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REVIEW ARTICLE

Roles of Non-Coding RNAs in Virus-Host Interaction About Pathogenesis of Hand-Foot-Mouth Disease

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Abstract

Noncoding RNAs (ncRNAs) represent the largest and main transcriptome products and play various roles in the biological activity of cells and pathological processes. Accumulating evidence shows that microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA) are important ncRNAs that play vital regulatory roles during viral infection. Hand-foot-mouth disease (HFMD) virus causes hand-foot-mouth disease, and is also associated with various serious complications and high mortality. However, there is currently no effective treatment. In this review, we focus on advances in the understanding of the modulatory role of ncRNAs during HFMD virus infection. Specifically, we discuss the generation, classification, and regulatory mechanisms of miRNA, lncRNA, and circRNA in the interaction between virus and host, with a particular focus on their influence with viral replication and infection. Analysis of these underlying mechanisms can help provide a foundation for the development of ncRNA-based antiviral therapies.

Introduction

Noncoding RNAs (ncRNAs) are the biggest RNA family accounting for 97 percent of gene regulators in mammalian cells and regulating around half of all human genes. NcRNAs do not code proteins and are initially considered "genomic noise". However, emerging evidence has shown that ncRNAs impact all cellular processes, including proliferation and development, apoptosis and differentiation, and cell signal transduction [1]. NcRNAs can be divided into housekeeping and regulatory RNAs. The housekeeping RNAs are ubiquitous in cells, including tRNAs and rRNAs, while regulatory RNAs can be classified into small and long noncoding RNAs (lncRNAs) according to their transcript length. Small RNAs are shorter than 200 nucleotides (nt) and include piwiRNAs (piRNAs), small interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs), and microRNAs (miRNAs) [2]. Recent technological advances in RNA-sequencing have allowed us to explore the roles of miRNAs, lncRNAs, and circular RNAs (circRNAs) in virus pathogenesis and investigate the relationship between the viral life cycle and immune escape [3–5]. In this review, we discuss the emerging roles of cellular-encoded ncRNAs in hand-foot-mouth disease (HFMD) virus-host regulation and offer potential strategies for antiviral therapies that target ncRNA regulation.

Hand-Foot-Mouth Disease (HFMD) Virus

The human genus *Enterovirus* (EV) of the family *Picornaviridae* is divided into seven species: Enterovirus species A to D and Rhinovirus species A to C. Infections by these diverse viruses can cause multiple diseases, such as HFMD, diabetes, paralysis, encephalitis, neurological diseases, and even result in death [6]. In recent years, HFMD has emerged as a major public health problem, with worldwide outbreaks frequently occurring in summer and early fall, especially in North America and Southeast Asia, including China, Singapore, Malaysia, and Japan [7–9]. Epidemiological investigations have shown that enterovirus 71 (EV71), coxsackievirus A (CVA16 and CVA6), and coxsackievirus B (CVB2 and CVB5) account for the majority of HFMD cases [7, 10]. HFMD virus spreads through the fecal–oral route and disseminates from the gastrointestinal tract to infect other

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tissues and organs, including the central nervous system. These infections result in clinical symptoms, including a low-grade fever, a maculopapular or papulovesicular rash on the hands, feet, and oral ulcerations. In most cases, the infection is self-limiting and patients recover in 7–10 days. However, growing evidence indicates a potential for more serious disease in children under 5 years of age, which permanent paralysis and central neurological complications, resulting in fatalities [11, 12]. Unfortunately, specific antiviral drugs are not available and vaccines (e.g., EV71 vaccines) are not cross-protective.

