 60 | 221 | 80.0 |
| *18S* | NR_033238.1 | Forward<br>Revers | 5′-ATCAGATACCGTCGTAGTTC-3′<br>5′-TTCCGTCAATTCCTTTAAG-3′ | 60 | 167 | 88.0 |

### *4.11. Statistical Analysis*

Statistical analysis was performed using STATISTICA PL Version 13 (StatSoft, Palo Alto, CA, USA). The data were evaluated as a mean ± standard error of the mean (SEM) for continuous variables. The normal distribution of the analyzed variables was tested using the Shapiro–Wilk test. For data analysis the Student's *t*-test was performed for data with a normal distribution, and for data with a non-parametric distribution, the Mann–Whitney U test was performed. To determine possible changes in all miRs or mRNAs, the one-way ANOVA was performed or Kruskal–Wallis test depending on the obtained distribution. Additionally, correlation analysis was performed using the test non-parametric Spearman's rank method. Spearman's rank correlation coefficient (R) was also calculated to measure the statistical dependence between expression of miRs and the expression of their target genes. In all statistical tests, the results were considered statistically significant if the *p*-value did not exceed 5% ($p \leq 0.05$).

## 5. Conclusions

Our report is the first to present the regulatory effect of miRs on innate immune and inflammatory response genes in rabbits infected with *L. europaeus*/GI.1 and GI.2 genotypes in four tissues (liver, lung, kidney, and spleen).

Our research provides new data for understanding the pathogenesis of rabbit hemorrhagic disease caused by *L. europaeus* and understanding the molecular regulation of the innate immune and inflammatory response.

The main regulators of the innate immune and inflammatory response in *L. europaeus*/GI.1 and GI.2 infection, as well as RHD, are miR-155, miR-223, and miR-146a. miR-125 expression was highly limited and affected only the liver and spleen. We have shown that during infection with *L. europaeus*/GI.1 and GI.2/RHD, (1) miR-155—has both pro- and anti-inflammatory effects in the liver and anti-inflammatory effects in the kidneys and spleen; (2) miR-146a has anti-inflammatory effects in the liver, lungs and kidneys; (3) miR-223 has anti-inflammatory effects in all tissues; (4) however, miR-125b has anti-inflammatory effects only in the liver. In each case, such an effect may be a determinant of

the pathogenesis of RHD. Our research shows that miRs may regulate three innate immune and inflammatory response pathways in *L. europaeus* infection: (1) TLR4-MyD88 signaling pathway; (2) NF-κB signaling pathway (p65); and (3) NRLP3 inflammasome pathway. However, the result of this regulation may be influenced by the tissue microenvironment, as observed in studies.

Our report is the first to show the expression profile of inflammatory biomarkers (IL-1β, IL-6, TNF-α, IL-18) in four tissues after infection with *L. europaeus*—with two genotypes simultaneously. Our research shows that infection of rabbits with *L. europaeus*/GI.1, and GI.2 genotypes causes an overexpression of two critical acute phase cytokines—*IL-6* in all examined tissues and *TNF-α* (in the liver, lungs, and spleen). Cytokine *IL-1β* was highly expressed only in the lungs after *L. europaeus* infection. These facts indicate a strong and rapid involvement of the local innate immune and inflammatory response in *L. europaeus* infection for both genotypes (GI.1 and GI.2) and in the pathogenesis of RHD. Profile biomarkers of inflammation in rabbits infected with *L. europaeus* by both genotypes are similar regarding the nature of changes but are different for individual tissues. Therefore, we propose three inflammation profiles for *L. europaeus* infection (pulmonary, renal, and liver and spleen).

The results of our research also have diagnostic (search for potential molecular biomarkers of inflammation/disease) and therapeutic potential (modulation of miR-dependent pathways, e.g., NF-κB and NLRP3 inflammasome) in the course of acute liver failure (ALF) and organ dysfunction in multi-organ failure (MOF) of a viral etiology that we encounter during *Lagovirus europaeus* infection.

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## References

1. Le Pendu, J.; Abrantes, J.; Bertagnoli, S.; Guitton, J.S.; Le Gall-Recule, G.; Lopes, A.M.; Marchandeau, S.; Alda, F.; Almeida, T.; Celio, A.P.; et al. Proposal for a unified classification system and nomenclature of lagoviruses. *J. Gen. Virol.* **2017**, *98*, 1658–1666. [CrossRef]
2. Liu, S.J.; Xue, H.P.; Pu, B.Q.; Qian, N.H. A new viral disease in rabbits. *Anim. Husb. Vet. Med.* **1984**, *16*, 253–255.
3. Abrantes, J.; van der Loo, W.; Le Pendu, J.; Esteves, P.J. Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): A review. *Vet. Res.* **2012**, *43*, 12. [CrossRef] [PubMed]
4. Rocchi, M.; Dagleish, M. Diagnosis and prevention of RHVD2 infection. *Vet. Rec.* **2018**, *182*, 604–605. [CrossRef]
5. Muller, C.; Hrynkiewicz, R.; Bebnowska, D.; Maldonado, J.; Baratelli, M.; Kollner, B.; Niedzwiedzka-Rystwej, P. Immunity against Lagovirus europaeus and the Impact of the Immunological Studies on Vaccination. *Vaccines* **2021**, *9*, 255. [CrossRef] [PubMed]
6. Trzeciak-Ryczek, A.; Tokarz-Deptula, B.; Deptula, W. The importance of liver lesions and changes to biochemical and coagulation factors in the pathogenesis of RHD. *Acta Biochim. Pol.* **2015**, *62*, 169–171. [CrossRef]
7. Park, J.H.; Lee, Y.S.; Itakura, C. Pathogenesis of acute necrotic hepatitis in rabbit hemorrhagic disease. *Lab. Anim. Sci.* **1995**, *45*, 445–449. [PubMed]

