Review Article

Potential Role of microRNAs in Response to *Aeromonas* Infection in Fish

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ABSTRACT

The genus *Aeromonas* is a widespread pathogen that includes more than 30 Gram-negative species, many of which are opportunistic bacteria. Aeromonas species are naturally distributed in various aquatic sources. Infectious processes in marine animals such as fish usually develop under stressful conditions, and when their immune systems are weakened. MicroRNAs (miRNAs/miRs) are short, non-coding RNAs that post-transcriptionally regulate gene expression. Their diverse biological functions, such as influencing cell development, proliferation, differentiation, tumorigenesis, metabolism, and apoptosis have been studied in various animals. Fish is the most important source of aquatic nutrients throughout the world, and its market is constantly growing. Overpopulation in aquaculture brings infectious diseases that threaten the development of aquaculture around the world. There is extensive evidence that microRNAs are involved in modulating infectious processes and regulating the inflammatory response to major bacterial fish infections, including *Aeromonas*. Here, we review the current literature on the fish microRNA repertoire and outline the physiological roles assigned to microRNAs to provide a foundation for future research during *Aeromonas* infection. Understanding the interaction between microRNAs and *Aeromonas* may provide clues to a remarkable strategy for preventing *Aeromonas* infections in fish.

Keywords: *Aeromonas*, Biomarker, Immunoregulation, MicroRNAs

1. Introduction

Infections in water are mainly caused by bacteria and result in high mortality rates (1). *Aeromonas* spp. are gram-negative bacteria that cause aquatic infections or even mortality in a variety of hosts (2). The genus *Aeromonas* consists of mesophilic and psychophilic bacteria that which can cause various diseases in warm- and cold-blooded animals (3). Recently, mesophilic *Aeromonas* has gained attention as a new causative agent of foodborne illness (4). Aeromonads possess several virulence factors, such as adhesins, hemolysins, cytotoxins, enterotoxins, lipases, proteases, II type secretion system (T2SS), III type secretion system (T3SS), VI type secretion system (T6SS), and biofilm production genes that enable them to overcome the host secretion system and infect various tissues (5). In humans, *Aeromonas* can cause extraintestinal diseases, especially in immunocompromised individuals, including septicemia, wound infections, urinary tract infections, hepatobiliary tract infections, and necrotizing fasciitis (6). Aeromonads are found naturally in the digestive tract of fish and do not cause disease under optimal conditions (7). The course of infectious diseases in fish depends on water temperature and other factors, including immune status. In general, the mortality rate is low (about 25%), depending on the quality of the water body and the population density of the fish (8, 9). Adverse factors such as stress, deterioration of water quality, or temperature fluctuations can affect hosts and result in aeromonads becoming primary pathogens or co-infecting microorganisms in immunocompromised fish. Many freshwater species such as carp, cyprinids, and pike (Esox lucius) are very sensitive to this bacteriosis (8). Therefore, it is particularly important to study the molecular details in the process of bacterial infection in fish. MicroRNAs (miRNAs/miRs) are endogenously small noncoding RNAs that can post-transcriptionally modulate gene expression and are involved in the maintenance of normal cellular functions (10).

Numerous microRNAs have been detected in various fish. For example, microRNA-192, which targets IL-1RI, may regulate immune and inflammatory responses in mussels upon contact with macrophages and Vibrio anguillarum (11). Furthermore, microRNA-200a-3p could regulate TLR signalling pathways by targeting TLR-1 in Vibrio anguillarum bacterially challenged blue mussel cancer (12). Therefore, the study of microRNA-related interactions between bacteria and hosts may help clarify the underlying mechanisms of bacterial infection and host response. In the present review, we summarized and discussed recent data on microRNAs in fishinfected with *Aeromonas* spp. to regulate and control host responses.