HFMD virus is a positive-strand RNA virus, which drives the translation of a polyprotein from a 7.4 kb genomic mRNA template. The virion translates its positive-strand (+) RNA genome into a single polyprotein which is cleaved into four structural proteins (VP1-VP4 capsid proteins) and seven non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D). Virus particles are formed by the assembly of the structural capsid proteins VP0, VP1, and VP3 into pentamers. VP1, VP2, and VP3 peptides are present on the surface of the viral capsid, and neutralizing antibodies mainly target VP1. VP4 is closely linked to the virus core and is embedded in the virus. Non-structural proteins are involved in the regulation of polyprotein processing, virus replication and host cell protein synthesis. Proteins of 2A and 3C cleave the polyprotein, 3B encodes a VPg protein, and 3D encodes the RNA-dependent RNA polymerase [13, 14].

Cellular miRNA Profile During HFMD Virus Infection

MicroRNA (miRNA) consists of approximately 22 nt and acts on endogenous RNAs involved in a wide range of biological functions, including cell proliferation, differentiation, apoptosis, and host–pathogen interactions through the regulation of gene expression at the post-transcriptional level [15]. In the classic pathway, the expression of miRNAs is typically mediated by RNA polymerase II which generates long primary transcripts (pri-miRNAs) that are cleaved by the Dorsha-DGCR8 complex, resulting in precursor miRNAs (pre-miRNAs). Pre-miRNAs contain a 5′ cap and a 3′ poly(A) tail, and are transferred from the nucleus to the cytoplasm, where they are recognized and spliced by the Dicer-TRBP complex to generate double-stranded miRNA duplexes. Mature miRNAs are associated with argonaut proteins targeted mRNAs [16]. To date, many studies have demonstrated the existence of an association between miRNAs and viral infection [5, 17, 18]. MiRNAs target the viral genome to regulate viral replication, participate in immune response and signaling pathways, and cell apoptosis. Therefore, we investigated the critical roles of cellular miRNAs in HFMD virus infection, especially EV71-induced HFMD infection.

Viral Replication

During viral infection, cellular miRNAs act as regulatory factors in the viral life cycle to suppress or promote virus replication by targeting the viral genome [5, 19]. Recently, several studies have suggested that host miRNAs can target viral genomes thereby regulating viral replication. Cell receptors are the viral entry points into cells. MiR-127-5p interacts with scavenger receptor class B member (SCARB2) to inhibit the entry and replication of EV71 [20]. Moreover, most miRNA can interact with UTRs or the whole genome to regulate viral replication. Let-7a was reported to target the EV71 RNA genome to inhibit viral replication both in vitro and in vivo. In the RD cells, the suppression of EV71 replication and viral load were demonstrated by luciferase activity, RT-PCR, western blotting, and plaque assay. Also, mice inoculated with let-7a showed decreased clinical scores and a prolonged survival time accompanied by decreased viral RNA, protein expression, and virus titer [21]. In EV71-infected RD and SK-N-SH cells, the expression of endogenous miR-296-5p decreases EV71 replication by targeting both the VP1 and VP3 regions of nt 2115–2135 and nt 2896–2920 of the viral genome [22]. MiRNA-2911 and miRNA-23b inhibit EV71 replication by directly targeting the VP1-coding sequence [23, 24]. Also, miR-876-5p was shown to be upregulated in EV71-infected neuroblastoma cells and directly targeted the host cyclic-AMP responsive element binding protein 5 (CREB5) to promote viral replication through the accumulation of the EV71 VP1 protein. The overexpression of miR-876-5p or CREB5 knockdown promoted EVA71 replication, whereas the downregulation of miR-876-5p inhibited viral accumulation. Interestingly, CREB5 overexpression also suppressed EV71 replication [25]. The miRNAs involved in the regulation of EV71 replication are listed in Fig. 1.

Innate Immune Response

Compared to adaptive immunity, the innate immunity serves as the first line of the host immune response against viral invasion. The innate immune system depends on the virus and the recognition of pattern recognition receptors (PRRs), including transmembrane Toll-like receptors (TLRs), retinoic acid inducible-gene I (RIG-I), and NOD-Like Receptors (NLRs). PRR signaling cascades immediately respond to pathogens and immune responses [26]. Most PRR signaling is mediated by adaptor molecules, such as myeloid differentiation factor 88 (MyD88), RIG-I, and activation and recruitment domain (ASC), which in turn are targeted by some viruses to regulate the innate immune pathway [27].

