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REVIEW

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The critical role of miRNAs in modulating PRRSV infection in swine: a review

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is a severe threat to pig farming worldwide and contributes to substantial financial losses. Endogenous short RNAs, known as microRNAs (miRNAs), play various roles in controlling viral infections in both human and animals through virus–host interactions, mediating immune-related gene responses in target cells despite their unknown precise roles in infectious illnesses. Thus, a comprehensive literature search was conducted in EMBASE, PubMed and Web of Science databases to compile this review, focusing on the function, role, and involvement of miRNAs in porcine reproductive and respiratory syndrome virus (PRRSV) infection. In addition to possible exogenous miRNAs such as miR2911 and miR168, our study provides strong evidence demonstrating the important effects of endogenous miRNAs such as miR-181, miR-26, and miR-145 on PRRSV infection, which were also anticipated to bind to certain locations within PRRSV genomes. Our study highlights that miRNAs can serve as a treatment strategy of PRRS and further research is needed to validate their clinical safety and efficacy.

Keywords MiRNAs, PRRS, Virus, Therapeutic, Immunity

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV), characterized as a single-stranded (+) RNA virus, is associated with global swine health issues. PRRSV is a member of the family *Arteriviridae* and genus *Betaarterivirus* originating from North American genotype 2, VR-2332 prototype, and European genotype 1, Lelystad prototype, which comprises at least 9 open reading frames (ORFs) of approximately 15 kb in length. Although there are similar clinical symptoms between these two genotypes, they only share approximately 60% nucleotide similarity (Han et al. 2019). A PRRSV strain was first reported and isolated in 1995 and identified as genotype type 2 in China (Tong et al. 2007), which was the main cause of high fever disease in pigs with high mortality across China (Tong et al. 2007). PRRS can cause enormous economic burdens in the pig industry due to reproductive dysfunction in sows and respiratory syndrome throughout the growth period whose clinical symptoms differ noticeably between herds, depending on the virulence of different strains. As the major cause of PRRS, PRRSV primarily targets and infects alveolar macrophages in the lungs, which play crucial roles in the immune response. The virus uses receptors such as CD163 and heparan sulfate to enter these cells. Once

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inside, PRRSV hijacks the host cellular machinery to replicate, causing cell damage and apoptosis. Cunningly, several strategies were developed by PRRSV to evade the host immune system, inhibiting the production of interferons and key antiviral cytokines and modulating the expression of various immune-related genes to suppress the innate immune response. Additionally, PRRSV induces immune suppression and reduces the effectiveness of both humoral and cellular immune responses, allowing the virus to persist in the host for extended periods (Peng et al. 2023).

miRNAs are small noncoding RNA molecules comprising approximately 22 nucleotides and can bind to mRNA target genes, regulating posttranscriptional gene expression via base complementarity. According to the origin, miRNAs can be divided into two types: exogenous and endogenous molecules. Plants in the diet have been shown to be a vital source of endogenous miRNAs that can be absorbed from the gastrointestinal tract to regulate host gene expression. In regulating host gene expression to resist viruses during viral infection, miRNAs can reach target organs through the bloodstream after absorption. Moreover, in the study of the biological activity of exogenous miRNAs, miRNAs from dietary sources have attracted much attention because of their availability and stability (Jia et al. 2021). And exogenous miRNAs derived from dietary sources are extremely prominent because they are stable, available, and bioactive following cross-kingdom absorption. Exogenous miRNAs participate in multiple gene expression and regulation processes. For example, the anti-inflammatory properties of curcumin are linked to the downregulation of microRNA-155 in the macrophages of mice (Al-Moghrabi et al. 2023).

Although the mechanisms of interaction between miRNAs (exogenous or endogenous RNAs) and viruses in the host are not fully understood, they have been reported to regulate viral replication in host cells. miRNAs are small, noncoding RNAs that influence gene expression through binding to complementary sequences on target mRNAs, leading to their degradation or inhibition of translation. This regulatory function of miRNAs can significantly impact viral replication through various pathways (Zhdanov 2022).

Direct targeting of viral mRNAs: Some miRNAs can directly bind to viral mRNAs, leading to their degradation or modulating translational repression. This can inhibit viral protein synthesis and reduce the replication efficiency of the virus. For example, cellular miRNAs can target hepatitis C virus (HCV) RNA to suppress its replication (Jopling 2008).

Modulation of host immune responses: miRNAs can regulate the expression of host genes involved in immune responses. For example, miRNAs can downregulate the expression of interferons and other cytokines that are crucial for antiviral defense, thereby facilitating viral replication and persistence. Also, miR-146a can modulate the host inflammatory response during viral infections (Pu et al. 2017).

Alteration of the host cell Environment: miRNAs can modulate the expression of genes that control cellular pathways and environments, such as those related to apoptosis, cell cycle progression and metabolic processes. By altering these pathways, miRNAs can create a more favorable environment for viral replication. An example is miR-21, which promotes cell survival during viral infection by targeting proapoptotic genes (Buscaglia and Li 2011).

Interaction with host antiviral proteins: miRNAs can regulate the expression of host antiviral proteins, such as those involved in the RNA interference (RNAi) pathway. By suppressing these proteins, miRNAs can decrease the host's ability to combat viral infections effectively. For example, miR-24 can suppress the expression of p53, a key protein in antiviral defense (Zhang et al. 2016). Scheel and Trobaugh reported that viral genome translation and replication could be triggered by the downregulation of endogenous miRNAs (Scheel et al. 2016; Trobaugh and Klimstra 2017). For instance, the wingless related-integration site (WNT), interferon (IFN), mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt and NOTCH pathways can be activated by miRNAs that regulate mRNA translation and contribute to the cellular responses during virus infection and inflammatory responses.