2. MicroRNAs

MicroRNAs are a type of conserved short single-stranded and noncoding RNA ~22 nucleotides in length (13). The biosynthesis process of these molecules has been elucidated. First, microRNAs are transcribed from genomic DNA to form long primary transcripts known as pri-microRNAs (Figure 1). Subsequently, they are cleaved in the nucleus by the enzyme Drosha into precursor RNAs of 60-70 nucleotides, known as pre-microRNAs (hairpin-shaped) (14). After pre-microRNAs are exported to the cytoplasm by Ran-GTP and exportin-5, they are cleaved into mature 22- nucleotide duplex RNAs, which are known as microRNA (14). Finally, one strand of the microRNA duplex binds to the untranslated region (3'UTR) of the target sequence in the RNA-induced silencing complex (RISC) and leads to degradation or inhibition of the target mRNA (15). Lin-4 is the first microRNA discovered in *Caenorhabditis elegans* in 1993. Since then, several microRNAs have been found in plant and animal branches of the Eukaryota (16).

3. MicroRNAs and Fish Bacterial Infections

MicroRNAs are involved in a wide variety of biological processes in eukaryotes. It is now clear that microRNAs are involved in bacterial infections. This

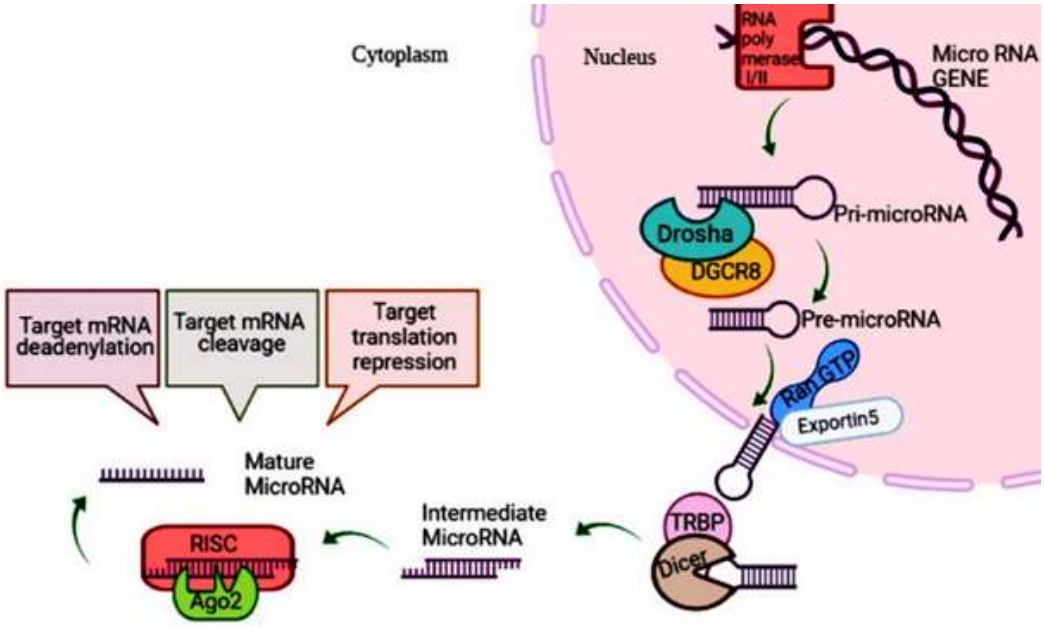

Figure 1. The pathway of microRNA biogenesis
During microRNA biogenesis, the pri-microRNA transcript is formed by RNA polymerase I/II and cleaved by DROSHA in the nucleus. The pre-microRNA, the precursor hairpin, is transported into the cellular cytoplasm by the exportin 5–Ran-GTP complex. RNase Dicer and RNA-binding protein (TRBP) cleave the pre-microRNA hairpin into the mature length. The functional strands of microRNAs, along with Argonaute proteins (Ago2), are loaded into the RNA-induced silencing complex (RISC), where they direct the RISC to repress target mRNAs by mRNA cleavage, deadenylation, and translational repression, while the passenger strands are degraded.

was first observed in plants, where microRNA-393 in Arabidopsis induced resistance to the extracellular pathogen Pseudomonas syringae by inhibiting auxin signalling (17).

In addition, there is evidence that invasive pathogenic bacteria activate microRNAs, which in turn affect a wide range of host cell functions including cell cycle, death or survival, immune response, and cytoskeletal organization. The regulatory role of microRNAs in innate signalling pathways provides new insights into the regulatory mechanisms of infection and inflammatory disease (18).