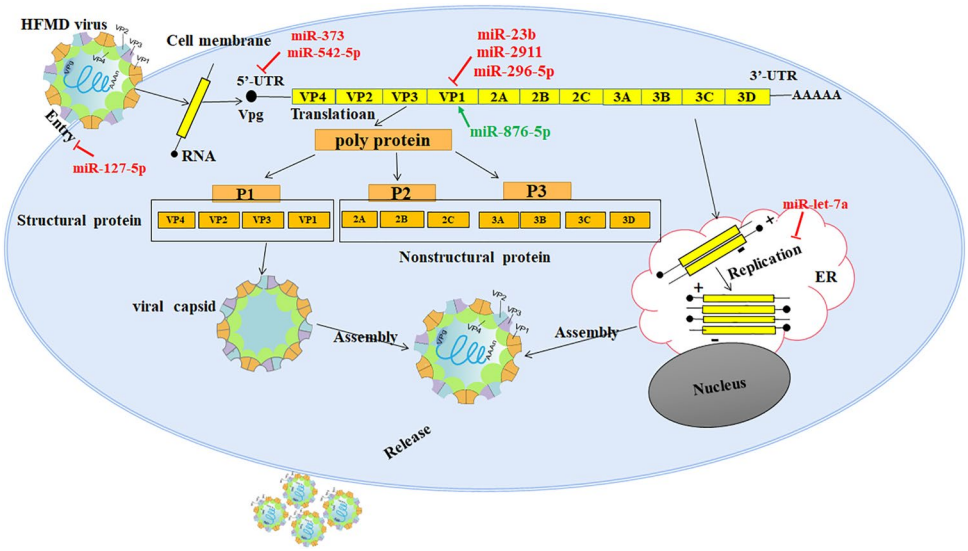

Fig. 1 The miRNAs involved in the regulation of EV71 replication. Dysregulated miRNAs target the viral entry, viral genome (UTR or structural protein VP1), and viral replication to influence EV71 life cycle (miRNAs, marked with red clippers, stands for inhibiting viral replication; miRNAs, marked with green clippers, stands for promoting viral replication) (Color figure online)

Critically, miRNAs play important roles in the regulation of the adaptor molecules. During EV71 infection, upregulated miR-21 directly targets MyD88 and interleukin-1 receptor-associated kinase 1 (IRAK1) to inhibit the production of type I interferon (IFN-I) [28]. Likewise, exosomal miR-30a is released from EV71-infected human oral epithelial cells. Compared to controls, treatment of cells with exo-miR-30a mimics significantly decreased the expression of IFN-β, increased the expression of viral structural protein VP1 and resulted in a decreased expression of MyD88 protein in macrophages. These results show that exo-miR-30a directly targets MyD88 to inhibit IFN-I production, thus promoting viral replication [29]. In RNA viral infection, the upregulation miR-526a provides resistance to EV71 infection in macrophages through interferon regulatory factor (IRF)-dependent pathways. The underlying mechanism involves miR-526a enhancement of RIG-I K-63 ubiquitination by inhibiting the expression of cylindromatosis (CYLD) [30]. Absent in Melanoma 2 (AIM2) is an intracellular microbial dsDNA sensor containing apoptosis associated speck-like protein which encompasses a caspase ASC and caspase-1. MiR-143 transfection into Jurkat cells can lead to an increase in the expression of AIM2 and ASC mRNAs [31]. Also, the TNF receptor-associated factor (TRAF) families are essential components of TLRs-activated signaling pathways [32]. EV71-induced miR-628-5p can target the 3'UTR of TRAF3 by luciferase reporter assays. Western blotting and qPCR results showed that overexpression of miR-628-5p reduced TRAF3 and inhibited IFN-β transcription to promote EV71 replication [33]. MiR-146a is upregulated during EV71 infection resulting in the suppression of two critical components in interferon production, IRAK1 and TRAF6, thereby promoting viral survival [34]. EV71 induced miR-545 expression in 293 T cells and RD cells. Overexpression of miR-545 promoted EV71 replication and attenuated the inhibitory effects of EV71 on the ability of these cell lines. Conversely, miR-545 knockdown significantly suppressed EV71 replication. The luciferase reporter assay showed that miR-545 directly targeted the 3'untranslated region of phosphatase and tensin homolog (PTEN) and tumor necrosis factor receptor-associated factor 6 (TRAF6) in HEK293 cells. Further results indicated that miR-545 promoted EV71 replication, at least partly by targeting PTEN and TRAF6. The overexpression of PTEN and TRAF6 suppressed EV71 replication and attenuated the enhanced effects of miR-545 overexpression on EV71 replication in HEK293 cells [35].

