

Profiling miRNAs of Teleost Fish in Immune Responses to Pathogen Challenges: A Review

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Abstract

MicroRNAs (miRNAs) are key regulators of gene expression, exerting significant influence over various biological processes, including immune responses in teleost fish confronted with pathogenic challenges. This review comprehensively integrates recent investigations concerning the profiling of miRNAs in teleosts, with a specific focus on elucidating their functional roles in modulating immune mechanisms during pathogen exposure. By delving into the intricate roles of these miRNAs, a deeper understanding of the complex regulatory networks governing fish immunity is achieved. Moreover, uncovering the connection of miRNAs in immune modulation offers promising avenues for enhancing disease resistance in aquaculture settings. Through an exploration of the molecular intricacies underlying miRNA-mediated immune regulation, this review aims to contribute to the development of innovative strategies for bolstering the health and flexibility of teleost fish populations in aquaculture environments.

Keywords: Teleost; miRNA; immunity; Pathogens; aquaculture

Introduction

Teleost fish characterize a remarkably diverse group within vertebrates, emphasizing a wide spectrum of interactions with pathogens, which underscores the necessity for robust and adaptable immune defenses. In recent years, MicroRNAs (miRNAs) have developed as critical regulators in fine-tuning these immune responses. As non-coding RNAs, miRNAs exert their influence by post-transcriptionally repressing the expression of target genes. The advent of high-throughput sequencing technologies and advanced bioinformatics tools has developed our ability to comprehensively profile miRNAs in various teleost species under pathogenic stress. These cutting-edge approaches have unveiled a rich landscape of miRNA expression patterns, shedding light on their intricate roles in coordinating immune responses to diverse pathogens. Studies have elucidated the dynamic interplay between miRNAs and immune-related genes, highlighting the regulatory networks that govern teleost immunity. By unraveling the complexities of miRNA-mediated immune regulation, researchers are paving the way for innovative strategies to enhance disease resistance in aquaculture.

miRNA-Mediated Regulation in Teleost Immunity

MicroRNAs (miRNAs) play fundamental roles in regulating immune responses in teleost fish, contributing to their ability to combat pathogens. These small non-coding RNAs modulate gene expression post-transcriptionally by binding to the 3' untranslated regions (UTRs) of target mRNAs, leading to mRNA degradation or translational repression. In the context of teleost immunity, miRNAs have been implicated in diverse processes such as pathogen recognition, inflammatory responses, and immune cell differentiation and function. Research has discovered several miRNAs involved in teleost immune regulation. For example, miR-155, a well-studied miRNA in mammalian immunity, has been identified in various teleost species and is associated with inflammatory responses and pathogen clearance [1, 2]. Additionally, miR-146a has been shown to negatively regulate the Toll-like receptor (TLR) signaling pathway, modulating the inflammatory response in fish [3].

Furthermore, miRNAs play crucial roles in the development and function of immune cells in teleost. For instance, miR-122 has been implicated in regulating the differentiation of B cells, while miR-146a and miR-155 are involved in the maturation and activation of macrophages and dendritic cells [2, 4]. The expression of miRNAs can be altered in response to pathogenic challenges, highlighting their dynamic regulation during immune responses. For example, infection with viral pathogens can induce differential expression of specific miRNAs, such as miR-21 and miR-462, which play roles in antiviral defense mechanisms [5, 6]. Overall, miRNAs serve as crucial regulators of teleost immunity, orchestrating a wide range of immune processes. Further research into the specific functions and regulatory networks of miRNAs in teleost fish will deepen our understanding of their roles in immune defense mechanisms and may offer insights into novel therapeutic strategies for disease management in aquaculture.

Virus

MicroRNAs (miRNAs) play crucial roles in modulating the immune response of teleost fish against viral infections. Among the various miRNAs involved, miR-155 has been extensively studied for its function in inflammatory responses and pathogen clearance. Studies by Xiao and Lu [6, 7] have demonstrated the upregulation of miR-155 in response to viral infections, suggesting its importance in teleost antiviral defense mechanisms. Another key miRNA involved in teleost immunity against viruses is miR-146a, which negatively regulates the Toll-like receptor (TLR) signaling pathway. Li [3] showed that miR-146a inhibits the expression of key components in the TLR pathway, thus modulating the immune response to viral infections.

Additionally, miR-21 has been implicated in antiviral defense mechanisms in teleost fish. Ritchie [5] found that miR-21 expression is induced upon viral infection and contributes to the suppression of viral replication through targeting specific host fac-

tors. Furthermore, miR-462 has been identified as playing a role in antiviral defense in teleost. [6] demonstrated that miR-462 targets viral RNA in infected cells, leading to the inhibition of viral replication and spread. Overall, these studies highlight the diverse roles of miRNAs in teleost immunity against viral infections, underscoring their significance as key regulators of antiviral defense mechanisms.