As for the regulatory function of microRNAs in fish in bacterial diseases, these valuable small RNAs are the focus of recent research to improve the health, productivity, and breeding of various strategic livestock. In addition, immune responses play an important role in fish during bacterial infections. Therefore, the study of microRNA-related interactions between bacteria and hosts could explain the major pathways and mechanisms of bacterial diseases and host responses. In addition, identifying the mechanisms of host immunity contributes significantly to improving disease control and prevention. Recent studies have investigated the various roles of microRNAs in *Aeromonas* spp. infected fish through experimental and computational approaches, which are discussed below.

4. The role of microRNAs in fish infected with *Aeromonas* spp.

4.1. The microRNAs in the context of the immune system against *Aeromonas* infections by targeting the *CiGadd* family

Gadd45 (growth arrest and DNA damage-inducible 45) consists of three members, including *Gadd45a*, *Gadd45b*, and *Gadd45g*. Gadd45 family members are involved in various signaling responses to genotoxic stressors, cell survival/arrest, DNA repair, apoptosis,

and innate immunity. Emerging evidence shows that proteins encoded by *Gadd45* genes play a critical role as sensors regulating the response of eukaryotic cells to a range of physiological and environmental stressors (18). Gadd45 family members are characterized by CiGadd45 in *Ctenopharyngodon idella* (19). In addition, CiGadd45 expression has been shown to be associated with bacterial infection (19). It should be noted that other studies have been performed in this field, which we will discuss below

4.1.1. MicroRNA-148

MicroRNA-148 belongs to the microRNA-148/152 family, which has been associated with the development and spread of various diseases in humans and aquatic animals. Recently, the microRNA-148/152 family has been shown to play a critical role in attenuating the innate immune response. For example, Liu et al. found that upregulation of microRNA-148/152 inhibited the biogenesis of cytokines on dendritic cells through *CaMKIIa* targeting (20). Chu et al. described a process by which overexpression of microRNA-148 attenuates activation of the NF-κβ pathway and production of inflammatory cytokines by myeloid differentiation factor 88 (*MyD-88*) targeting in teleost fish (21). A study by Fang et al. indicated that microRNA-148 was significantly less -expressed in *Ctenopharyngodon idella* kidney (CIK) cells within 2 hours after infection with A. hydrophila. Fang et al. also showed that upregulation of microRNA-148 by a mimic significantly inhibited the expression of immune genes such as *JNK*, *P38*, *ERK*, *IFN*, and *TNF-α* (22). It is noteworthy that the *JNK*, *P38*, and *ERK* genes encode cytokines relevant to the mitogen-activated protein kinase (MAPK) signaling pathway, which play an important role in inflammation and immune responses (23). In their study, microRNA-148 controlled the inflammatory response by decreasing the expression of proinflammatory genes such as *CiGadd45ba* and *CiGadd45bb*, subtypes of *Gadd45b* (22). Taken together, these studies suggest that microRNA-148 may be involved as an immunogenic factor in *Aeromonas* spp. infection and inflammatory response.

4.1.2. MicroRNA-23a-3p and microRNA-23a-5p

The microRNA-23a belongs to the microRNA-23a~27a~24-2 cluster, which shows altered expression in various diseases (22). Expression of microRNA-23a is regulated mainly by transforming growth factor-β (TGF-β) signaling (24). In a study by Fang et al. the role of microRNA-23a-3p and microRNA-23a-5p was investigated in detail in grass carp. Based on the microRNA expression profile and a dual-luciferase reporter assay, they indicated for the first time that *CiGadd45ab* may be a target gene for microRNA-23a-3p and microRNA-23a-5p (25). Expression of microRNA-23a-3p and microRNA-23a-5p in response to exposure to *A. hydrophila* decreased after 2 h. However, the expression of CiGadd45ab increased significantly after infection of CIK cells with A. hydrophila. It was confirmed that microRNA-23a-3p and microRNA-23a-5p are involved in apoptosis and inflammatory response of grass carp after infection with *A. hydrophila* by targeting *CiGadd45a* (23, 26). In addition, overexpression of microRNA-23a-3p and microRNA-23a-5p decreased apoptosis (caspase-3 and caspase-7) and immune-associated genes (*TNFα* and *IL-8*) and MAPK signaling in CIK cells infected with *A. hydrophila*. IL-8 plays an important role in neutrophil-induced acute inflammation. TNF-α is an important mediator of immune defense and inflammatory response (27). Thus, these data suggest that these microRNAs play an important role in many regulatory phases of the response of fish to bacterial infections, allowing suppression of inflammatory signals.