In addition, miRNAs are also associated with cytokines and cells of the innate immune system. The miR-548 family was shown to target the 3'UTR of IFN-λ1, highlighting an important innate response mechanism to viral infection [36]. MiR-155-5p was shown to be significantly upregulated in serum obtained from patients with EV71 infection. The overexpression of miR-155-5p suppressed EV71 titers and VP1 protein level, and also enhanced EV71 triggered IFN I production and the expression of IFN-stimulated genes (ISGs). Conversely, miR-155-5p inhibition had the opposite effect. The luciferase reporter assay demonstrated that miR-155-5p directly targeted forkhead box protein O3 (FOXO3) and negatively regulated the FOXO3/IRF7 axis, an important regulatory pathway for IFN-I production. In EV71-infected mice, agomir-155-5p injection, a small RNA mimic, resulted in a significant reduction of viral VP1 protein expression in brain and lung tissues, and increased IFN-α/β production; this results in a higher survival rate of the experimental mice. Collectively, the inhibition of miR-155-5p facilitated EV71 replication by suppressing of type I IFN response via the FOXO3/IRF7 pathway in vivo and vitro [37]. EV71 can also evade host IL-6R and STAT3-mediated antiviral activities through EV71-induced miR-124 [38]. Dendritic cells (DCs) connect innate and adaptive immune responses. The

miR-148 family (miR-148a, miR-148b, and miR-152) is a negative regulator of the DCs innate response, involved in the production of cytokines. They can fine-tuner in regulating the innate response and the antigen presenting capacity of DCs [39].

Signaling Pathways

MiRNAs can affect HFMD virus-induced innate immune responses either by directly targeting cytokine production and adaptor molecules, or by indirectly interacting with other regulators that influence innate immune signaling pathway. MiRNAs have been characterized as critical players in host signaling transduction. During viral infection, the IFN-I and NF-κB singling pathways are critical steps in innate immune responses and thus play major roles in the host defense mechanism [40]. During EVA71 infection, most differentially expressed miRNAs can indirectly interact with host signaling pathways and regulate viral replication. MiR-103 and miR-107 were downregulated in serum from patients with EV71. Overexpression of miR-103 and miR-107 suppressed EV71 titers and VP1 expression and triggered the production of type I IFNs. Moreover, miR-103 and miR-107 directly targeted the suppressor of cytokine signaling 3 (SOCS3) which upregulation reversed the effects of miR-103 and miR-107 on EV71 replication and the IFN-I pathway [41]. Some molecules associated with IFN-producing signaling, such IRAK-1, TLR-7, IRF1, TBK1-binding protein, and NF-κB repressing factor were also found to be targeted by some miRNAs, including has-miR-142-3p, has-miR-525-5p, has-miR-205, has-miR-202, and has-miR-124 [42]. The overexpression of miR-9-5p suppressed RIG-I-mediated NF-κB signaling [43].