8. Alonso, C.; Oviedo, J.M.; Martin-Alonso, J.M.; Diaz, E.; Boga, J.A.; Parra, F. Programmed cell death in the pathogenesis of rabbit hemorrhagic disease. *Arch. Virol.* **1998**, *143*, 321–332. [CrossRef]
9. Jung, J.Y.; Lee, B.J.; Tai, J.H.; Park, J.H.; Lee, Y.S. Apoptosis in rabbit haemorrhagic disease. *J. Comp. Pathol.* **2000**, *123*, 135–140. [CrossRef]
10. San-Miguel, B.; Alvarez, M.; Culebras, J.M.; Gonzalez-Gallego, J.; Tunon, M.J. N-acetyl-cysteine protects liver from apoptotic death in an animal model of fulminant hepatic failure. *Apoptosis* **2006**, *11*, 1945–1957. [CrossRef]
11. Ni, Z.; Wei, W.; Liu, G.Q.; Chen, L.; Yu, B.; Yun, T.; Hua, J.G.; Li, S.M. Studies on the apoptosis of RK13 cells induced by rabbit hemorrhagic disease virus. *Bing. Du. Xue Bao* **2009**, *25*, 316–317. [PubMed]
12. Garcia-Lastra, R.; San-Miguel, B.; Crespo, I.; Jorquera, F.; Alvarez, M.; Gonzalez-Gallego, J.; Tunon, M.J. Signaling pathways involved in liver injury and regeneration in rabbit hemorrhagic disease, an animal model of virally-induced fulminant hepatic failure. *Vet. Res.* **2010**, *41*, 2. [CrossRef]
13. Marques, R.M.; Costa, E.S.A.; Aguas, A.P.; Teixeira, L.; Ferreira, P.G. Early acute depletion of lymphocytes in calicivirus-infected adult rabbits. *Vet. Res. Commun.* **2010**, *34*, 659–668. [CrossRef]
14. Chen, M.; Liu, X.; Hu, B.; Fan, Z.; Song, Y.; Wei, H.; Qiu, R.; Xu, W.; Zhu, W.; Wang, F. Rabbit Hemorrhagic Disease Virus Non-structural Protein 6 Induces Apoptosis in Rabbit Kidney Cells. *Front. Microbiol.* **2018**, *9*, 3308. [CrossRef]
15. Vallejo, D.; Crespo, I.; San-Miguel, B.; Alvarez, M.; Prieto, J.; Tunon, M.J.; Gonzalez-Gallego, J. Autophagic response in the Rabbit Hemorrhagic Disease, an animal model of virally-induced fulminant hepatic failure. *Vet. Res.* **2014**, *45*, 15. [CrossRef] [PubMed]
16. Ostrycharz, E.; Fitzner, A.; Kesy, A.; Siennicka, A.; Hukowska-Szematowicz, B. MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with Lagovirus europaeus GI.1 and GI.2 genotypes. *Front. Microbiol.* **2024**, *15*, 1349535. [CrossRef] [PubMed]
17. Bebnowska, D.; Hrynkiewicz, R.; Rzeszotek, S.; Freus, M.; Poniewierska-Baran, A.; Niedzwiedzka-Rystwej, P. Apoptotic Cell Death in an Animal Model of Virus-Induced Acute Liver Failure-Observations during Lagovirus europaeus/GI.2 Infection. *Int. J. Mol. Sci.* **2024**, *25*, 798. [CrossRef]
18. Bebnowska, D.; Hrynkiewicz, R.; Wisniewska, K.; Zabinska, M.; Rintz, E.; Pierzynowska, K.; Niedzwiedzka-Rystwej, P. Apoptosis activation during Lagovirus europaeus/GI.2 infection in rabbits. *Front. Microbiol.* **2023**, *14*, 1308018. [CrossRef]
19. Niedzwiedzka-Rystwej, P.; Deptula, W. Apoptosis of peripheral blood leukocytes from rabbits infected with non-haemagglutinating strains of rabbit haemorrhagic disease virus (RHDV). *Vet. Immunol. Immunopathol.* **2012**, *149*, 54–57. [CrossRef]
20. Hukowska-Szematowicz, B. Genetic and immunological characteristic of European strains of RHD (rabbit heamorrhagic disease) virus. *Pol. J. Environ. Stud.* **2013**, *2*, 1–114.
21. Niedzwiedzka-Rystwej, P.; Hukowska-Szematowicz, B.; Tokarz-Deptula, B.; Trzeciak-Ryczek, A.; Dzialo, J.; Deptula, W. Apoptosis of peripheral blood leucocytes in rabbits infected with different strains of rabbit haemorrhagic disease virus. *Acta Biochim. Pol.* **2013**, *60*, 65–69. [CrossRef] [PubMed]
22. Hu, B.; Wei, H.; Song, Y.; Chen, M.; Fan, Z.; Qiu, R.; Zhu, W.; Xu, W.; Wang, F. NF-kappaB and Keap1 Interaction Represses Nrf2-Mediated Antioxidant Response in Rabbit Hemorrhagic Disease Virus Infection. *J. Virol.* **2020**, *94*, e00016-20. [CrossRef]
23. Hukowska-Szematowicz, B.; Ostrycharz, E.; Dudzinska, W.; Roszkowska, P.; Siennicka, A.; Wojciechowska-Koszko, I. Digital PCR (dPCR) Quantification of miR-155-5p as a Potential Candidate for a Tissue Biomarker of Inflammation in Rabbits Infected with Lagovirus europaeus/Rabbit Hemorrhagic Disease Virus (RHDV). *Viruses* **2023**, *15*, 1578. [CrossRef] [PubMed]
24. Hukowska-Szematowicz, B.; Maciejak-Jastrzebska, A.; Blatkiewicz, M.; Maciak, K.; Gora, M.; Janiszewska, J.; Burzynska, B. Changes in MicroRNA Expression during Rabbit Hemorrhagic Disease Virus (RHDV) Infection. *Viruses* **2020**, *12*, 965. [CrossRef] [PubMed]
25. Ostrycharz, E.; Hukowska-Szematowicz, B. Micro-Players of Great Significance-Host microRNA Signature in Viral Infections in Humans and Animals. *Int. J. Mol. Sci.* **2022**, *23*, 10536. [CrossRef]
26. Bartel, D.P. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* **2004**, *116*, 281–297. [CrossRef]
27. Hrynkiewicz, R.; Bebnowska, D.; Niedzwiedzka-Rystwej, P. Myeloperoxidase and Lysozymes as a Pivotal Hallmark of Immunity Status in Rabbits. *Animals* **2020**, *10*, 1581. [CrossRef]
28. Tokarz-Deptuła, B. Immunity phenomena in rabbits infected with the RHD virus (rabbit haemorrhagic disease). *Pol. J. Environ. Sci.* **2009**, *7*, 1–81.
29. Tokarz-Deptula, B.; Kulus, J.; Baraniecki, L.; Stosik, M.; Deptula, W. Characterisation of Lagovirus europaeus GI-RHDVs (Rabbit Haemorrhagic Disease Viruses) in Terms of Their Pathogenicity and Immunogenicity. *Int. J. Mol. Sci.* **2024**, *25*, 5342. [CrossRef]
30. Niedźwiedzka-Rystwej, P.; Deptuła, W. Non-specific immunity in rabbits infected with 10 strains of the rabbit haemorrhagic disease virus with different biological properties. *Cent. Europ. J. Biol.* **2010**, *5*, 613–632. [CrossRef]
31. Hukowska-Szematowicz, B.; Deptuła, W. Non-specific cellular in rabbits experimentlaly infected with four Czech strains of the Rabbit Haemorrhagic Disease Virus with Different patho-genicity. *Pol. J. Environ. Stud.* **2012**, *21*, 879–892.
32. Niedzwiedzka-Rystwej, P.; Tokarz-Deptula, B.; Deptula, W. Reactivity of selected markers of innate and adaptive immunity in rabbits experimentally infected with antigenic variants of RHD (Lagovirus europaeus/GI.1a). *Vet. Res. Commun.* **2022**, *46*, 233–242. [CrossRef] [PubMed]
33. Hukowska-Szematowicz, B. Changes in innate and adaptive immune response to Lagovirus europaeus GI.1 (Rabbit Haemorrhagic Disease Virus-RHDV) infection. *Acta Biol.* **2020**, *27*, 77–91. [CrossRef]