4.1.3. MicroRNA-731

MicroRNA-731 is a flounder microRNA involved in the early stages of viral infection, type I interferon response, viral replication, apoptosis, and cell cycle arrest (28). To date, few studies have investigated the

role of microRNA-731 in immune mechanisms. Therefore, the molecular basis and function of microRNA-731 in inflammation need to be further explored. A study conducted by Feng et al. showed that microRNA-731 targeted the *CiGadd45aa* gene (in CIK cells infected with *A. hydrophila*), which was associated with confirmation of the luciferase test (a luciferase enzyme reporter vector containing the wild-type 3′-UTR fragment of *CiGadd45aa*, which enhanced the binding sites of mir-731 for genomic DNA detection). After infection with, *A. hydrophila* in CIK cells, microRNA-731 expression decreased and CiGadd45aa expression increased significantly. After studying the immune and apoptosis response of microRNA-731 , the results showed the crucial immunogenic role of microRNA-731 in regulating inflammatory response through MAPK signaling pathway. Their data showed that downregulated microRNA-731 led to a decrease in immune genes such as p38, ERK JNK, IFN, TNF-α, and IL-8. Down-regulated microRNA-731 also increased the activity of caspase-3, caspase-7, and CiGadd45aa, which are considered pro-apoptotic genes (29). Thus, microRNA-731 may modulate the pro-inflammatory response, promote apoptosis and inhibit the cell cycle in CIK cells infected with *A. hydrophila* to prevent the progression of *Aeromonas* infection.

4.1.4. MicroRNA-429b

MicroRNA-429b is known as a member of the microRNA-200 family, which has been associated with the determination of cell growth, metastasis, and cell phenotype (30). MicroRNA-200 can also regulate immune responses by driving Th17 cell differentiation, leading to inhibition of T lymphocyte differentiation into Tregs and release of IL-17 and IL-22 (31). MicroRNA-429b has been studied in grass carp (32), but in general, there is little information about it in fish. A recent study reported that microRNA-429b can regulate the expression of *CiGadd45g* and downstream genes in CIK cells infected with *A. hydrophila* (33). Downstream genes of *CiGadd45g* ,including proinflammatory - cytokines (p38, JNK, IL-8, IFN-1, and TNF-α) and the MAPK pathway, were activated by *CiGadd45g* after infection with *A. hydrophila*. The results of this study showed that infection of grass carp with *A. hydrophila* downregulated microRNA-429 and conversely upregulated *CiGadd45g* expression (33). Moreover, *CiGadd45g* is mainly expressed in various tissues and is involved in innate antibacterial immunity in grass carp. By inhibiting the activity of *CiGadd45g* by microRNA-429b, the function of pro-inflammatory cytokines can be suppressed in grass carp infected with A. hydrophila.

4.2. The microRNAs in the context of the related to immune system against *Aeromonas* infections infection by targeting other genes

4.2.1. MicroRNA-146a

The limited number of microRNAs such as microRNA-146 is intensively promoted by bacterial endotoxin and shows prolonged expression, which has been associated with immune tolerance. Indeed, it acts as a fine-tuning system to curb the high motivation of the inflammatory response (34). Some bacterial infections such as *Mycobacterium Bovis* (*M. Bovis*) in macrophages and *Helicobacter pylori* in gastric epithelial cells could lead to the expression of microRNA-146a (35). Increased expression of microRNA-146a targeting *IRAK-1*, *TRAF-6*, and *PTGS-2* suppresses the inflammation and associated chemokines (MCP-1) and cytokines (IL-6) in macrophages after infection with M . *Bovis* (36). In a study evaluating the dietary immunostimulant CpG in salmon, microRNA-146a-1-2-3p, was identified as one of the most important microRNAs in response to injection of formalin-killed typical *Aeromonas salmonicida* (ASAL), indicating the importance of these microRNAs in enhancing the immune response of salmon to infectious diseases (37). Therefore, microRNA-146a appears to be a critical regulator of genes related to development, organogenesis, growth, tissue differentiation, and physiological processes. In contrast to mammals, the expression of microRNA-146a and its role in the response to bacterial infection