Moreover, the phosphoinositide 3-kinase (PI3K)/Akt and the mitogen-activated protein kinase (MAPK), are two important signaling pathways that are evolutionarily conserved and play roles in the control of cell differentiation, autophagy, and survival [44, 45]. EV71-induced miR-494-3p activates the PI3K/Akt signaling pathway by targeting PTEN [46]. Also, MAPK kinase 4 (MAP4K4) is the target of suppression by hsa-let-7c-5p, which facilitates the replication of EV71 by regulating the MAPK signaling pathway [47]. During severe EV71 infection, the altered expression of upregulated-miR-876-5p regulates genes that are targeted by the PI3K-Akt signaling pathways [48]. In addition, the miR-302 family can reduce EV71-induced innate immune responses by targeting the 3'UTR of Karyopherin 2 (KPNA2), which indirectly affects MAPK signaling pathways [49]. Among the regulatory mediators, miR-1246 is involved in cell death signaling, which might be associated with EV71-induced neurological pathogenesis through the targeting of disc-large homolog 3 (DLG3) [50]. The epidermal growth factor receptor (EGFR) signaling also plays a crucial role in EV71 replication in the human neuroblastoma cell line, SK-N-SH cells. The downregulated miR-27a suppresses EGFR expression and reduces Akt and ERK phosphorylation, which act as an inhibitor of EV71 replication [51].

The miRNAs that are involved in innate immune responses and signaling pathways (IFN-I and NF-κB) that play a role in the regulation of EV71 replication are listed in Fig. 2.

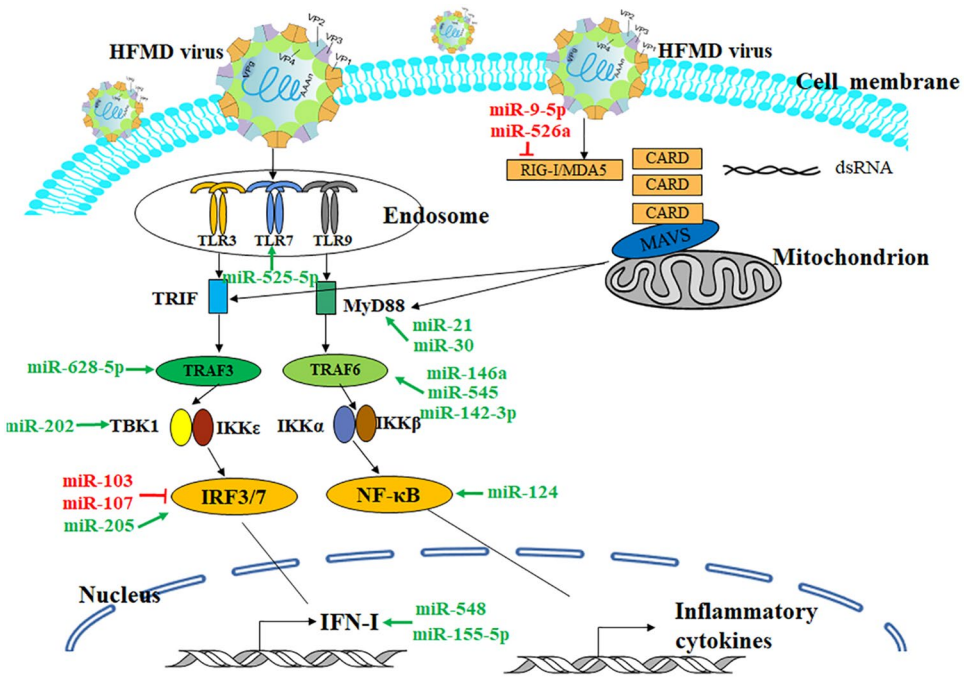

Fig. 2 The miRNAs involved in innate immune response and signaling pathways (IFN-I and NF-κB) during EV71 infection. Dysregulated miRNAs regulate by targeting PPRs, adaptor molecules, transcription factor, and cytokines that affect innate signals (miRNAs, marked with red clippers, stands for inhibiting viral replication; miRNAs, marked with green clippers, stands for promoting viral replication) (Color figure online)

