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Virus-Encoded MicroRNAs Reveal How Ranavirus Interacts with Amphibian Immune Defense

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ABSTRACT

Ranaviruses are harmful viruses that infect amphibians, fish, and reptiles, and have caused particularly devastating declines in amphibian populations. One particular type of ranavirus, called Frog Virus 3 (FV3), has been extensively studied due to its prevalence and impact on amphibians. Previous research has primarily focused on the virus's genes, but little attention has been given to the non-coding regions of its genome. This article reviews recent studies that reveal the ability of ranaviruses, including FV3, to encode microRNA (miRNA), a type of regulatory RNA. These viral miRNAs play a crucial role in suppressing frog immune genes, modulating the virus-host interaction, and promoting viral infection. Understanding how ranaviruses use miRNAs to control disease progression is essential for addressing the health threat they pose to wildlife and ecosystems.

1. INTRODUCTION

Frog Virus 3 (FV3) is a large virus (measuring approximately 150 nm in diameter with a large single linear dsDNA genome of roughly 105 kbp) that belongs to the Ranavirus family [1]. It infects amphibians at different stages of their life cycle, causing lethal infections in tadpoles but often remaining unnoticed in adult frogs [1-7]. Due to a broad virulence among virus isolates and particular variation of immune status of animals, FV3 has been found in both sick and seemingly healthy frogs, indicating its ability to adapt to its host and effectively spread [8-16]. This ability is similar to what we see in other successful viruses that have caused epidemics in animals and humans. The impact of ranaviruses on amphibians is a significant concern, as they contribute to the global decline of these species, threatening biodiversity and ecological balance by disrupting food webs, reducing biological diversity, and impairing ecosystem functions such as pest control and nutrient cycling [2-7]. FV3 is the most commonly reported ranavirus worldwide, found in wild amphibians and turtles in North America. Additionally, there have been reports of genetic recombination between FV3 and another ranavirus called common midwife toad virus (CMTV) in Canada and Europe [2,

11-16]. Reports of genetic recombination between FV3 and other ranaviruses are important because they could lead to the emergence of new, more virulent strains, making it crucial to understand genomic and molecular mechanisms to better predict and manage potential outbreaks [11, 12]. To better understand the biology of ranaviruses and their impact, further research is needed at the genomic and molecular levels. In this context, the recent studies about if ranaviruses encode microRNAs and how these virus-derived microRNAs (v-miR) play roles in the virus-host interaction are crucial to understand ranaviral pathogenesis in amphibians [8-10].

2. BRIEF ABOUT VIRUS-DERIVED MICRORNA (V-MIR)

Viruses employ various strategies to evade the host's immune response. In addition to viral proteins, some viruses, particularly DNA viruses, also produce microRNA (miRNA) to regulate gene expression. miRNAs are small non-coding RNA molecules that target specific regions of cellular transcripts, silencing or modulating their expression [8-10]. Virus-derived miRNAs (v-miRNAs) can mediate the expression of both host and viral genes, shaping the interaction between the virus and its host. Several DNA viruses, including ranaviruses, have been found to encode miRNAs [8-10]. These miRNAs play a crucial role in viral pathogenesis for some DNA viruses by interfering with host defense mechanisms and impacting cellular processes such as cell death and proliferation. The presence and role of v-miRNAs in ranaviruses, including FV3, are not well understood and require further investigation [8-10]. As illustrated in **Figure 1**, some DNA viruses, including human hepatitis B virus and papillomavirus, can adopt cell miRNA biosynthesis complex (Drosha-Dicer as shown) to produce virus-derived miRNA (v-miR) and targeting viral particularly host gene expression. Our question is if ranaviruses like FV3 have similar capacity to modulate disease progress in amphibians [8, 9].