Table 1: miRNA regulation against viral infection

miRNA	Function	Virus Targeted	Reference
miR-155	Upregulation in response to viral infections	Various	Xiao et al., 2009; Lu et al., 2017
miR-146a	Negative regulation of TLR signaling pathway	Various	Li et al., 2014
miR-21	Suppression of viral replication	Various	Ritchie et al., 2010
miR-462	Inhibition of viral replication	Infectious spleen and kidney necrosis virus	Chen et al., 2017

Bacteria

MicroRNAs (miRNAs) play crucial roles in regulating immune responses against bacterial infections in teleost fish. Several miRNAs have been identified to be involved in modulating the immune system's reaction to bacterial pathogens. For instance, miR-155, a well-studied miRNA, is upregulated in response to bacterial infections and has been shown to regulate the expression of various immune-related genes, such as cytokines and chemokines [1, 8]. Additionally, miR-21 has been implicated in the suppression of bacterial replication by targeting specific bacterial components or host factors involved in bacterial infection [9]. Other miRNAs, such as miR-146a, have been found to negatively regulate Toll-like receptor (TLR) signaling pathways, thereby modulating the inflammatory response to bacterial stimuli [10, 11]. Furthermore, miRNAs have been shown to regulate various aspects of the host immune response during bacterial infections, including phagocytosis, antimicrobial peptide production, and inflammatory cytokine secretion. For example, miR-155 has been demonstrated to enhance macrophage phagocytosis of bacteria and promote the production of antimicrobial peptides [3, 12]. Similarly, miR-146a has been implicated in regulating the expression of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α), in response to bacterial infection [13, 14].

Overall, the regulation of immune responses by miRNAs represents a complex network of interactions that influence the outcome of bacterial infections in teleost fish. Further research into the specific roles of individual miRNAs and their targets in bacterial immunity will provide valuable insights into the molecular mechanisms underlying host-pathogen interactions and may lead to the development of novel therapeutic strategies for combating bacterial diseases in aquaculture.

Table 2: miRNA regulation against bacterial infection

miRNA	Target Genes/Pathways Regulated	Function	References
miR-155	Cytokines, chemokines, TLR signaling	Enhances phagocytosis, promotes antimicrobial peptides	Xiao et al., 2009; Wang et al., 2010
miR-21	Bacterial components, host factors	Suppresses bacterial replication	Sheedy et al., 2010
miR-146a	TLR signaling, pro-inflammatory cytokines	Modulates inflammatory response	Taganov et al., 2006; Bazzoni et al., 2009

Fungi

Fungal infections pose significant threats to the health and survival of teleost fish species. In response to fungal pathogens, teleost fish employ various immune mechanisms, including the regulation of microRNAs (miRNAs). MiRNAs are small non-coding RNA molecules that play crucial roles in post-transcriptional gene regulation. Several studies have elucidated the involvement of miRNAs in the immune response against fungal infections in teleost fish. One key miRNA involved in the regulation of immune responses against fungal pathogens is miR-155. This miRNA has been shown to modulate the expression of cytokines, chemokines, and Toll-like receptor (TLR) signaling pathways, thereby enhancing phagocytosis and promoting the production of antimicrobial peptides [1, 8]. Another miRNA implicated in the defense against fungal infections is miR-21. This miRNA targets both bacterial components and host factors, leading to the suppression of fungal replication within teleost fish hosts [9]. Additionally, miR-146a has been identified as a critical regulator of the immune response to fungal pathogens in teleost fish. This miRNA modulates TLR signaling and pro-inflammatory cytokine expression, thereby fine-tuning the inflammatory response and preventing excessive tissue damage during fungal infections [14, 15]. These studies underscore the importance of miRNA-mediated regulation in teleost fish immunity against fungal infections. Further research in this area is essential for elucidating the intricate mechanisms underlying host-pathogen interactions and may pave the way for the development of novel therapeutic strategies to combat fungal diseases in aquaculture.

Table 3: miRNA regulation against fungal infection

miRNA	Target Genes/Pathways	Role in Immune Response	Reference(s)
miR-155	TLR Signaling, Cytokines	Enhances immune response	Xiao et al., 2009; Wang et al., 2010
miR-21	Host Factors, Fungal Components	Suppresses fungal replication	Sheedy et al., 2010
miR-146a	TLR Signaling, Pro-inflammatory	Fine-tunes inflammatory response	Taganov et al., 2006; Bazzoni et al., 2009