In host cells, endogenous miRNAs can attach to the RNA viral genome and prevent the viral genome from being translated, or change the amount of free miRNAs in the cell. By evading immune responses, enhancing viral replication, or even altering miRNA-mediated host gene regulation, miRNAs contribute to the pathogenesis of viruses (Diggins and Hancock 2023). Through direct binding to the RNA virus genome or virus-mediated alterations in the host transcriptome, miRNAs also have the abilities to affect RNA virus replication and pathogenesis (Wickramage et al. 2023). As a result, exogenous miRNAs have been employed thus far to prevent the growth of numerous viruses. Although exogenous miRNAs have numerous benefits, permanently silencing target genes might be challenging because of the rapid mutation of viruses. The difference of the binding sites of miRNAs in the viral genome affects the function of miRNAs. Therefore, in the study of exogenous miRNA-targeted therapy for PRRS, it is necessary to focus on miRNAs that bind to highly-conserved regions of the viral genome.

Advancements in understanding the regulatory function of miRNAs in PRRSV infection and their potential targets for treatment are crucial, as PRRSV is responsible for the deadly swine disease, causing significant economic losses in the global pig industry (Meulenberg 2000). Consequently, the related research on the regulatory function of miRNAs in PRRSV infection has intensified in recent years, with the aim of developing novel treatment approaches and strategies for this disease. In particular, miRNAs such as miR-181, miR-26, and miR-145 have garnered attention because of their special roles in PRRSV infection (Ostrycharz and Hukowska-Szematowicz 2022).

In this review, we explored the roles of various miRNAs in PRRSV replication, pathogenesis and regulation of antiviral responses in swine. The core objective of most related studies in the current review was to summarize biomarkers that could be associated with miRNAs to improve PRRS disease.

The endogenous role of miRNAs in PRRSV infection

As described in Table 1, 41 studies investigated the effects of miRNAs in PRRSV infections via different methodologies. Among these studies, miR-181 was mentioned 4 times (Table 1). These findings were divided into different groups according to the main contents. G1 is the key host miRNAs targeting the PRRSV genome. G2 is the expression of porcine miRNAs in PRRSV infection, and G3 represents miRNA host and their mRNA gene targets. A summary of these miRNAs and their corresponding target mRNAs involved in PRRSV infection is shown in Table 1.

On the basis of these studies, miR-181 appears to play a main role in PRRSV prevention by targeting the PRRSV receptor CD163 to suppress PRRSV replication and affect the growth of pigs. The miR-26 family has also been reported many times, and miR-26a suppresses PRRSV replication by upregulating type I interferons. Additionally, miR-145 and miR-30 have been reported as key host miRNAs that target the PRRSV genome. The characteristics and mechanisms of the above miRNAs are described in this review.

miR-181

Various miRNAs are involved in virus infection, including various RNA and DNA viruses in humans and animals (Fig. 1) (Yoshikawa et al. 2024). miR-181 is an astounding antiviral agent RNA that has been proved to target CD163 and SRP14 dually after PRRSV infection (Guo et al. 2013). miR-181 was reported to prevent PRRSV replication by targeting PRRSV genomic RNA whose binding site was located at the junction of approximately 96% of the highly conserved regions at the bottom of Open Reading Framework 4 (ORF4). Moreover, miR-181 can also prevent PRRSV infection by degrading CD163, which is the PRRSV receptor in agranulocytes and PAMs. Previous publications have shown that activating miR-181 can reduce the viral load and fever symptoms caused by PRRSV infection (Guo et al. 2013). Porcine macrophage sequencing further revealed that miR-181a and miR-181b inhibit PRRSV infection by targeting CD163 (Zhen et al. 2018). These studies indicate that the miR-181 exosome may be a potential activator of PRRSV infection.

Target gene scanning revealed that miR-181 had excellent complementarity to Ddx3x, Nfat5, Foxp1, and Mpp5, which have low caloric value, in various pig cells. Interestingly, miR-181 could also bind to PRRSV structures such as the ORF1a polyprotein, which contains 4 binding sites; and ORF2 and ORF4, which contains one binding site, according to the RNAhybrid software (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid) in Table 2. These results showed that miR-181 might directly affect the replication of PRRSV with a low minimum free energy. Therefore, miR-181 could be a potential therapeutic target for PRRSV replication through the regulation of cellular immunity and viral replication.

miR-26

The miR-26 family comprises miR-26-1, miR-26-2 and miR-26b, which inhibit the mRNA expression of the carboxyl-terminal domain RNA polymerase II polypeptide A small phosphatase family (CTDSP) that includes CTDSP1, CTDSP2 and CTDSPL. All of these features can downregulate RNA polymerase II (RNAPII) to impede neural genome transcription (Li et al. 2015b). The regulatory mechanism of miR-26 against inflammation is shown in Fig. 2 (Chen et al. 2016). Previous reports have shown that miR-26 has inhibitory effects on carcinogenesis and tumor progression and it can inhibit tumor proliferation and progression by inducing cell apoptosis even at low expression levels. Jia reported that miR-26a can markedly enhance antiviral ability, including activating the type I IFN signaling pathway (Jia et al. 2015). Moreover, miR-26 can target IL-6 and NF-κB to reduce inflammation and tumorigenicity. Similarly, miR-26 can also bind to the PRRSV genome, including GP4, nsp12, GP2 and nsp3, with a low minimum free energy (Table 2). These results demonstrated that the miR-26 family might be potential therapeutic targets for PRRSV infection.