Cell Apoptosis

Apoptosis is considered as one of the major pathogenic features of viral infection that result in host cell death and tissue damage [52]. In the early phase of infection, apoptosis acts as an important cellular defense mechanism against viral proliferation. During EV71 infection, host miRNA regulates the key elements involved in cellular apoptosis, thus affecting viral replication. In SHSY-5Y cells, EV71 infection induces endogenous miR-let-7b expression which directly targets the 3'-UTR of CCND1 (cyclin D1) to modulate the host cell cycle and proliferation. CCND1 is a key factor in cellular apoptosis, and the inhibition of miR-let-7b expression restores CCND1 expression, thereby reducing the cell G2/M cycle phase and SH-SY5Y proliferation [53]. These results indicated that miRNAs mediated the proliferation and apoptosis of EV71-infected cells. It has also been shown that miR-16-5p is involved in the regulation of cyclin E1 (CCNE1) and CCND1, both important cell cycle regulators that suppress EV71 replication. Moreover, another study showed that miR-16-5p is a positive feedback regulator in EV71-induced apoptosis and an inhibitor of viral replication [54]. Downregulated miR-874 was shown to facilitate EV71-induced apoptosis in a granzyme B (GZMB) dependent manner in Jurkat T cells [55]. The downregulated miR-206 interacts with Chemokine (C-C motif) ligand 2 (CCL2) 3'-UTR to reduce the apoptosis of NPC cells [56]. Recently, it was found that during EV71 infection, the upregulated miR-146a targeted the son of sevenless homolog 1 (SOS1), while the downregulated miR-370 targeted DNA-damage-inducible (GADD45). The silencing of miR-146a restores SOS1 expression and partially attenuates EV71-induced apoptosis, while the ectopic expression of miR-370 decreases EV71-induced GADD45 expression and diminishes apoptosis. In addition, the co-expression of miR-146a and miR-370 was shown to attenuate EV71-induced apoptosis [57]. These results imply that miRNAs might attenuate EV71 infection-induced apoptosis, and thus suggest that they are therapeutic candidates.

In addition to the above relatively well-studied miRNA mechanisms, other miRNAs have also been shown to influence EV71 infection. However, the details of the underlying molecular mechanisms still require further investigation, such as miR-134 which regulates poliovirus replication via the nuclear transport system and inhibits EV71 replication [58]; miR-195 which has also been shown to suppress EV71-induced pyroptosis in human neuroblastoma cells [59].

Cellular lncRNA Profile in HFMD Virus Infection

Long non-coding RNAs (lncRNAs) are transcribed by RNA polymerase II and are more than 200 nucleotides in length. Based on the position of target genes on the host chromosome, they are classified into five groups including sense, antisense, bidirectional, intronic, and intergenic lncRNAs. The majority of lncRNAs are 5'-capped, spliced, and polyadenylated to form a structure similar to that of mRNAs, but they do not contain a protein-coding open reading frame [60]. Each lncRNA develops a final stable structure that shapes its important roles in the cell. Previous studies have shown that lncRNAs participate in the cell cycle and differentiation, chromatin re-modeling through epigenetic mechanisms, and that they act as "sponges" by regulating mRNAs. LncRNAs are also versatile molecules that physically and functionally interact with DNA, other RNAs and proteins to regulate transcription or translation [61, 62]. Using high throughput RNA sequencing, Peng et al. identified for the first time approximately 500 known and more than 100 unknown lncRNAs in mouse model of SARS-CoV virus, thus revealing that lncRNAs were closely related to virus infection [63]. Additionally, lncRNAs have been shown to regulate and participate in virus-host interactions, such as the viral life cycle, immune cells, host transcription, and downstream signaling pathways [64].