34. Marcato, P.S.; Benazzi, C.; Vecchi, G.; Galeotti, M.; Della Salda, L.; Sarli, G.; Lucidi, P. Clinical and pathological features of viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev. Sci. Technol.* **1991**, *10*, 371–392. [CrossRef]
35. Ferreira, P.G.; Costa, E.S.A.; Oliveira, M.J.; Monteiro, E.; Aguas, A.P. Leukocyte-hepatocyte interaction in calicivirus infection: Differences between rabbits that are resistant or susceptible to rabbit haemorrhagic disease (RHD). *Vet. Immunol. Immunopathol.* **2005**, *103*, 217–221. [CrossRef]
36. Ostrycharz, E.; Blatkiewicz, M.; Hukowska-Szematowicz, B. *Młodzi naukowcy 2.0. Tom 1. Expression of the Master Anti-Inflammatory Regulator IL-10 during Lagovirus Europaeus GI.1/RHDV (Rabbit Hemorrhagic Disease Virus) Infection.*; Fundacja Centrum Badań Socjologicznych (Centre of Sociological Research): Szczecin, Poland, 2021; pp. 59–72.
37. Trzeciak-Ryczek, A.; Tokarz-Deptula, B.; Deptula, W. Expression of IL-1Ra, IL-6, IL-8, IL-18, TNF-alpha and IFN-gamma genes in peripheral blood leukocytes of rabbits infected with RHDV (Rabbit Haemorrhagic Disease Virus). *Dev. Comp. Immunol.* **2017**, *76*, 310–315. [CrossRef]
38. Trzeciak-Ryczek, A.; Tokarz-Deptula, B.; Deptula, W. Expression of IL-1beta, IL-2, IL-10, TNF-beta and GM-CSF in peripheral blood leukocytes of rabbits experimentally infected with rabbit haemorrhagic disease virus. *Vet. Microbiol.* **2016**, *186*, 71–81. [CrossRef]
39. Semerjyan, A.B.; Sargsyan, M.A.; Arzumanyan, H.H.; Hakobyan, L.H.; Abroyan, L.O.; Semerjyan, Z.B.; Avetisyan, A.S.; Karalova, E.M.; Manukyan, D.M.; Matevosyan, H.S.; et al. Immune cell pathology in rabbit hemorrhagic disease. *Vet. World* **2019**, *12*, 1332–1340. [CrossRef]
40. Teixeira, L.; Marques, R.M.; Aguas, A.P.; Ferreira, P.G. Regulatory T cells are decreased in acute RHDV lethal infection of adult rabbits. *Vet. Immunol. Immunopathol.* **2012**, *148*, 343–347. [CrossRef]
41. Marques, R.M.; Costa, E.S.A.; Aguas, A.P.; Teixeira, L.; Ferreira, P.G. Early inflammatory response of young rabbits attending natural resistance to calicivirus (RHDV) infection. *Vet. Immunol. Immunopathol.* **2012**, *150*, 181–188. [CrossRef]
42. O'Toole, A.D.; Mohamed, F.M.; Zhang, J.; Brown, C.C. Early pathogenesis in rabbit hemorrhagic disease virus 2. *Microb. Pathog.* **2022**, *173*, 105814. [CrossRef] [PubMed]
43. Yu, J.; Li, Y.; Xiao, L.; Xie, J.; Guo, Z.; Ye, Y.; Lin, Y.; Cao, Y.; Wu, X.; Mao, C.; et al. Neglected Spleen Transcriptional Profile Reveals Inflammatory Disorder Conferred by Rabbit Hemorrhagic Disease Virus 2 Infection. *Viruses* **2024**, *16*, 495. [CrossRef] [PubMed]
44. Hukowska-Szematowicz, B.; Deptuła, W. Peripheral blood lymphocytes in rabbits infected with Czech strains, CAMPV-562 and CAMPV-558 of RHD virus. *Cent. Eur. J. Immunol.* **2008**, *33*, 8–13.
45. Niedźwiedzka-Rystwej, P.; Tokarz-Deptuła, B.; Hukowska-Szematowicz, B.; Działo, J.; Trzeciak-Ryczek, A. Peripheral blood lymphocytes B and T and their subpopulation in rabbits infected with rabbit haemorrhagic diseaase virus (RHDV). *Med. Weter.* **2013**, *59*, 484–487.
46. Nejad, C.; Stunden, H.J.; Gantier, M.P. A guide to miRNAs in inflammation and innate immune responses. *FEBS J.* **2018**, *285*, 3695–3716. [CrossRef]
47. Mahesh, G.; Biswas, R. MicroRNA-155: A Master Regulator of Inflammation. *J. Interferon Cytokine Res.* **2019**, *39*, 321–330. [CrossRef] [PubMed]
48. Jafarzadeh, A.; Naseri, A.; Shojaie, L.; Nemati, M.; Jafarzadeh, S.; Bannazadeh Baghi, H.; Hamblin, M.R.; Akhlagh, S.A.; Mirzaei, H. MicroRNA-155 and antiviral immune responses. *Int. Immunopharmacol.* **2021**, *101*, 108188. [CrossRef]
49. Das, K.; Rao, L.V.M. The Role of microRNAs in Inflammation. *Int. J. Mol. Sci.* **2022**, *23*, 15479. [CrossRef]
50. Lee, H.M.; Kim, T.S.; Jo, E.K. MiR-146 and miR-125 in the regulation of innate immunity and inflammation. *BMB Rep.* **2016**, *49*, 311–318. [CrossRef]
51. Marques-Rocha, J.L.; Samblas, M.; Milagro, F.I.; Bressan, J.; Martinez, J.A.; Marti, A. Noncoding RNAs, cytokines, and inflammation-related diseases. *FASEB J.* **2015**, *29*, 3595–3611. [CrossRef] [PubMed]
52. Sonkoly, E.; Stahle, M.; Pivarcsi, A. MicroRNAs and immunity: Novel players in the regulation of normal immune function and inflammation. *Semin. Cancer Biol.* **2008**, *18*, 131–140. [CrossRef] [PubMed]
53. Szumna, M.; Hukowska-Szematowicz, B. MicroRNAa as new immunity regulators in viral and bacterial infection. *Acta Biol.* **2020**, *27*, 93–108. [CrossRef]
54. Gruys, E.; Toussaint, M.J.; Niewold, T.A.; Koopmans, S.J. Acute phase reaction and acute phase proteins. *J. Zhejiang Univ. Sci. B* **2005**, *6*, 1045–1056. [CrossRef] [PubMed]
55. Jain, S.; Gautam, V.; Naseem, S. Acute-phase proteins: As diagnostic tool. *J. Pharm. Bioallied Sci.* **2011**, *3*, 118–127. [CrossRef] [PubMed]
56. Pirhonen, J. Regulation of IL-18 expression in virus infection. *Scand. J. Immunol.* **2001**, *53*, 533–539. [CrossRef]
57. Kaplanski, G. Interleukin-18: Biological properties and role in disease pathogenesis. *Immunol. Rev.* **2018**, *281*, 138–153. [CrossRef]
58. Slaats, J.; Ten Oever, J.; van de Veerdonk, F.L.; Netea, M.G. IL-1beta/IL-6/CRP and IL-18/ferritin: Distinct Inflammatory Programs in Infections. *PLoS Pathog.* **2016**, *12*, e1005973. [CrossRef]
59. Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X.; Zhao, L. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* **2018**, *9*, 7204–7218. [CrossRef]
60. Tanaka, T.; Narazaki, M.; Kishimoto, T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb. Perspect. Biol.* **2014**, *6*, a016295. [CrossRef]
61. Kang, S.; Kishimoto, T. Interplay between interleukin-6 signaling and the vascular endothelium in cytokine storms. *Exp. Mol. Med.* **2021**, *53*, 1116–1123. [CrossRef]