Table 1 Mechanisms of action of miRNAs against PRRSV

Articles No	Aims	miRNA	Pathways	References
1	PRRSV 3'UTR	miRNA let-7 family	NEAT1 and ARID3A/NF-κB	You et al. 2022
2	LC3B	miR-204	LC3B-mediated autophagy	Yao et al. 2023
3	FAM134B	miR-142-5P	ER-phagy	Guan et al. 2022
4	TRIM22	miR-376b-3p	lysosomal pathway	Chen et al. 2022
5	Rac1	miR-142-3p	downregulating Rac1 expression	Yao et al. 2022
6	IRF 7	miR-541-3p	negatively regulate the transcription of type I interferon	Shi et al. 2022
7	IRF 1	miR296-3p	IRF1/TNF-α	Zhang et al. 2021b
8	IFNs	miR218	↓miR218 → ↓ IFNs → ↑PRRSV	Zhang et al. 2021a
9	CD163 receptor	miR181	↑miR181 → ↓CD163	Kang et al. 2021
10	IFNs	miR218	↓miR218 → ↓ IFNs	Zhang et al. 2021a
11	SPARC/CLIC1/cofilin-1/COX7A2	miR-27a-5p and 21-3p	↑miR-27a-5p and 21-3p → ↑PRRSV → ↓SPARC/CLIC1/cofilin-1/COX7A2	Liang et al. 2020
12	IFNs	miR-382-5p	↑miR-382-5p → ↑PRRSV → ↓IFNs	Chang et al. 2020
13	The host RXRB	miR-c89	↑ miR-c89 → ↓ RXRB → ↓ PRRSV	Zhang et al. 2019b
14	THBS/SLC3A1	miR-339-5p, miR-181d-5p	↑miR-339-5p → ↓THBS1 → ↓PRRSV ↑miR-181d-5p → ↓SLC3A1 → ↓PRRSV	Zhang et al. 2019a
15	PI3K/AKT pathway	miR-27b	↑ miR-27b → ↓ PI3K/AKT	Wu et al. 2019
16	PI3K/AKT	miR-27b	↑miR-27b → ↓ PI3K/AKT → ↓PRRSV	Wu et al. 2019
17	CD151	miR-199a-3p	↑miR-199a-3p → ↓ CD151	Zhen et al. 2018
18	IFN	miR-132-3p/miR-335-5p/miR-7-5p	↑miR-132-3p/miR-335-5p/miR-7-5p → ↓ IFN	Dhorne-Pollet et al. 2019
19	IFNAR2	miR-30c	↑miR-30 → ↓IFNAR2 → ↓PRRSV	Liu et al. 2018
20	The host miRNAs	miR-140-3p	↑miR-140-3p → ↓PRRSV	Li et al. 2018
21	As a biomarker	miR-24-3P	↓miR-24-3P → ↓PRRSV	Le and Seo 2018
22	IFN-1, MX1 and ISG15	miR-26, miR-30d	↑miR-26 → ↓ IFN-1/MX1/ISG1 -↓PRRSV ↑miR-26 → ↓ autophagy/apoptosis -↓PRRSV	Calcatera et al. 2018
23	SRP 14	miR-10a-5p	↑miR-10a-5p → ↓SRP14 -↓PRRSV	Zhao et al. 2017
24	IFN-β	miR-373	↑miR-373 → ↓IFN-β -↓PRRSV	Chen et al. 2017
25	AKT3	miR-29	↑miR-29 → ↓ AKT3 → ↑ PRRSV	Zhou et al. 2016
26	IFNβ	miR-26a/34a/145	↑miR-26a/34a/145 → ↓IFN-β-↓PRRSV	Wang et al. 2018
27	ORF5 of PRRSV	amirGP5-370	↑amirGP5-370 → ↓ORF5-↓PRRSV	Xiao et al. 2011
28	TLR4/MyD88/NF-κB	miR-30d-R_1	↑miR-30d-R_1 → ↓ TLR4/MyD88/NF-κB-↓PRRSV	Wang et al. 2016
29	The NSP2 sequence	vsRNA1	↑ vsRNA1 → ↓ NSP	Li et al. 2016b
30	MYH9	miR-let-7f-5p	↑ miR-let-7f-5p → ↓ MYH9-↓PRRSV	Li et al. 2016a
31	PRRSV 3'UTR	amiRNA	↑amiRNA → ↓PRRSV UTR	Zhu et al. 2015
32	The host factor HO-1	miR-22	↑miR-22 → ↓HO-1 → ↑PRRSV	Xiao et al. 2016
33	IFN-I	miR-26a	↑miR26a → ↓IFN-I → ↓PRRSV	Li et al. 2015b
34	PRRSV 5'UTR	miR130b	↑miR130b → PRRSV 5'UTR	Li et al. 2015a
35	IFN	miR26a	↑miR130b → ↑IFN	Jia et al. 2015
36	CD163	artificial miRNAs	↑ artificial miRNAs → ↑ CD163 → ↓PRRSV	Zhu et al. 2014
37	TLRS and IFN	miR-145 and 127	↑miR-145 → ↓TLRS and IFN → ↑PRRSV ↓miR-127 → ↓MARPK4/Bcl6/CD64 → ↑PRRSV	Zhou et al. 2014
38	IFN	miR-23	↑miR-23 → ↓PRRSV → ↑ IFN	Zhang et al. 2014
39	CD151	miR-506	↑ miR-506 → ↓CD151 → ↓PRRSV	Wu et al. 2014
40	Associated with H- and N-PRRSV	miR-125a	miR-125 were high expressed in PAMS during H- and N-PRRSV infection	Cong et al. 2014