In 2011, Cabili et al. characterized lncRNAs as having over 30 characteristics and found that 78% of lncRNAs were tissue-specific across 24 tissue and cell types when compared with protein-coding genes [65]. LncRNAs demonstrate poor evolutionary conservation and are expressed in specific cell types or tissues and different cell locations. Therefore, we summarize the expression profiles of lncRNAs in different conditions, including cell lines or tissues, host factor virus strains, and other factors infected by HFMD viruses [66–69] (Table 1). To date, a few lncRNAs have been demonstrated to have a specific role in HFMD virus infection. During EV71 infection, lncRNAs can regulate cell apoptosis and host cell signaling pathways. Liao et al. identified that the downregulated lnc-IRAK3-3 was involved in EV71 infection-induced apoptosis. Lnc-IRAK3-3 post-transcriptionally governed growth arrest and DNA damage-inducible (GADD) 45β which expression was increased and triggered the cleavage of caspase3 and PARP, resulting in lnc-IRAK3-3 mediated promotion of cell apoptosis during EV71 infection [70]. In RD cells and in the blood from HFMD patients infected with EV71, the novel lncRNAAK097647 was shown to be upregulated, resulting in increased EV71 replication by decreasing IFN-λ expression and inhibiting NF-κB phosphorylation

Table 1 The expression profiles of lncRNAs in host cell infected with HFMD virus

Virus type	Cell/tissue	Total lncRNAs	Different-expressed lncRNAs		methods	Refs
			Up	Down		
EV71	RD	2216	18	5	RNA-seq	66
EV71	Skeletal muscle/mice	1585	72	32	RNA-seq	66
EV71	PBMCs	4866	2990	1876	microarray	67
EV71	RD	477	287	190	microarray	68
CVA16	RD	1970	760	1210	RNA-seq	69

[71]. In a previous study, our group revealed the expression profile of lncRNAs after CVB5 infection of RD and SHSY5Y cells, however, the function of lncRNAs in coxsackievirus infection is still under study. [72].

Cellular circRNA Profile in HFMD Virus Infection

Circular RNA (circRNA) is a special endogenous ncRNA that is formed by mRNA splicing in reverse sequence during post-transcriptional processing. It is widely expressed in mammalian cells. Unlike linear RNA, circRNA is covalently linked to form a closed ring structure, without 5' end caps or 3' Poly (A) tails. CircRNAs are divided into exonic circRNA, exonic-intronic circRNA, and circular intronic RNA. Several biological processes, including gene regulation, protein assembly and trafficking, and cell division are tightly regulated by circRNAs [73, 74]. More importantly, many studies have explored the potential function of circRNAs in host-virus interactions [75]. In most cases, circRNAs act as sponge molecules of miRNA, serving as competing endogenous RNAs (ceRNA) that have been identified in many viral diseases, especially in oncogenic DNA viral infection [76, 77]. Increasingly it has become clear that circRNAs play important roles in immune regulation and participate in the occurrence and development of a variety of diseases. Upon viral infection, the immune factor, NF90/NF110, translates to the cytoplasm, and suppresses the expression of circRNA. The dissociation of NF90/NF110 from circRNPs can inhibit viral replication, indicating that circRNA production is associated with the activation of the innate immune response [78]. The relationship between circRNAs and immune responses in viral infections has gradually been demonstrated. Zhan et al. investigated circRNAs expression profiles upon EV71 and CVA16 infection with SHSY5Y cells. In CVA16-infected cells, a total of 8726, 8611, and 6826 circRNAs were identified at 0, 12, and 24 h post-infection, respectively. About all, 1769 and 1192 circRNAs were differentially expressed in the CVA16-12 h and CVA16-24 h groups. In EV71-infected cells, 10,405 and 4710 circRNAs were detected at 12 h and 24 h post-infection, of which 1851 and 951 circRNAs were differentially expressed in the EV71-12 h and EV71-24 h groups [79, 80]. Our group identified the expression profile of circRNAs after CVB5 infection of RD cells [81]. Nonetheless, the underlying mechanisms of circRNAs in HFMD virus infection have not yet been elucidated.