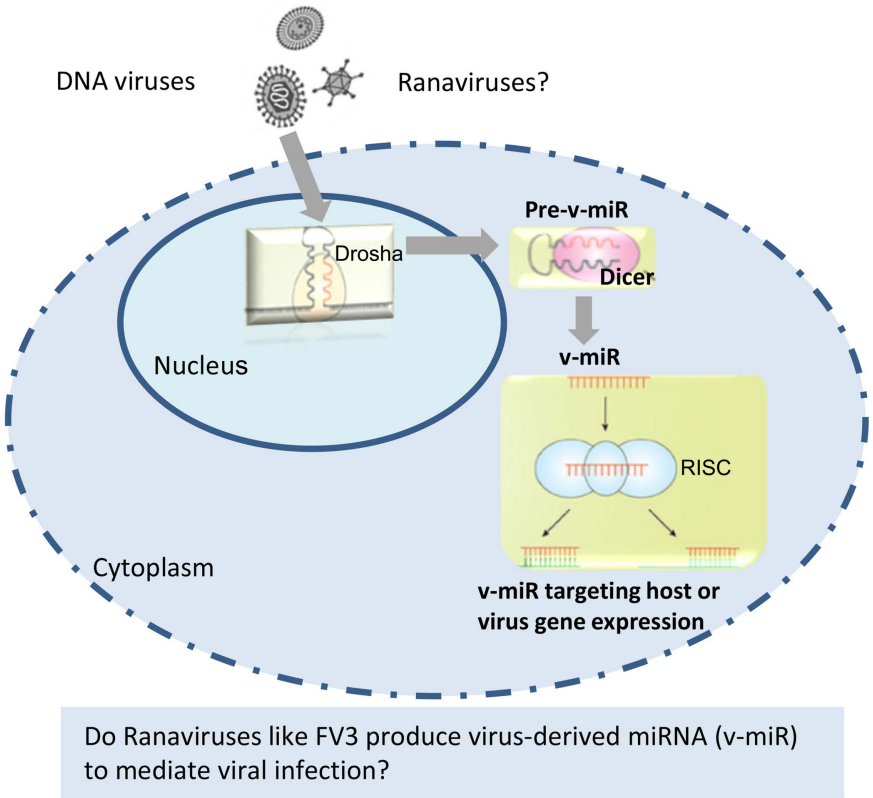

Figure 1. Do ranaviruses like FV3 produce miRNA to modulate disease progress in amphibians? Reproduced from the major idea of the references [8-10]. See Abbreviations in the associated text.

3. VIRUS-ENCODED MIRNA IN RANAVIRUSES OTHER THAN FV3

Viral miRNAs, may function like the host counterpart, to regulate the expression of both viral and host genes and play a role in evading the host's antiviral immune response. For example, in other ranaviruses like Singapore grouper iridovirus (SGIV) and tiger frog virus (TFV), several miRNAs have been identified and shown to influence virus replication and infection [8-10]. These miRNAs target host genes involved in antiviral signaling pathways. The identification and study of these viral miRNAs contribute to our understanding of ranavirus biology and their interactions with host organisms [8-10].

4. VIRUS-ENCODED MIRNA IN FV3 AND ITS POTENTIAL ROLE IN MODULATING THE IMMUNE RESPONSE

Recent research has analyzed the entire transcriptome of FV3-infected frog tissues to explore the presence of both viral and cellular RNA [10]. This study revealed the presence of viral miRNAs in the intergenic regions of the FV3 genome (**Figure 2**) [10]. These miRNAs are particularly concentrated in specific intergenic regions located near highly transcribed genes. Analysis of these viral miRNAs showed that they target important components of the frog's immune system, such as interferon receptor subunits and interferon regulatory factors that represent key antiviral immune regulators in high vertebrates (**Table 1**) [17-19]. The differential expression of these target genes in infected tissues further confirms the potential regulatory role of these viral miRNAs. Further study identified additional FV3-encoded miRNAs and found that they target genes involved in antiviral signaling pathways and other cellular functions [10]. These findings provide valuable insights into the non-coding regulatory mechanisms employed by ranaviruses, shedding light on how they manipulate host gene expression and evade immune responses.

Table 1. Enrichment of predicted FV3 miRNA targeting sites in the mRNA 3-UTR regions of interferon receptors, especially the beta subunits [10].

mRNA (GenBank Acc. #)	3'-UTR length (kb)	Target sites/kb by predicted FV3 miRNA	No. of FV3 miRNA/Group
Ifnar1.L (XM_018245928)	0.163	0	0
Ifnar1.S (XM_018248888)	0.406	2.46	1/1 (1AT)
Ifnar2.L (XM_018245430)	0.439	**84.28**	26/9 (11C, 4AF, 4AT, …)
Ifnar2.S (NM_001095360)	2.305	**76.79**	69/14 (30C, 15AT, 6R, 5AF, …)
Ifnar2.2S (XM_018248427)	0.495	**68.69**	27/6 (14C, 4R, 3AF, 3AT, …)
ifngr1.S (XM_018265300)	0.138	7.25	1/1 (1C)
ifngr2.L (XM_018245930)	0.656	25.91	16/5 (9C, 4AT, …)
ifngr2.S (XM_018248887)	1.241	**45.93**	42/7 (19C, 8AT, 4R, 4AF, …)
ifnlr1.L (XM_018242320)	0.156	0.00	0
il10rb.L (XM_018245931)	0.438	25.11	11/6 (3C, 3AT, 2AF, …)
il10rb.S (NM_001093545)	0.955	**77.49**	42/12 (17C, 10AT, 4AF, …)
	Ave: 0.672	Ave: 37.63	

Abbreviations: Acc., Accession; Ave., Average; kb, kilobase; UTR, Untranslated Region.