Table 1 (continued)

Articles No	Aims	miRNA	Pathways	References
41	PRRSV UTR	amiR5UTR, amiR3UTR	↑amiR5/3UTR → ↓PRRSV UTR	Xia et al. 2013
42	NF-κB	miR-125b	↑ miR-125b → ↓ NF-κB -↓PRRSV	Wang et al. 2013
43	PRRSV UTR	miR-147	↑ miR-147 → ↑PRRSV	J et al. 2013
44	SRP14	miR-181	↑ miR-181 → ↓SRP14 -↓PRRSV	Guo et al. 2013
45	CD163	miR-181	↑ miR-181 → ↓CD163 -↓PRRSV	Gao et al. 2013
46	Heme Oxygenase-1	miR-24-3p	↑ miR-24-3p → ↓HO-1 -↑ PRRSV	Xiao et al. 2015

miR-145

The high expression of miR-145 during PRRSV infection suggests that it may play a role in promoting infection. MiR-145 has been shown to be effective as an antiviral host factor against human tumor virus (HPV), and it can also suppress the growth of tumor cells by inducing apoptosis and controlling the cell cycle (Ostrycharz and Hukowska-Szematowicz 2022). Specifically, miR-145 plays a major proinflammatory role in allergic inflammation mediated by Th2 cells (Wang et al. 2018). According to recent studies, miR-145 levels are dramatically downregulated, accompanied by the activation of TLR and RIG-I innate signaling. Furthermore, PRRSV infection can disrupt the RIG-I signaling pathway by inducing IPS-1 junction inactivation, which reduces the level of IFN-β. In PRRSV-infected cells, the TLR3 signaling pathway is suppressed (Okoye et al. 2021). Furthermore, miR-145 directly targets the epigenetic IL-10 gene silencer HDAC11, which can increase the expression of IL-10 in TLR-triggered macrophages. To be more precise, IL-10 is a regulatory cytokine that is crucial in viral infections and is known to block the production of many proinflammatory cytokines, including TNF-α and IL-1. Simultaneously, a number of investigations have demonstrated that PRRSV infection can dramatically increase the production of IL-10 in a variety of cell lines at both the mRNA and protein levels, depending on a number of immune-related signaling pathways (Gambardella et al. 2023). Furthermore, miR-145 can target immune response regulators TRAM1, IFNGR2, IKBKB, and MAP2K1. These findings suggest that miR-145-mediated gene regulation may be a crucial component of the host response to PRRSV infection, as evidenced by its upregulation in PRRSV-infected MARC-145 cells (Heawchaiyaphum et al. 2021).

The role of exogenous miRNAs

Research has recently demonstrated that certain exogenous miRNAs can be identified by a wide variety of receptors in host eukaryotic cells and these tiny non-coding RNAs can be transferred across species to cause transboundary signal interference. This type of cross-species communication may mediate symbiotic or harmful connections between different organisms, just like bacteria and hosts do. Exogenous miRNAs can be selectively packed into microvesicles and actively transported to destination cells after they enter the host body. Exogenous plant miRNAs can be digested, according to previous studies, and can subsequently be found in the blood, organs, and tissues of experimental animals (Del Pozo-Acebo et al. 2021). Furthermore, breast milk also contain certain plant-derived miRNAs that can enter the circulation and control the production of endogenous mRNAs after being absorbed by gastrointestinal cells through SITT1. Plant miRNAs have also been found in the blood, gastrointestinal tract, and organs following the direct addition of plant RNA to food particles. For example, broccoli-derived miRNAs are resistant to typical food processing and digestive conditions (Zhou et al. 2020).

Exogenous miRNAs can regulate multiple pathways, such as blocking GSK-3β-mediated NF-κB and altering the TGF-β1 (growth factor β1) pathway (Zhou et al. 2020). Exogenous miRNAs produced from food have the capacity to enter the mammalian circulatory system and accumulate in several organs. Once inside organs, these plant miRNAs function similarly to endogenous miRNAs in regulating target genes, which can affect related physiological processes. Honeysuckle-derived miR2911 has antiviral efficacy against SARS-CoV-2 and influenza A viruses (H1N1, H5N1, and H7N9) in both cells and animals (Zhou et al. 2020). Because of its high GC content in plant 26S ribosomal RNA, miR2911 is stable even after decoctions and oral administration. miR168a and miR2911 were detected in honeysuckle whereas miR168a and miR156 were not detected in cooked rice or mouse serum. On the other hand, mature miR168a and miR156 were present at very high levels. These findings implied that the stomach can absorb only mature miRNAs and miRNAs derived from ribosome fragments may be more stable and plentiful after frying. Besides, strong therapeutic effects have been demonstrated by miRNAs. For example, miR159 suppresses breast cancer by targeting the TCF7 gene and miR156a, miR168, and miR168a