has been less studied in fish. A study by Ordas et al. found the induction of TRAF-6 and MyD-88-dependent expression of microRNA146a /b in zebrafish embryos after infection with *Salmonella typhimurium* (38). Previous studies have reported the tissue distribution of microRNA-146a in adult fish and the expression of microRNA-146a in larval fish (34). Similarly, a study by Liyanage et al. found that induction of *A. hydrophila* and *E. piscicida* infections in larval and adult zebrafish increased the expression of microRNA-146a and negatively affected its likely targets such as *MyD-88*, *TLR-4ba*, *TRAF-6*, *RELA, IL-1β*, *TNF-α*, *CXCL-18b*, and *CCL-34a.4*, which are mainly involved in regulating the inflammatory response. Moreover, overexpressed microRNA-146a can downregulate chemokines (MCP-1b and MMP-9) and cytokines (IL-6 and RELA) after *A. hydrophila* infection (39). Overall, the upregulation of microRNA-146a plays an important role in modulating genes involved in inflammation to reduce inflammatory responses.

4.2.2. MicroRNA-155–5p

It is widely believed that bacterial infections can alter the expression of host microRNAs. It has also been demonstrated that these differentially expressed microRNAs (DEMs) from fish play a role in regulating immune responses and signaling triggered by bacterial infections. Cao et al. examined rainbow trout (*Oncorhynchus mykiss*) as a model to evaluate host microRNA responses to *A. salmonicida subsp salmonicida* infection using Solexa deep sequencing of rainbow trout spleens infected with/without a clinical isolate of *A. salmonicida subsp salmonicida*. After analyzing Gene Ontology (GO) and Kyoto Encyclopedia (KEGG), they showed that microRNA-155–5p could promote *A. salmonicida*-induced inflammation and its overexpression increased A. salmonicida-induced production of IL-2 and IL-1β.. Based on their results, microRNA-155–5p could also be used as a potential molecular adjuvant for the bacterial vaccine in rainbow trout (40).

4.2.3. MicroRNA-122

It is well documented that microRNA-122 is the most abundant liver-specific microRNA (41-43) and contains approximately 70% of total liver microRNAs (44). Research has increasingly shown that microRNA-122 is involved in the regulation of hepatocyte proliferation, apoptosis, carcinogenesis, and immune response in the liver by targeting the signaling pathways of cyclin-G1, BCL-W, WNT-1, and TLR-4. In addition, microRNA-122 has been reported to play a role in hepatitis C virus (HCV) infection (45). A study conducted by Liu et al. showed that microRNA-122 triggers the response to *A. hydrophila* infection in *Epinephelus coioides* grouper (GSC) spleen cells . They presented that upregulation/downregulation of microRNA-122 in GSC cells inhibits/induces the expression of *IL-6*, *IL-15*, and *IL-1β*.. *IL-15* can be considered as a potential target of microRNA-122, which was confirmed by LUC assay, and microRNA-122 regulates the immune response of *E. coioides* to bacterial infection by triggering IL -15 (46).

4.2.4. MicroRNA-375

According to studies, microRNAs also play an important role in regulating basic biological processes and the immune response in the liver of *M. amblycephala* after infection with *A. hydrophila* (47, 48). In addition, GO and KEGG analyses of microRNA target prediction revealed that several biological pathways could be affected by *A. hydrophila* infection. Therefore, most of the differentially expressed microRNAs might contribute to the immune response of M. *amblycephala* to *A. hydrophila* infection by regulating the expression of associated genes in various immune-related pathways. In addition, recent studies show that microRNAs play a critical role in the regulation of iron metabolism (49). The results of the present studyshow that the *transferrin* (*TF*) and *transferrin receptor* (*TFR*) genes are direct targets of microRNA-375, which are central to iron

metabolism and homeostasis (50). Cui et al. demonstrated that after infection with *A. hydrophila* in *M. amblycephala*, downregulation of microRNA-375 could be an antibacterial mechanism by allowing expression of *TF* and *TFR* to create a bacteriostatic environment. These findings highlight the important role of microRNA-375 in regulating iron homeostasis during bacterial infection (51).