62. Jang, D.I.; Lee, A.H.; Shin, H.Y.; Song, H.R.; Park, J.H.; Kang, T.B.; Lee, S.R.; Yang, S.H. The Role of Tumor Necrosis Factor Alpha (TNF-alpha) in Autoimmune Disease and Current TNF-alpha Inhibitors in Therapeutics. *Int. J. Mol. Sci.* **2021**, *22*, 2719. [CrossRef]
63. Mohd Zawawi, Z.; Kalyanasundram, J.; Mohd Zain, R.; Thayan, R.; Basri, D.F.; Yap, W.B. Prospective Roles of Tumor Necrosis Factor-Alpha (TNF-alpha) in COVID-19: Prognosis, Therapeutic and Management. *Int. J. Mol. Sci.* **2023**, *24*, 6142. [CrossRef] [PubMed]
64. Lai, W.Y.; Wang, J.W.; Huang, B.T.; Lin, E.P.; Yang, P.C. A Novel TNF-alpha-Targeting Aptamer for TNF-alpha-Mediated Acute Lung Injury and Acute Liver Failure. *Theranostics* **2019**, *9*, 1741–1751. [CrossRef]
65. Haneklaus, M.; Gerlic, M.; Kurowska-Stolarska, M.; Rainey, A.A.; Pich, D.; McInnes, I.B.; Hammerschmidt, W.; O'Neill, L.A.; Masters, S.L. Cutting edge: miR-223 and EBV miR-BART15 regulate the NLRP3 inflammasome and IL-1beta production. *J. Immunol.* **2012**, *189*, 3795–3799. [CrossRef] [PubMed]
66. Morales, L.; Oliveros, J.C.; Enjuanes, L.; Sola, I. Contribution of Host miRNA-223-3p to SARS-CoV-Induced Lung Inflammatory Pathology. *mBio* **2022**, *13*, e0313521. [CrossRef] [PubMed]
67. Houshmandfar, S.; Saeedi-Boroujeni, A.; Rashno, M.; Khodadadi, A.; Mahmoudian-Sani, M.R. miRNA-223 as a regulator of inflammation and NLRP3 inflammasome, the main fragments in the puzzle of immunopathogenesis of different inflammatory diseases and COVID-19. *Naunyn Schmiedebergs Arch. Pharmacol.* **2021**, *394*, 2187–2195. [CrossRef] [PubMed]
68. Yuan, S.; Wu, Q.; Wang, Z.; Che, Y.; Zheng, S.; Chen, Y.; Zhong, X.; Shi, F. miR-223: An Immune Regulator in Infectious Disorders. *Front. Immunol.* **2021**, *12*, 781815. [CrossRef]
69. Gu, J.; Xu, H.; Chen, Y.; Li, N.; Hou, X. MiR-223 as a Regulator and Therapeutic Target in Liver Diseases. *Front. Immunol.* **2022**, *13*, 860661. [CrossRef]
70. Wan, L.; Yuan, X.; Liu, M.; Xue, B. miRNA-223-3p regulates NLRP3 to promote apoptosis and inhibit proliferation of hep3B cells. *Exp. Ther. Med.* **2018**, *15*, 2429–2435. [CrossRef]
71. Shi, M.; Lu, Q.; Zhao, Y.; Ding, Z.; Yu, S.; Li, J.; Ji, M.; Fan, H.; Hou, S. miR-223: A key regulator of pulmonary inflammation. *Front. Med.* **2023**, *10*, 1187557. [CrossRef]
72. Zheng, Q.; Hua, C.; Liang, Q.; Cheng, H. The NLRP3 inflammasome in viral infection (Review). *Mol. Med. Rep.* **2023**, *28*, 160. [CrossRef] [PubMed]
73. Peng, Q.; Yin, R.; Zhu, X.; Jin, L.; Wang, J.; Pan, X.; Ma, A. miR-155 activates the NLRP3 inflammasome by regulating the MEK/ERK/NF-kappaB pathway in carotid atherosclerotic plaques in ApoE(-/-) mice. *J. Physiol. Biochem.* **2022**, *78*, 365–375. [CrossRef]
74. Sanada, T.; Sano, T.; Sotomaru, Y.; Alshargabi, R.; Yamawaki, Y.; Yamashita, A.; Matsunaga, H.; Iwashita, M.; Shinjo, T.; Kanematsu, T.; et al. Anti-inflammatory effects of miRNA-146a induced in adipose and periodontal tissues. *Biochem. Biophys. Rep.* **2020**, *22*, 100757. [CrossRef] [PubMed]
75. Escoubet-Lozach, L.; Benner, C.; Kaikkonen, M.U.; Lozach, J.; Heinz, S.; Spann, N.J.; Crotti, A.; Stender, J.; Ghisletti, S.; Reichart, D.; et al. Mechanisms establishing TLR4-responsive activation states of inflammatory response genes. *PLoS Genet.* **2011**, *7*, e1002401. [CrossRef] [PubMed]
76. Yuan, K.; Zhang, X.; Lv, L.; Zhang, J.; Liang, W.; Wang, P. Fine-tuning the expression of microRNA-155 controls acetaminophen-induced liver inflammation. *Int. Immunopharmacol.* **2016**, *40*, 339–346. [CrossRef] [PubMed]
77. Sul, O.J.; Sung, Y.B.; Rajasekaran, M.; Ke, K.; Yu, R.; Back, S.H.; Choi, H.S. MicroRNA-155 induces autophagy in osteoclasts by targeting transforming growth factor beta-activated kinase 1-binding protein 2 upon lipopolysaccharide stimulation. *Bone* **2018**, *116*, 279–289. [CrossRef]
78. Yang, B.; Yang, R.; Xu, B.; Fu, J.; Qu, X.; Li, L.; Dai, M.; Tan, C.; Chen, H.; Wang, X. miR-155 and miR-146a collectively regulate meningitic Escherichia coli infection-mediated neuroinflammatory responses. *J. Neuroinflammation* **2021**, *18*, 114. [CrossRef]
79. Blevins, H.M.; Xu, Y.; Biby, S.; Zhang, S. The NLRP3 Inflammasome Pathway: A Review of Mechanisms and Inhibitors for the Treatment of Inflammatory Diseases. *Front. Aging Neurosci.* **2022**, *14*, 879021. [CrossRef]
80. Fitzner, A.; Kesy, A.; Bulenger, K.; Niedbalski, W. Evidence of independent introductions of RHDV2 strains in Poland based on the genome analysis of viral isolates from 2016–2018. *Acta Biochim. Pol.* **2021**, *68*, 255–263. [CrossRef]
81. Regulation of the Minister of Agriculture and Rural Development of 10 March 2006 on Detailed Conditions for Maintenance of Laboratory Animals in Experimental Units and Breeding Units. Available online: https://leap.unep.org/countries/pl/national-legislation/regulation-detailed-conditions-keeping-laboratory-animals (accessed on 25 July 2024).
82. Percie du Sert, N.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; Dirnagl, U.; Emerson, M.; et al. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* **2020**, *18*, e3000411. [CrossRef]
83. Percie du Sert, N.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; Dirnagl, U.; et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol.* **2020**, *18*, e3000410. [CrossRef]
84. Chou, C.H.; Shrestha, S.; Yang, C.D.; Chang, N.W.; Lin, Y.L.; Liao, K.W.; Huang, W.C.; Sun, T.H.; Tu, S.J.; Lee, W.H.; et al. miRTarBase update 2018: A resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res.* **2018**, *46*, D296–D302. [CrossRef]
85. Chen, Y.; Wang, X. miRDB: An online database for prediction of functional microRNA targets. *Nucleic Acids Res.* **2020**, *48*, D127–D131. [CrossRef] [PubMed]