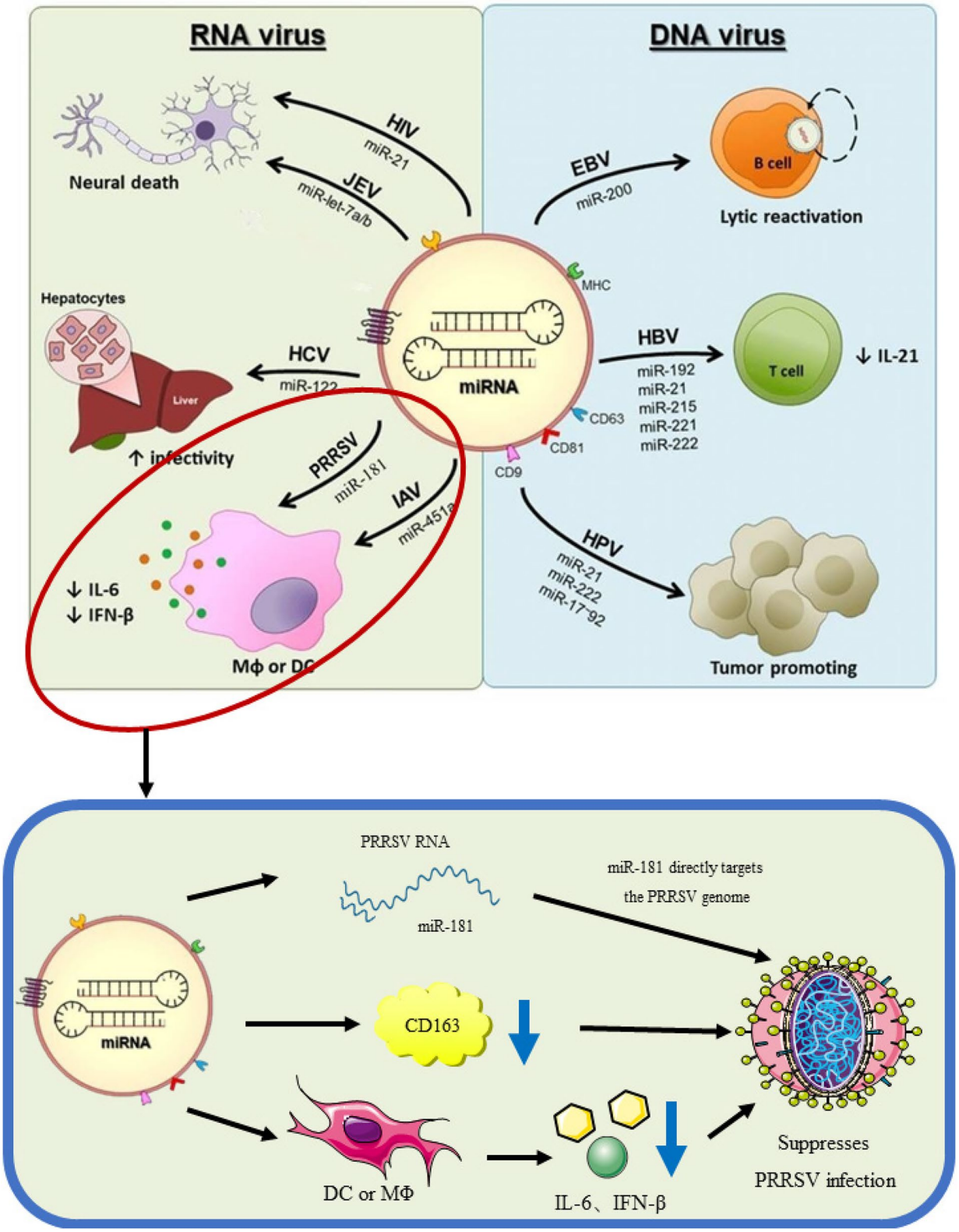
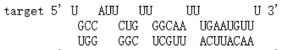
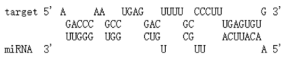
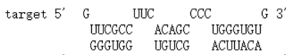
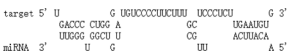
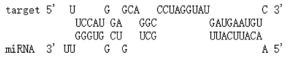
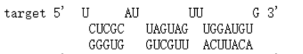
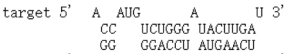
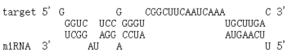
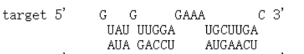
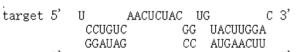
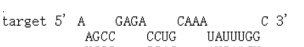
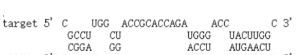
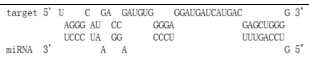
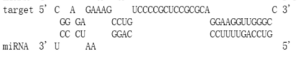
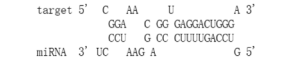
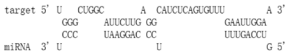
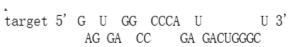
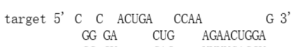