4.2.5. MicroRNA-142a-3p AND cid-miRn-115

Numerous studies over the years have underscored that microRNA-142 plays the major role in various biological processes and immune regulation, and inflammatory responses in diseases (52, 53). MicroRNA-142a-3p is a member of the microRNA-142 family. This family is highly conserved between vertebrates and invertebrates, representing its protected function (54). Apoptosis and polarization of macrophages involved in immune diseases and infections are important factors regulated by microRNAs (55). Accordingly, Tao et al. investigated the apoptosis- and macrophage-related regulatory mechanism of microRNA-142a-3p in grass carp infected with A. hydrophila. They found that microRNA-142a-3p was overexpressed in immune organs such as the trunk kidney, whereas its expression was significantly decreased in cells infected with *A. hydrophila* (56). The trunk kidney is the main organ that triggers innate immune system responses in grass carp (57). In addition, *TNFα-induced protein 2* (*TNFAIP-2*) and *glucose transporter 3* (*GLUT-3*) were identified as direct targets of microRNA-142a-3p, and expression correlation analysis, gene overexpression, and the dual-luciferase reporter assay (56). TNFAIP-2 and *GLUT-3* are involved in various biological processes such as cell proliferation, apoptosis, and inflammation (for *TNFAIP-2*) (58) and cAMP, NF-κβ, and p53 signalling pathways (for *GLUT-3*) (59). In the present study, overexpression of microRNA-142a-3p by agomir (antagonist of microRNA) decreased cell viability, and increased anti-inflammatory factors and cell apoptosis by regulating macrophage polarization via *TNFAIP-2* and *GLUT-3* in grass carp infected with A. hydrophila (56). Furthermore, a previous study by the same research team identified microRNA-142a-3p and cid-miRn-115 as two of 21 DEMs expressed in the kidneys of grass carp between susceptible and resistant types of *A. hydrophila* (54). These data suggest an effective microRNA in the immunogenic regulatory process that enables inhibition of opportunistic bacterial pathogens.

4.2.6. cid-miRn-118 AND Let-7i

The most serious threat to aquaculture is bacterial septicemia as, indicated by previous findings; the presence of *A.hydrophila* in natural waters has been the most important septic factor in recent years, especially motile aeromonad septicemia (MAS) in Chinese aquatic animals.

Because of the importance of grass carp in the Chinese fish farming industry, Lu et al. investigated microRNAs associated with grass carp infection with *A. hydrophila* in another study. They were identified using next-generation sequencing of microRNAs during immune activation. They identified 185 microRNAs in *A. hydrophila*-susceptible and resistant grass carp using Illumina next-generation sequencing during immune activation. 21 of them showed different expression between susceptible and resistant grass carp. This study showed that *cid-miRn-118* and *let-7i* were significantly upregulated in grass carp susceptible to *A. hydrophila* in contrast to *A. hydrophila*-resistant fish. The TLR *-4* and *NFIL-3,6* genes, which are direct targets of let-7i and cid-miRn-118, also play important roles in antibacterial immune processes (52). Table 1 summarizes the role of important microRNAs involved in *Aeromonas* infection of fish. The results of such studies support the discovery of target genes of microRNAs involved in bacterial septicemia outbreaks during *Aeromonas* infection in fish. On the other hand, knowledge and understanding of tissue-specific expression patterns of microRNAs may be helpful in functional studies.

Table 1. Involved microRNAs in the immune response of fish with *Aeromonas* spp. Infection