86. Ashburner, M.; Ball, C.A.; Blake, J.A.; Botstein, D.; Butler, H.; Cherry, J.M.; Davis, A.P.; Dolinski, K.; Dwight, S.S.; Eppig, J.T.; et al. Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* **2000**, *25*, 25–29. [CrossRef] [PubMed]
87. Agarwal, V.; Bell, G.W.; Nam, J.W.; Bartel, D.P. Predicting effective microRNA target sites in mammalian mRNAs. *Elife* **2015**, *4*, e05005. [CrossRef] [PubMed]
88. Tang, B.; Xiao, B.; Liu, Z.; Li, N.; Zhu, E.D.; Li, B.S.; Xie, Q.H.; Zhuang, Y.; Zou, Q.M.; Mao, X.H. Identification of MyD88 as a novel target of miR-155, involved in negative regulation of Helicobacter pylori-induced inflammation. *FEBS Lett.* **2010**, *584*, 1481–1486. [CrossRef]
89. Bandyopadhyay, S.; Long, M.E.; Allen, L.A. Differential expression of microRNAs in Francisella tularensis-infected human macrophages: miR-155-dependent downregulation of MyD88 inhibits the inflammatory response. *PLoS ONE* **2014**, *9*, e109525. [CrossRef]
90. Imaizumi, T.; Tanaka, H.; Tajima, A.; Yokono, Y.; Matsumiya, T.; Yoshida, H.; Tsuruga, K.; Aizawa-Yashiro, T.; Hayakari, R.; Inoue, I.; et al. IFN-gamma and TNF-alpha synergistically induce microRNA-155 which regulates TAB2/IP-10 expression in human mesangial cells. *Am. J. Nephrol.* **2010**, *32*, 462–468. [CrossRef]
91. Zhou, H.; Huang, X.; Cui, H.; Luo, X.; Tang, Y.; Chen, S.; Wu, L.; Shen, N. miR-155 and its star-form partner miR-155* cooperatively regulate type I interferon production by human plasmacytoid dendritic cells. *Blood* **2010**, *116*, 5885–5894. [CrossRef]
92. Wei, T.T.; Cheng, Z.; Hu, Z.D.; Zhou, L.; Zhong, R.Q. Upregulated miR-155 inhibits inflammatory response induced by C. albicans in human monocytes derived dendritic cells via targeting p65 and BCL-10. *Ann. Transl. Med.* **2019**, *7*, 758. [CrossRef]
93. Shen, S.M.; Jiang, H.; Zhao, J.N.; Shi, Y. Down-regulation of miR-155 inhibits inflammatory response in human pulmonary microvascular endothelial cells infected with influenza A virus by targeting sphingosine-1-phosphate receptor 1. *Chin. Med. J.* **2020**, *133*, 2429–2436. [CrossRef] [PubMed]
94. Zhang, X.; Guo, Y.; Xu, X.; Tang, T.; Sun, L.; Wang, H.; Zhou, W.; Fang, L.; Li, Q.; Xie, P. miR-146a promotes Borna disease virus 1 replication through IRAK1/TRAF6/NF-kappaB signaling pathway. *Virus Res.* **2019**, *271*, 197671. [CrossRef] [PubMed]
95. Li, T.; Morgan, M.J.; Choksi, S.; Zhang, Y.; Kim, Y.S.; Liu, Z.G. MicroRNAs modulate the noncanonical transcription factor NF-kappaB pathway by regulating expression of the kinase IKKalpha during macrophage differentiation. *Nat. Immunol.* **2010**, *11*, 799–805. [CrossRef] [PubMed]
96. Bauernfeind, F.; Rieger, A.; Schildberg, F.A.; Knolle, P.A.; Schmid-Burgk, J.L.; Hornung, V. NLRP3 inflammasome activity is negatively controlled by miR-223. *J. Immunol.* **2012**, *189*, 4175–4181. [CrossRef]
97. Ma, X.; Tian, D.; Lv, W.; Gao, B.; Ma, Z.; Zheng, X. Anti-inflammatory effects of microRNA-223 on sepsis-induced lung injury in rats by targeting the Toll-like receptor signaling pathway. *Exp. Ther. Med.* **2021**, *22*, 964. [CrossRef] [PubMed]
98. Peng, C.; Wang, H.; Zhang, W.J.; Jie, S.H.; Tong, Q.X.; Lu, M.J.; Yang, D.L. Inhibitory effect of miR-125b on hepatitis C virus core protein-induced TLR2/MyD88 signaling in THP-1 cells. *World J. Gastroenterol.* **2016**, *22*, 4354–4361. [CrossRef]
99. Primer-BLAST. Computational Tools for Online. Available online: https://www.ncbi.nlm.nih.gov/tools/primer-blast/ (accessed on 25 July 2024).
100. Beacon-Designer. Computationaly Tools for Online. Available online: http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1 (accessed on 25 July 2024).


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# Załącznik 2

# Oświadczenia współautorów publikacji naukowych wchodzących w skład rozprawy doktorskiej wraz z określeniem ich indywidualnego udziału

**Ewa Ostrycharz-Jasek**

**Rozprawa doktorska**

Ekspresja wybranych cząsteczek mikroRNA i ich genów docelowych

w zakażeniu *Lagovirus europaeus* – genotypami GI.1 i GI.2

Szczecin, 2.09.2024r.

dr hab. Beata Hukowska-Szematowicz, prof. US

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**Oświadczenie**

Oświadczam, że jestem współautorem prac wchodzących w skład rozprawy doktorskiej mgr Ewy Ostrycharz-Jasek:

**[P-1]** Ostrycharz E., Hukowska-Szematowicz B. (2022). Micro-players of great significance-host microRNA signature in viral infections in humans and animals. *International Journal of Molecular Science* 23 (18), 10536. DOI: 10.3390/ijms231810536.

Mój wkład w powstanie tej pracy polegał na współudziale w opracowaniu koncepcji i metodologii pracy, ocenie merytorycznej przeglądu literatury z zakresu omawianego tematu, współudziale w napisaniu oraz przygotowaniu manuskryptu, wysłaniu manuskryptu do redakcji oraz pełnienie roli autora korespondencyjnego, a także na pozyskiwaniu funduszy i nadzorze.