Fig. 1 Various miRNAs involved in virus infection including RNA and DNA viruses in humans and animals. Copyright © 2019 by the Fabio Seiti Yamada Yoshikawa, Franciane Mouradian Emidio Teixeira, Maria Notomi Sato, and Luanda Mara da Silva Oliveira. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

Table 2 Prediction of target genes of miRNAs in PRRSV infection

miRNA	Position	Target	Function	Binding structure	Minimum free energy (kcal/mol)
miR-181	13756	GP4	ORF4		-27.1
	688	nsp1	Comprises papain-like cysteine proteinase domains 1a (PCP1a) and 1b (PCP1b)		-25.2
	906	nsp1	Comprises papain-like cysteine proteinase domains 1a (PCP1a) and 1b (PCP1b)		-24.8
	4009	nsp2	CP2 which mediates cleft at the C-terminus		-24.3
	1721	nsp2	CP2 which mediates cleft at the C-terminus		-22.0
	7575	nsp9	RNA-dependent RNA polymerase		-24.2
	12481	E protein	An ion-channel protein in the unfolding procedure during virus entry and penetration		-22.9
miR-26	187	nsp1	Comprises papain-like cysteine proteinase domains 1a (PCP1a) and 1b (PCP1b)		-23.2
	5756	nsp4	3C-like serine proteinase		-22.1
	13786	GP4	ORF4		-20.9
	11663	nsp12	PRRSgp1		-20.7
	12536	GP2	ORF2, GP2 glycosylated envelope protein		-20.5
	5508	nsp3	Hydrophobic protein		-20.4
miR-145	12925	ORF3	GP3 envelope protein		-26.2
	2681	ORF1a	ORF1a polyprotein		-24.5
	11930	ORF1b	ORF1b polyprotein		-23.5
	15261	ORF7 3'UTR	Nucleocapsid protein N		-22.9
	1571	nsp2	CP2 which mediates cleft at the C-terminus.		-22.4
	3939	nsp2	CP2 which mediates cleft at the C-terminus.		-22.3
	1179	nsp2	CP2 which mediates cleft at the C-terminus.		-21.4
	7611	ORF1a	ORF1a polyprotein		-21.0

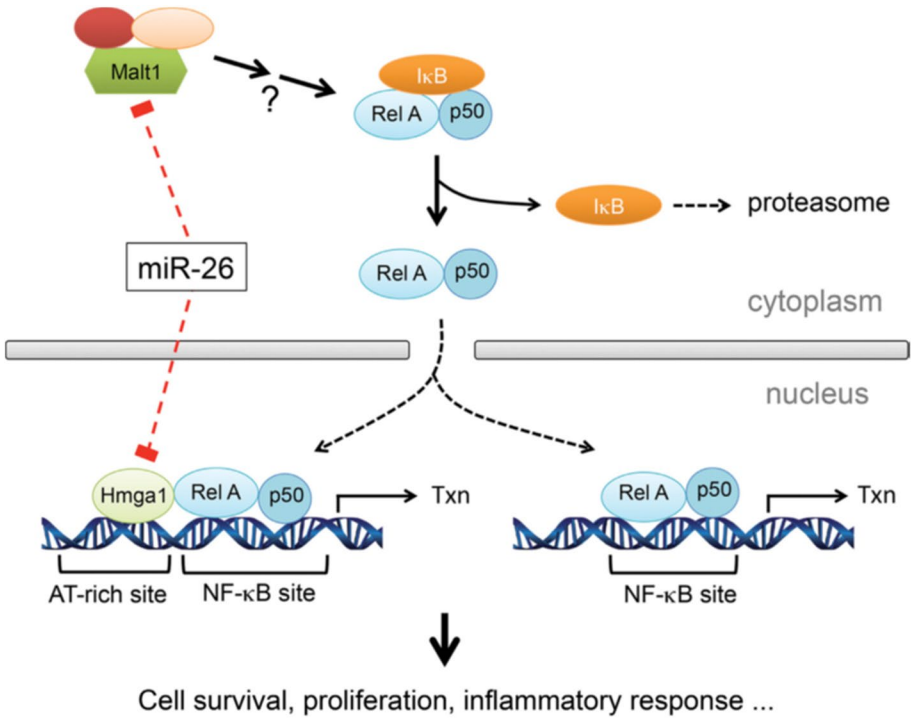


Fig. 2 The regulatory mechanism of miR-26 against inflammation. Copyright © The Chyi-Ying A Chen, Jeffrey T Chang, Yi-Fang Ho, and Ann-Bin Shyu, 2016. Published by Oxford University Press on behalf of Nucleic Acids Research. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc/4.0/)

activate low-density lipoprotein receptor adaptor protein 1 (LDLRAP1). Therefore, these characteristics decrease cytokine-induced monocyte adhesion and serve as molecules that protect blood vessels. As for the antiviral function, miR2911 exhibited high potential antiviral activity by binding to multiple targets in PRRSV, including GP4, nsp2, and nsp12 (Table 3).

High-temperature frying does not affect highly conserved miR-2911, which is found in large quantities in honeysuckle. After cooking, the majority of the miRNAs enriched in honeysuckle soup, including miR-166a, miR-2914, and miR-156, were quickly downregulated, but the expression of miR-2911 remained stable. The unusual miRNA ribosomal RNA (rRNA) of miR-2911 has a high GC content, which could be a factor in its remarkable stability (Fig. 3) (Del Pozo-Acebo et al. 2021). These findings imply that rRNA-derived herbal miRNAs with high GC concentrations might be important in TCM. More investigations are needed to fully utilize this potential.