The Network of ncRNA Profile in HFMD Virus Infection

MiRNAs, lncRNAs and circRNAs are the most important elements of ncRNAs and their complex regulatory relationships have attracted significant attention. LncRNAs and circRNAs can act as miRNAs "sponges" or precursors, and miRNAs can regulate lncRNAs, circRNAs, or compete for mRNAs during viral infection. Consequently, the interactions between miRNAs, lncRNAs, and circRNAs play important roles in viral infection [77, 82].In HFMD virus infection, some studies have found mutual regulatory mechanisms between lncRNAs and miRNAs. In EV71 infection, lnc-IRAK3-3 acts as miRNA "sponges" that capture miR-891b to restrict GADD45β expression, which eventually promotes cell apoptosis [70]. Also, the ceRNA network involves lncRNAs that act as sponges for miRNAs to regulate mRNA expression. The MALAT1/miR-194-5p/DUSP1 ceRNA regulatory axis during EV71 infection was involved in cell apoptosis, but no association was found with autophagy in that study [80]. During CV-A16 infection, the three key circRNA-associated ceRNA networks pointed out to the immune system and inflammation mediated by the chemokine and cytokine signaling pathway, including hsa_circ_0004447/hsa-miR-942-5p/MMP2, hsa_circ_0078617/hsa-miR-6780b-5p/ MMP2, and hsa_circ_0078617/hsa-miR-5196-5p/MMP2 [79].

Future Perspectives

The role of ncRNAs in cancer has been well established, however, their function during infection has only been reported for specific viruses [83, 84]. In this review, we

discussed current advances regarding the roles of miRNAs, lncRNAs, and circRNAs in HFMD virus infection, and described the novel and diverse mechanisms through which ncRNAs serve as important regulators in the virus life cycle, and how the host cell immune response, signaling pathways, and apoptosis are affected by the viral infection.

Therapeutic targeting of ncRNAs represents a promising approach for the treatment of cancers, now accumulating evidence supports a link between ncRNAs and almost all human pathophysiological processes of human diseases which offer a potential diagnostic and therapeutic target for HFMD-associated diseases. The clinical potential of miRNAs mimics has been indirectly explored in human studies. Patients with CVA16 infection show an increased permeability of the blood–brain barrier (BBB) that is accompanied by an upregulation of matrix metalloproteinase 9 (MMP9) expressions. The downregulated miR-1303 directly regulates BBB by targeting MMP9 which ultimately causes pathological CNS changes [85]. MiR-3473a could also regulate focal adhesion and leukocyte trans-endothelial migration, suggesting a role in EV71-induced blood–brain barrier disruption [86]. The upregulated expression of has-miR-3605-5p during CVA16 and EV71 infections has been shown to modulate the cellular receptor SCARB2, resulting in aphthous ulcers, cough, myocarditis, and somnolence of meningoencephalitis [87].These results have highlighted the potential development of preventive and therapeutic strategies against HFMD-associated infections by manipulating miRNA expressions. Mimic therapeutics are also in development, including the major receptor of enteroviruses [88]. Unfortunately, sensitivity, specificity, clinical development cost, and unpredictable immune responses remained to be addressed in clinical applications becomes practical. In conclusion, the use of ncRNA or other mimics to treat HFMD is still a long way.

NcRNAs are also an emerging source of biomarkers. Notably, during EV71 infection, the specific co-regulation of miR-133b and miR-206 in Th17-type immune reactions can be used as novel biomarkers [89]. In colorectal cancer cell (CRC), the overexpression of miR-362-3p, which is observed during EV71 infection, can reduce cell viability, and proliferation mainly through cell cycle arrest. MiR-362-3p may also be used as a novel prognostic marker in CRC [90].

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Declarations

Conflict of Interest All authors have read and approved the final version of the manuscript and no conflicts of interest to disclose.

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