Mój udział procentowy szacuję na 35%.

**[P-2]** Ostrycharz E., Fitzner A., Kęsy A., Siennicka A., Hukowska-Szematowicz B. (2024). MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *Frontiers in Microbiology* 15:1349535. DOI: 10.3389/fmicb.2024.1349535.

Mój wkład w powstanie tej pracy polegał na zaplanowaniu i przygotowaniu eksperymentu, uzyskaniu zgody Lokalnej Komisji Etycznej ds. doświadczeń na zwierzętach, współudziale w przeprowadzeniu eksperymentu (główny prowadzący eksperyment) i pozyskaniu materiału biologicznego do badań, współudziale w opracowaniu koncepcji i metodologii badań, współudziale w walidacji metod, wykonaniu analiz in silico, współudziale w analizie i opracowaniu uzyskanych wyników badań, współudziale w napisaniu oraz przygotowaniu manuskryptu, wysłaniu manuskryptu do redakcji oraz pełnienie roli autora korespondencyjnego, a także na pozyskaniu funduszy i nadzorze.

Mój udział procentowy szacuję na 24%.

**[P-3]** Ostrycharz-Jasek E., Fitzner A., Siennicka A., Budkowska M., Hukowska-Szematowicz B. (2024). MicroRNAs regulate the expression of genes related to the innate immune and inflammatory response in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *International Journal of Molecular Science* 25 (17), 9531. DOI: 10.3390/ijms25179531.

Mój wkład w powstanie tej pracy polegał na zaplanowaniu i przygotowaniu eksperymentu, uzyskaniu zgody Lokalnej Komisji Etycznej ds. doświadczeń na zwierzętach, współudziale w przeprowadzeniu eksperymentu (główny prowadzący eksperyment) i pozyskaniu materiału biologicznego do badań, współudziale w opracowaniu koncepcji i metodologii badań, współudziale w walidacji metod, wykonaniu analiz in silico, współudziale w analizie i opracowaniu uzyskanych wyników badań, współudziale w napisaniu oraz przygotowaniu manuskryptu, wysłaniu manuskryptu do redakcji oraz pełnienie roli autora korespondencyjnego, a także na pozyskiwaniu funduszy oraz nadzorze.

Mój udział procentowy szacuję na 24%.

Podpis współautora

UNIWERSYTET SZCZECIŃSKI
Instytut Biologii
dr hab. Beata Hukowska-Szematowicz
prof. US

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dr hab. Beata Hukowska-Szematowicz, prof. US

Zduńska Wola, 2.09.2024r.

dr hab. Andrzej Fitzner, prof. PIWet-PIB
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Państwowy Instytut Weterynaryjny- Państwowy Instytut Badawczy
Zakład Pryszczycy PIWet-PIB w Zduńskiej Woli
Krajowe Laboratorium Referencyjne ds. krwotocznej choroby królików (RHD)
Ul. Wodna 7
98-220 Zduńska Wola

**Oświadczenie**

Oświadczam, że jestem współautorem prac wchodzących w skład rozprawy doktorskiej mgr Ewy Ostrycharz-Jasek:

**[P-2]** Ostrycharz E., Fitzner A., Kęsy A., Siennicka A., Hukowska-Szematowicz B. (2024). MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *Frontiers in Microbiology* 15:1349535. DOI: 10.3389/fmicb.2024.1349535.

Mój wkład w powstanie tej pracy polegał na przygotowaniu antygenów wirusowych do eksperymentu oraz współudziale w przygotowaniu pierwszej wersji manuskryptu.

Mój udział procentowy szacuję na 5%.

**[P-3]** Ostrycharz-Jasek E., Fitzner A., Siennicka A., Budkowska M., Hukowska-Szematowicz B. (2024). MicroRNAs regulate the expression of genes related to the innate immune and inflammatory response in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *International Journal of Molecular Science* 25 (17), 9531. DOI: 10.3390/ijms25179531.

Mój wkład w powstanie tej pracy polegał na przygotowaniu antygenów wirusowych do eksperymentu oraz współudziale w analizie wyników.

Mój udział procentowy szacuję na 5%.

Podpis współautora
dr hab. Andrzej Fitzner
Profesor Instytutu

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dr hab. Andrzej Fitzner, prof. PIWet-PIB

Zduńska Wola, 2.09.2024r.

dr Andrzej Kęsy
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Państwowy Instytut Weterynaryjny- Państwowy Instytut Badawczy
Zakład Pryszczycy PIWet-PIB w Zduńskiej Woli
Krajowe Laboratorium Referencyjne ds. krwotocznej choroby królików (RHD)
Ul. Wodna 7
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**Oświadczenie**

Oświadczam, że jestem współautorem pracy wchodzącej w skład rozprawy doktorskiej mgr Ewy Ostrycharz-Jasek:

**[P-2]** Ostrycharz E., Fitzner A., Kęsy A., Siennicka A., Hukowska-Szematowicz B. (2024). MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *Frontiers in Microbiology* 15:1349535. DOI: 10.3389/fmicb.2024.1349535.

Mój wkład w powstanie tej pracy polegał na współudziale w przygotowaniu pierwszej wersji manuskryptu.

Mój udział procentowy szacuję na 3%.

Podpis współautora

KIEROWNIK
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dr n. wet. Andrzej Kęsy

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dr Andrzej Kęsy

Szczecin, 12.09.2024r.

dr hab. n. med. Aldona Siennicka
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Wydział Farmacji, Biotechnologii Medycznej i Medycyny Laboratoryjnej
Pomorski Uniwersytet Medyczny w Szczecinie
Al. Powstańców Wielkopolskich 72
70-111 Szczecin

**Oświadczenie**

Oświadczam, że jestem współautorem prac wchodzących w skład rozprawy doktorskiej mgr Ewy Ostrycharz-Jasek:

**[P-2]** Ostrycharz E., Fitzner A., Kęsy A., Siennicka A., Hukowska-Szematowicz B. (2024). MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *Frontiers in Microbiology* 15:1349535. DOI: 10.3389/fmicb.2024.1349535.

Mój wkład w powstanie tej pracy polegał na współudziale w analizie wyników oraz współudziale w przygotowaniu pierwszej wersji manuskryptu.

Mój udział procentowy szacuję na 3%.

**[P-3]** Ostrycharz-Jasek E., Fitzner A., Siennicka A., Budkowska M., Hukowska-Szematowicz B. (2024). MicroRNAs regulate the expression of genes related to the innate immune and inflammatory response in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *International Journal of Molecular Science* 25 (17), 9531. DOI: 10.3390/ijms25179531.

Mój wkład w powstanie tej pracy polegał na współudziale w analizie wyników.

Mój udział procentowy szacuję na 3%.

Podpis współautora

KIEROWNIK
Zakładu Analityki Medycznej
dr hab. n. med. Aldona Siennicka

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dr hab. n. med. Aldona Siennicka

Szczecin, 12.09.2024r.