According to a study in mouse, miR-2911 can be found in a variety of organs and can enter mice's gastrointestinal tract, make its way to the bloodstream, and then enter the lungs. Afterwards, dietary miRNAs, such as miR-2911, are taken up by the gastric fossa cell membrane through the action of SID1 transmembrane family member 1, which facilitates their absorption. This process has antiviral, anti-tumor, and anti-inflammatory effects on the cell. Similarly, the H1N1 virus replication can be completely and directly inhibited by miR-2911. miR-2911 also has the ability to suppress H5N1 and H7N9 viral replication in cells both in vitro and in vivo, indicating that it may be a special natural product that successfully stops the spread of viral infections. Moreover, it has the ability to specifically target the VP1 gene to inhibit enterovirus type 71, as well as the IE62 gene to directly block varicella-zoster virus replication and it induces TGF-β1 to stop tumor growth.

Conclusion and future prospects

This study describes multiple mechanisms mediating the interaction of host-derived miRNAs in PRRSV infection and predicts new target binding sites on the basis of the computational prediction analysis presented in Table 2. This review comprehensively discusses the interplay between host and viral miRNAs and their active contributions to the response to PRRSV infection. However,

Table 3 Prediction of target genes of miR2911 in PRRSV infection

Position	Target	Function	Binding structure	Minimum free energy (kcal/mol)
14122	GP4	ORF4	target 5' A G G G 3' C CGGUCUGU CCCUGGCU G GUCAGGCA GGGGCCGG miRNA 3' A G G 5'	-37.4
1882	nsp2	CP2 which mediates cleft at the C-terminus.	target 5' A G 3' UCCCAG CCG CUCUGGCC AGGGUC GGC GGGGCCGG miRNA 3' A AG 5'	-34.8
5048	nsp4	3C-like serine proteinase	target 5' A UUUG A 3' CCCG CCGU CCCUGGCU GGGU GGCA GGGGCCGG miRNA 3' A CA G 5'	-33.5
7029	nsp9	RNA-dependent RNA polymerase	target 5' G GACAUUGUUG A 3' CCCGGU UCG CUCUCGGCC GGGUCA GGC GGGGGCCGG miRNA 3' A A 5'	-33.5
6340	nsp4	3C-like serine proteinase	target 5' C C 3' UCCCAG CCG UCCUGGUC AGGGUC GGC GGGGCCGG miRNA 3' A AG 5'	-32.2
3647	nsp2	CP2 which mediates cleft at the C-terminus.	target 5' A GAA U 3' CCAG CCCUCGGCC GGUC GGGGGCCGG miRNA 3' AG AGGCA 5'	-31.3
2261	ORF1a	ORF1a polyprotein	target 5' A G G C 3' CCAGU UCGU CUCUGGUC GGUCA GGCA GGGGCCGG miRNA 3' AG G 5'	-31.3
10177	nsp12	PRRSgp1	target 5' G AA G G 3' CCGGUUC C UCCCGGCU GGUCAGG G GGGGCCGG miRNA 3' AG CA 5'	-30.4
1913	nsp2	CP2 which mediates cleft at the C-terminus.	target 5' A AUC GC A 3' UUCCG GUUCG UUCUCCGGUC AGGGU CAGGC AGGGGGCCGG miRNA 3' 5'	-30.3
14763	GP4	ORF4	target 5' A CGCAUUUGUC GCG C 3' CCA GUCCG UCCCGGCU GGU CAGGC GGGGCCGG miRNA 3' AG AG 5'	-30.3

more evidence is needed to explore the mechanism by which PRRSV induces inflammation.

miR-181 is a significant regulator of PRRSV infection, as it targets specific regions in the PRRSV genome to prevent or modify virus–host cell interactions, reducing harm to pigs and increasing their resistance to PRRSV (Ninsuwon et al. 2020). miR-26 plays a crucial role in suppressing PRRSV replication and spread by affecting virus entry, gene expression, and cell death and mitigating tissue damage and inflammatory responses (Li et al. 2015b). miR-145 specifically targets and suppresses the expression of PRRSV genes, halting virus replication and improving antiviral immune responses in pigs (Wang et al. 2018).

While these results provide compelling evidence for the regulatory function of miRNAs in PRRSV infection, many mechanisms remain unclear, and ongoing research is in its early stages. Future investigations will focus on elucidating the precise mechanisms of action of miRNAs in disease onset and progression, as well as their interactions with PRRSV. miRNAs showed promising prospect as therapeutic targets for PRRSV, and further clinical trials and experimental validation are needed to ensure their safety and efficacy.