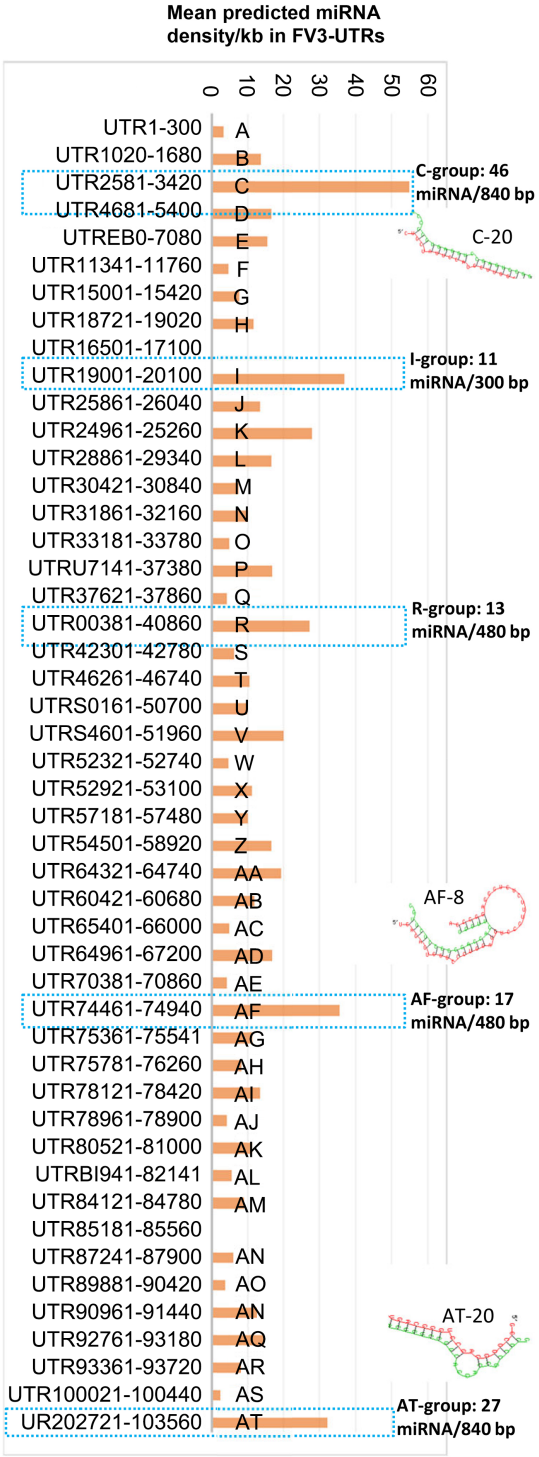

Figure 2. Virus-derived miRNA (v-miR) sequences in intergenic regions of FV3 genome. The prediction of miRNA-like sequences in most intergenic regions (marked as UTR start-end site along FV3 reference genome including the 5'- and 3'-untranslated regions), which are especially enriched in five regions (named as C, I, R, AF and AT per putative miRNA density/Kb) as marked using blue dash line. Some v-miR have identified function against frog antiviral immune genes are shown with sequence-paired structure with their targets. Adapted from Tian *et al.*, 2021 with authors' permission [10].

5. CONCLUSION

While many viruses have been found to encode miRNAs to modulate disease progression, their presence and role in ranaviruses have been less studied. Recent research on ranaviruses, particularly FV3, has demonstrated their ability to encode miRNAs that regulate both viral and host gene expression. Understanding how ranaviruses utilize miRNAs to modulate disease progression is crucial for addressing the health threats they pose to wild animals and ecosystems. By unraveling the intricate mechanisms of virus-host interactions, we can develop strategies to mitigate the impact of ranaviruses and protect vulnerable amphibian populations [2-7].

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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