Dr n. med. Marta Budkowska
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Katedra Diagnostyki Laboratoryjnej
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Al. Powstańców Wielkopolskich 72
70-111 Szczecin

**Oświadczenie**

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**[P-3]** Ostrycharz-Jasek E., Fitzner A., Siennicka A., Budkowska M., Hukowska-Szematowicz B. (2024). MicroRNAs regulate the expression of genes related to the innate immune and inflammatory response in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *International Journal of Molecular Science* 25 (17), 9531. DOI: 10.3390/ijms25179531.

Mój wkład w powstanie tej pracy polegał na współudziale w analizie wyników.

Mój udział procentowy szacuję na 3%.

Podpis współautora
ADIUNKT
Zakładu Analityki Medycznej
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dr n. med. Marta Budkowska

# Załącznik 3

# Aktywność naukowa Doktoranta
# wraz z sumarycznym zestawieniem dorobku naukowego

**Ewa Ostrycharz-Jasek**

**Rozprawa doktorska**

Ekspresja wybranych cząsteczek mikroRNA i ich genów docelowych

w zakażeniu *Lagovirus europaeus* – genotypami GI.1 i GI.2

# Mgr Ewa Ostrycharz-Jasek

## 1. Edukacja

**11.2020 – 09.2024** Szkoła Doktorska Uniwersytetu Szczecińskiego

**10.2023 – 07.2024** Polsko – Japońska Akademia Technik Komputerowych w Warszawie, studia podyplomowe, bioinformatyka

**10.2017 – 06.2019** Pomorski Uniwersytet Medyczny w Szczecinie, studia magisterskie, biotechnologia, spec. biotechnologia medyczna

**10.2014 – 07.2017** Pomorski Uniwersytet Medyczny w Szczecinie, studia licencjackie, biotechnologia, spec. biotechnologia medyczna

## 2. Dorobek naukowy

## 2.1 Publikacje naukowe wchodzące w skład rozprawy doktorskiej

Praca przeglądowa

1) **Ostrycharz E.**, Hukowska-Szematowicz B. (2022). Micro-players of great significance-host microRNA signature in viral infections in humans and animals. *International Journal of Molecular Science* 23 (18), 10536. DOI: 10.3390/ijms231810536.

**IF- 5,6; pkt. MNiSW – 140**

Prace oryginalne

2) **Ostrycharz E.**, Fitzner A., Kęsy A., Siennicka A., Hukowska-Szematowicz B. (2024). MicroRNAs participate in the regulation of apoptosis and oxidative stress-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *Frontiers in Microbiology* 15:1349535. DOI: 10.3389/fmicb.2024.1349535.

**IF- 4,0; pkt. MNiSW – 100**

3) **Ostrycharz-Jasek E.**, Fitzner A., Siennicka A., Budkowska M., Hukowska-Szematowicz B. (2024). MicroRNAs regulate the expression of genes related to the innate immune and inflammatory response in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. *International Journal of Molecular Science* 25 (17), 9531. DOI: 10.3390/ijms25179531.

**IF- 4,9; pkt. MNiSW – 140**

## 2.2. Publikacje stanowiące pozostały dorobek naukowy

Prace przeglądowe

1) **Ostrycharz E.**, Hukowska-Szematowicz B. (2022). New insights into the role of the complement system in human viral diseases. *Biomolecules* 12 (2), 226. DOI: 10.3390/biom12020226.

**IF- 5,5; pkt. MNiSW – 100**

2) Roszkowska P., Klimczak E., **Ostrycharz E.**, Rączka A., Wojciechowska-Koszko I., Dybus A., Cheng Y. H., Yu Y. H., Mazgaj S., Hukowska-Szematowicz B. (2024). Small intestinal bacterial overgrowth (SIBO) and twelve groups of related diseases-current state of knowledge. *Biomedicines* 12 (5), 1030. DOI: 10.3390/biomedicines12051030.

**IF- 3,9; pkt. MNiSW – 100**

Prace oryginalne

3) Budkowska M., **Ostrycharz E.**, Wojtowicz A., Marcinowska Z., Woźniak J., Ratajczak M.Z., Dołęgowska B. (2018). A circadian rhythm in both complement cascade (ComC) activation and sphingosine-1-phosphate (S1P) levels in human peripheral blood supports a role for the ComC-S1P axis in circadian changes in the number of stem cells circulating in peripheral blood. *Stem Cell Reviews and Reports* 14: 677-685. DOI: 10.1007/s12015-018-9836-7.

**IF- 4,7; pkt. MNiSW – 100**

4) **Ostrycharz E.**, Wasik U., Kempińska-Podhorodecka A., Banales J.M., Milkiewicz P., Milkiewicz M. (2020). Melatonin protects cholangiocytes from oxidative stress-induced proapoptotic and proinflammatory stimuli via miR-132 and miR-34. *International Journal of Molecular Science* 21 (24), 9667. DOI: 10.3390/ijms21249667.

**IF- 5,9; pkt. MNiSW – 140**

5) Kempińska-Podhorodecka A., Adamowicz M., **Ostrycharz E.**, Chmielarz M., Wójcicki M., Milkiewicz P., Milkiewicz M. (2021). Role of miR-506 in ulcerative colitis associated with primary sclerosing cholangitis. *Scientific Reports* 11:10134. DOI: 10.1038/s41598-021-89631-4.

**IF- 4,4; pkt. MNiSW – 140**

6) **Ostrycharz E.**, Blatkiewicz M., Hukowska-Szematowicz B. (2021). Ekspresja głównego regulatora przeciwzapalnego IL-10 w przebiegu zakażenia wirusem krwotocznej choroby królików (RHDV – Rabbit Hemorrhagic Disease Virus). *Młodzi naukowcy 2.0. Tom 1*. Pod red. Korpysa J., Niedźwiedzka-Rystwej P. Wydawnictwo Fundacja Centrum Badań Socjologicznych, Szczecin, Polska, 2021: pp. 59-72. DOI: 10.14254/ 978-83-959336-8-4/2021.

**pkt. MNiSW – 20**

7) Hukowska-Szematowicz B., **Ostrycharz E.**, Dudzińska W., Roszkowska P., Siennicka A., Wojciechowska-Koszko I. (2023). Digital PCR (dPCR) quantification of miR-155-5p as a potential candidate for a tissue biomarker of inflammation in rabbits infected with *Lagovirus europaeus*/Rabbit Hemorrhagic Disease Virus (RHDV). *Viruses* 15 (7), 1578. DOI: 10.3390/v15071578.

**IF- 3,8; pkt. MNiSW – 100**

8) Budkowska M., **Ostrycharz E.**, Serwin N.M., Nazarewski Ł., Cecerska-Heryć E., Poręcka M., Rykowski P., Pietrzak R., Zieniewicz K., Siennicka A., Hukowska-Szematowicz B., Dołęgowska B. (2023). Biomarkers of the complement system activation (C3a, C5a, sC5b-9) in serum of patients before and after liver transplantation. *Biomedicines* 11(7), 2070. DOI: 10.3390/biomedicines11072070.