N	miRNA	Species/ Type of study	Infection with	miR expression after infection	Target	Function	Ref
1	miR-148	Grass carp (CIK cells)/ In *vitro*	*A. hydrophila*	Down	*Ci Gadd45b (bb,ba)*	Induces the inflammatory response by increasing JNK, P38, ERK, IFN, TNF-α, and MAPK pathway	(22)
2	miR-23a-3p and miR-23a-5p	Grass carp (CIK cells)/ In *vitro*	*A. hydrophila*	Down	*Ci Gadd45ab*	Increases apoptosis by caspase3/7 and inflammatory response by TNFα, IFN, IL-8, and MAPK pathway	(25)
3	miR-731	Grass carp (CIK cells)/ In *vitro*	*A. hydrophila*	Down	*CiGadd45aa*	Promotes the proinflammatory response by p38, ERK JNK, IFN, TNF-α, IL-8, MAPK pathway, and apoptosis by caspase3/7	(29)
4	miR-429b	Grass carp (CIK cells)/ In *vitro*and in *vivo*	*A. hydrophila*	Down	*CiGadd45g*	Induces the inflammatory response by p38, JNK, IL-8, IFN-1, TNF-α, and MAPK pathway	(33)
5	miR-146a	Zebrafish(larvae and adult)/In *vivo*	*A.hydrophila* and *E. piscicida*	Up	*MyD-88, TLR-4ba, TRAF-6, IL-1β, TNF-α, CXCL-18b, and CCL-34aN*	Reduces the inflammatory responses by inhibiting the chemokines (MCP-1b and MMP-9) and cytokines (IL-6 and RELA)	(39)
6	miR-155-5p	rainbow trout (RTG-2 cells)/ In silicoand In *vitro*	*A.salmonicida*	Up	NM	Induces the inflammatory response by enhancing IL-2, IL-1β, IL-2, and IL-1β	(40)
7	miR-122	Epinephelus coioides (GSC cells)/In *vitro*	*A. hydrophila*	NA	*IL-15*	regulates the immune response by triggering IL-15, IL-6, and IL-1β	(46)
8	miR-375	Blunt Snout Bream/In silicoandIn *vivo*	*A. hydrophila*	Down	*TF* and *TFR*	Increases the antibacterial mechanism by TF and TFR expression and creation of a bacteriostatic environment	(51)
9	miR-142a-3p	Grass carp/ In silico andIn *vivo*	*A. hydrophila*	Down	*TNFAIP-2* and *GLUT-3*	Promotes the proinflammatory response by regulation of macrophage polarization and decreases apoptosis by caspase3/7	(56)
10	142a-3p and cid-miRn-115	Grass carp (CIK cells)/ In silico andIn *vitro*	*A. hydrophila*	NM	*TLR-5*	Affects the immune functions by a modulation of IL-1β, IL-8 and TNF-α expression	(54)
11	cid-miRn-118 and Let-7i	Grass carp/ In silico andIn *vivo*	*A. hydrophila*	Up	*TLR-4* and *NFIL-3,6*	Modulates motile aeromonad septicemia	(52)

(NA: Not Available, NM: Not Mentioned)

5. Discussion

Bacteria are one of the most important pathogens that harm aquaculture. Despite the importance of *Aeromonas* in aquatic infections, the mechanisms of bacterial survival against the host immune system are not well understood. Given the importance of microRNAs in regulating immunity and inflammation, researchers' attention has been drawn to exploring the role of microRNAs in inducing an immune response during infection with *Aeromonas* spp in fish. The various *in silico*, *in vitro*, and *in vivo* studies over the past decade have recognized many dysregulated microRNAs including miR-148, miR-23a-3/5p, miR-731, miR-429b, miR-146a, miR-155-5p, miR-122, miR-375, miR-142a-3p, 142a-3p, cid-miRn-115, cid-miRn-118, and Let-7i, showing different patterns in key steps of immune response regulation in *Aeromonas*-infected fish. These studies suggest that microRNAs may play a potential role in regulating the immune response fish , as they are part of the interacting network of biomolecules that protect fish from *Aeromonas* infection. They also suggest that the bacteria use microRNAs during infection, as microRNAs can control gene expression at the post-transcriptional level. However, identification of effective microRNAs in Aeromonas infected fish requires a-deep understanding of their physiological significance in a specific context in living systems. Moreover, profiling and identification of microRNAs in infected fish is the beginning of a longer path to reveal the functional mechanisms and variations of microRNA regulatory pathways to identify key targets in infected fish. Finally, further research on microRNAs can be expected to guide the broader application of microRNAs in the therapeutic strategies of *Aeromonas* -infected fish.

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Authors' Contribution

literature review and research, conceptualization, methodology, supervision, project administration, writing-reviewing and editing, methodology, investigation, studies analysis: S.G.K., H.S.

Writing original draft preparation, writing-reviewing and editing, and methodology: F.K.D., I.D. investigation.

Validation and Reviewing: D.A., S.H., N.GH.

Ethics

Not Applicable.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

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