Current PRRSV vaccines, including modified live vaccines (MLVs) and inactivated vaccines, have several limitations. MLVs can revert to virulence and cause disease in vaccinated animals, leading to safety concerns. On the other hand, inactivated vaccines often provide insufficient protection because of their inability to induce strong cell-mediated immune responses (Lunney and Chen 2010). Additionally, the high genetic variability of PRRSV strains poses a tough challenge to vaccine efficacy, as vaccines may not provide cross-protection against diverse viral strains (Charerntantanakul 2009). Given these limitations, miRNA-based strategies offer promising alternatives. miRNAs can be designed to target conserved regions of the viral genome, potentially overcoming the issue of viral genetic variability. Furthermore, miRNAs can be engineered to modulate host

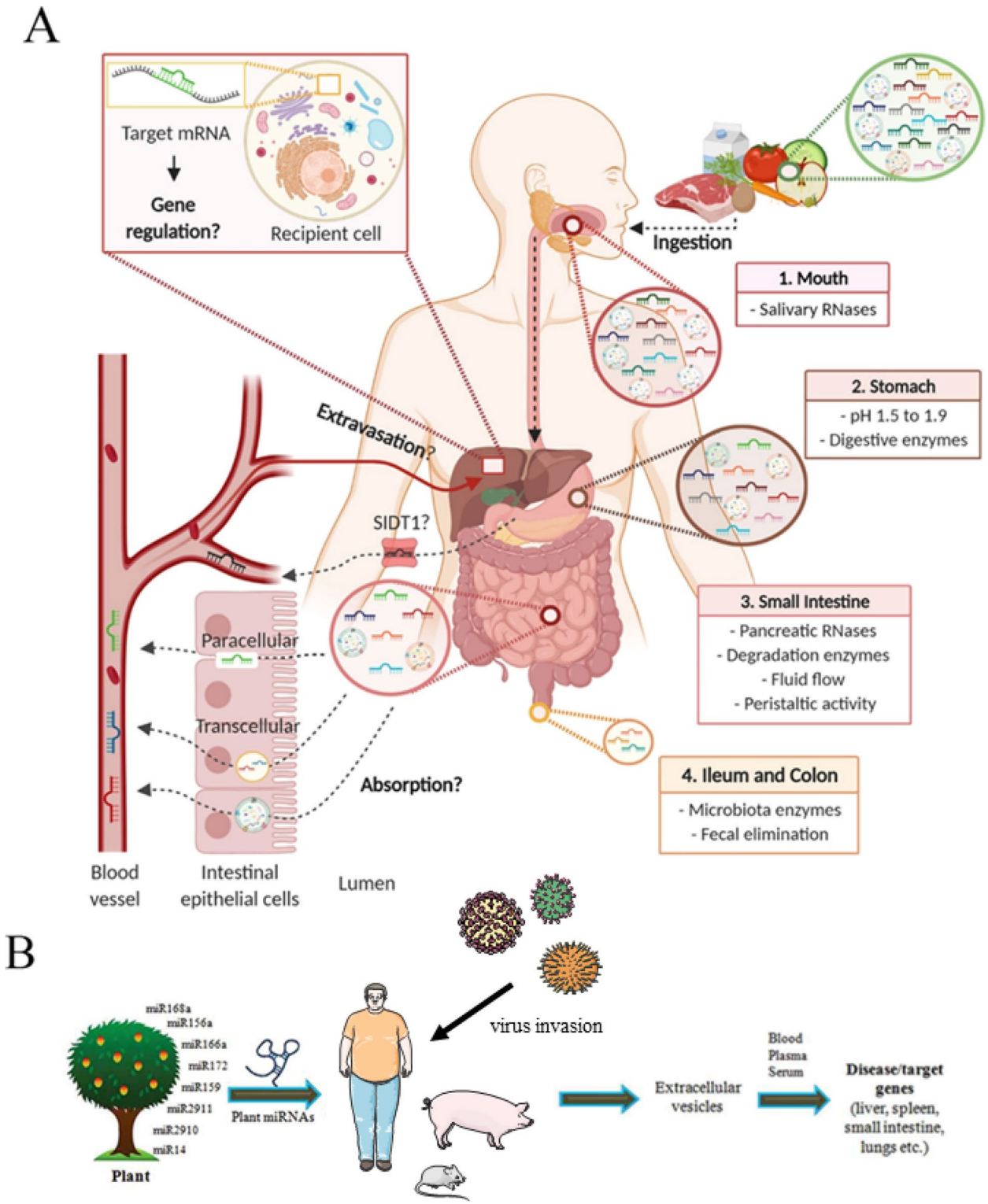


Fig. 3 Cross-kingdom regulatory mechanisms of plant miRNAs. **A** Absorption of miRNAs after oral administration; **B** Regulatory mechanisms of miRNAs derived from plants. Copyright © 2017 Published by British Journal of Pharmacology

immune responses, enhancing antiviral defenses without the safety risks that MLVs may raise (Mishra et al. 2020).

In conclusion, miRNAs represent novel therapeutic targets with significant regulatory roles in PRRSV infection. Exogenous miRNAs can function similarly to endogenous miRNAs, regulating various aspects of biological process. Future research will enable deeper insights into miRNA–PRRSV interactions and explore their therapeutic potential.

Abbreviations

miRNA	MicroRNAs
PRRSV	Porcine reproductive and respiratory syndrome virus
IFN	Interferon
WNT	Wingless/Integrated
MAPK	Mitogen-activated protein kinase
PIK3	Phosphatidylinositol 3 kinase
AKT	RAC-α serine/threonine-protein kinase, Akt
NOTCH	Notch signaling pathway
NF-κB	Nuclear factor kappa-light chain enhancer of activated B cells
IL-6	Interleukin-6
TNF	Tumor necrosis factor

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Authors' contributions
Y.W. and Z.S conceived this study. W.Y., L.W., and W.L. designed and performed this research. X.C. and R.C. wrote this review. B.H. improved the language. All the authors reviewed this manuscript.

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Availability of data and materials
The database and items included in the review are available upon request to the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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