**IF- 3,9; pkt. MNiSW – 100**

**Łączny IF – 46,6**

**Łączna liczba punktów MNiSW – 1180 pkt**

**H-index – 5**

## 2.3. Doniesienia konferencyjne

1) **Ostrycharz E.**, Blatkiewicz M., Hukowska-Szematowicz B. (2021). Diffrent expression patterns of transforming growth factor-β (TGF-β) during Rabbit Hemorrhagic Disease Virus (RHDV) infection. 8th Summer School of Immunology 2021 (Grecja, wydarzenie online, 24-27.05.2021).

2) **Ostrycharz E.**, Blatkiewicz M., Hukowska-Szematowicz B. (2021). MikroRNA (miRNA) jako nowe biomarkery w ochronie zdrowia ludzi i zwierząt. Konferencja środowisko, a zdrowie i dobrostan ludzi i zwierząt 2021 (Szczecin, wydarzenie online, 11.06.2021).
3) **Ostrycharz E.**, Budkowska M., Hukowska-Szematowicz B. (2021). Wpływ czynnika wzrostu hepatocytów (HGF) oraz czynnika wzrostu śródbłonka naczyniowego (VEGF) na proces przeszczepu wątroby. Konferencja środowisko, a zdrowie i dobrostan ludzi i zwierząt 2021 (Szczecin, wydarzenie online, 11.06.2021).
4) **Ostrycharz E.**, Blatkiewicz M., Hukowska-Szematowicz B. (2021). Ekspresja głównego regulatora przeciwzapalnego IL-10 w przebiegu zakażenia wirusem krwotocznej choroby królików (RHDV – Rabbit Hemorrhagic Disease Virus). I Międzynarodowa Multidyscyplinarna Konferencja Doktorantów Uniwersytetu Szczecińskiego (MKDUS 2.0) 2021 (Szczecin, wydarzenie online, 23-25.06.2021).
5) **Ostrycharz E.**, Blatkiewicz M., Hukowska-Szematowicz B. (2021). The anti-inflammatory regulator IL-10 in the course of Rabbit Hemorrhagic Disease Virus (RHDV) infection. 6th European Congress of Immunology 2021 (Belgrad, Serbia, wydarzenie online, 1-4.09.2021).
6) **Ostrycharz E.**, Blatkiewicz M., Hukowska-Szematowicz B. (2022). Ekspresja genów przeciwzapalnych i mikroRNA w tkankach królików zakażonych *Lagovirus europaeus* GI.1/RHDV (Rabbit Hemorrhagic Disease Virus). II Międzynarodowa Multidyscyplinarna Konferencja Doktorantów Uniwersytetu Szczecińskiego (MKDUS 2.0) 2022 (Szczecin, wydarzenie online, 22-24.06.2022).
7) Hukowska-Szematowicz B., **Ostrycharz E.**, Szumna M., Blatkiewicz M. (2022). Study of microRNA and anti-inflammatory genes in tissues of rabbit infected with *Lagovirus europaeus* GI.1/RHDV (Rabbit Hemorrhagic Disease Virus). Viruses 2022: At the Leading Edge of Virology Research (wydarzenie online, 5-8.04.2022).
8) **Ostrycharz E**., Fitzner A., Kęsy A., Siennicka A., Hukowska-Szematowicz B. (2024). MicroRNAs participate in the regulation of apoptosis-related gene expression in rabbits infected with *Lagovirus europaeus* GI.1 and GI.2 genotypes. World Congress on Virology and Infectious Diseases 2024 (Praga, Czechy, 24-25.06.2024).

## 3. Udział w projektach badawczych

1) 1.01.2020 – 31.12.2020: Wykonawca projektu „Ocena ekspresji i roli PPAR-α w dyslipidemiach indukowanych przewlekłą cholestazą". Regionalna Inicjatywa Doskonałości nr PUM-002/RID/2018/19. Kierownik projektu: prof. dr hab. n. med. Małgorzata Milkiewicz.

## 4. Szkolenia i warsztaty

1) 15.02.2021 – 3.03.2021: szkolenie w zakresie: szkolenia dla osób odpowiedzialnych za planowanie procedur i doświadczeń na zwierzętach oraz za ich prowadzenie; szkolenia dla osób wykonujących procedury; szkolenia dla osób uśmiercających zwierzęta wykorzystywane w procedurach-prowadzone przez Polskie Towarzystwo Nauk o Zwierzętach Laboratoryjnych PolLASA (wydarzenie on-line).
2) 15-17.10.2021: Warsztaty z pisania artykułów naukowych w języku angielskim *Academic Writing in English Made Painless* prowadzone przez Centrum Studiów Amerykańskich (wydarzenie on-line).
3) 16-19.11.2021: Szkolenie w zakresie obsługi aparatu do dPCR (digital PCR) QlAcuity One 5plex wraz z przygotowaniem reakcji i analizą wyników, Qiagen. Szczecin, Instytut Biologii US, Laboratorium Biologii Infekcyjnej i Molekularnej oraz Immunologii.
4) 6.09.2022: Szkolenie w zakresie obsługi i eksploatacji aparatu hematologicznego BC30 VET. Szczecin, Instytut Biologii US, Laboratorium Biologii Infekcyjnej i Molekularnej oraz Immunologii.
5) 19-20.10.2023: Szkolenie w zakresie obsługi aparatu ddPCR, Bio-Rad. Szczecin, Instytut Biologii US.

## 5. Praktyki i staże

1) 1-30.07.2021: Staż w Zakładzie Biologii Medycznej Pomorskiego Uniwersytetu Medycznego w Szczecinie z zakresu technik Real-Time PCR, Western Blot, metod ELISA i BCA oraz pracy z hodowlami komórkowymi.
2) 16-24.05.2022: Staż w Zakładzie Badawczo-Wdrożeniowym Ośrodka Salmonella Immunolab w Gdańsku z zakresu technik immunologicznych, mikrobiologicznych z modułem obliczeń laboratoryjnych oraz biologii molekularnej.

## 6. Inne

1) 15.06.2021: Udział w sympozjum naukowym „Szczepienia przeciw COVID-19 – bezpieczeństwo, skuteczność i znaki zapytania" organizowanym przez EUROIMMUN, (wydarzenie on-line).
2) 20.04.2022: Udział w spotkaniu naukowym „Cytometria przepływowa wczoraj, dziś, jutro…" organizowanym przez Polskie Towarzystwo Cytometrii oraz Becton Dickinson Polska, Szczecin.
3) 28.01.2022: Udział w sympozjum „COVID-19 – raport z oblężonego świata" organizowanym przez Polskie Towarzystwo Mikrobiologów (wydarzenie on-line).
4) 31.03.2023: Udział w konferencji naukowej „Od homeostazy do sepsy – układ immunologiczny, a mikroorganizmy" prowadzonej przez Polskie Towarzystwo Immunologii Doświadczalnej i Klinicznej, oddział Szczecin, IB US, Szczecin.
5) 9.03.2024: Udział w konferencji „Mikrobiota jest kobietą" organizowanej przez Fundację MediSzczecin oraz Sanprobi, Szczecin.
6) 14.06.2024: Udział w konferencji naukowej „Szczecińskie Spotkania z Mikrobiologią – Teoria i Praktyka" organizowanej przez Polskie Towarzystwo Immunologii Doświadczalnej i Klinicznej oraz Polskie Towarzystwo Mikrobiologów, ZUT, Szczecin.