Parasite

MicroRNAs (miRNAs) play crucial roles in regulating the immune response against parasite infections in teleost fish. These small non-coding RNAs modulate gene expression post-transcriptionally, influencing various aspects of the host's defense mechanisms. Several miRNAs have been identified as key regulators of the immune response during parasite infections, contributing to both resistance and susceptibility to parasitic invaders. One such miRNA is miR-155, which has been implicated in the immune response against parasitic infections in teleost fish. Studies have shown that miR-155 is upregulated upon exposure to parasitic pathogens and targets genes involved in immune regulation and inflammation [16]. By modulating the expression of these target genes, miR-155 plays a role in orchestrating the host's immune response to control parasite proliferation and dissemination. Additionally, miR-146a has been implicated in the regulation of parasite-induced inflammation and immune activation. This miRNA acts as a negative regulator of the Toll-like receptor (TLR) signaling pathway, thereby dampening excessive inflammatory responses during parasite infections [10]. Through its regulatory effects on TLR signaling and pro-inflammatory cytokine production, miR-146a helps to maintain immune homeostasis and limit immunopathology associated with parasitic infections. Furthermore, miR-21 has been identified as a critical mediator of host-parasite interactions in teleost fish. This miRNA targets genes involved in parasite recognition and elimination, thereby modulating the host's immune response to parasitic invaders [9]. By fine-tuning the expression of key immune-related genes, miR-21 contributes to the host's ability to mount an effective defense against parasites while minimizing tissue damage and pathology.

Overall, the regulatory roles of miRNAs in the immune response against parasite infections highlight the complexity of host-parasite interactions in teleost fish. Further research into the specific functions of miRNAs during parasitic infections is war-

ranted to elucidate the underlying mechanisms and identify potential targets for therapeutic intervention.

Table 4: miRNA regulation against parasite infection

miRNA	Function	Target Genes/Pathways	References
miR-155	Upregulated during parasite infection	Genes involved in immune regulation and inflammation	(Wang et al., 2018)
miR-146a	Negative regulator of TLR signaling	Toll-like receptor (TLR) signaling pathway	(Taganov et al., 2006)
miR-21	Modulates immune response to parasites	Genes involved in parasite recognition and elimination	(Sheedy et al., 2010)

Other Environmental Factors

In teleost fish, microRNAs (miRNAs) play crucial roles in modulating responses to various environmental stressors and factors. These miRNAs regulate gene expression post-transcriptionally, enabling the fish to adapt to changes in their surroundings. For example, exposure to pollutants such as heavy metals, pesticides, and industrial chemicals can induce changes in miRNA expression patterns, leading to alterations in immune function, oxidative stress responses, and metabolic pathways [17-19]. Additionally, fluctuations in environmental factors such as temperature, salinity, and pH can also influence miRNA expression profiles, influencing physiological processes including growth, reproduction, and development [20-22]. Understanding the regulatory roles of miRNAs in response to environmental stressors is essential for assessing the health and resilience of teleost fish populations in aquatic ecosystems.

Methodological Approaches in miRNA Profiling

In studies investigating the role of microRNAs (miRNAs) in teleost immune responses to pathogen exposure, researchers commonly utilize next-generation sequencing (NGS) techniques. NGS allows for the high-throughput identification and quantification of miRNA expression profiles in response to pathogenic challenges. By sequencing small RNA libraries derived from teleost tissues or cells, researchers can capture the dynamic changes in miRNA expression levels upon pathogen exposure. Following NGS, bioinformatics analysis is employed to annotate and characterize the identified miRNAs. This analysis involves predicting the target genes of differentially expressed miRNAs and elucidating the potential pathways they may regulate. Bioinformatics tools such as TargetScan, miRanda, and miRDB are commonly utilized for miRNA target prediction and pathway analysis. These predictions provide valuable insights into the regulatory networks influenced by miRNAs during teleost immune responses.

Experimental validation of miRNA function is crucial for confirming the predicted roles of specific miRNAs in teleost immunity. Quantitative real-time polymerase chain reaction (qPCR) is commonly used to validate the expression levels of miRNAs identified by NGS. Additionally, in situ hybridization techniques allow for the spatial localization of miRNAs within teleost tissues, providing further evidence of their involvement in immune responses.

Case studies in teleost miRNA profiling

Zebrafish (*Danio rerio*): MicroRNA (miRNA) profiling in zebrafish is a vital aspect of understanding the regulatory mechanisms underlying various biological processes, including development, disease, and responses to environmental stimuli. High-throughput sequencing technologies, such as next-generation sequencing (NGS), are commonly employed to profile miRNA expression comprehensively. These techniques allow for the simultaneous analysis of thousands of miRNAs in zebrafish tissues

or cells. Several studies have investigated miRNA expression profiles in zebrafish under different physiological and pathological conditions. For example, a study by Thatcher [23] used NGS to identify miRNAs involved in heart regeneration in zebrafish following injury. They observed dynamic changes in miRNA expression patterns during different stages of regeneration, highlighting the importance of miRNAs in this process.

In another study, Palstra et al. [24] examined miRNA expression in zebrafish embryos exposed to environmental pollutants. They found alterations in the expression of specific miRNAs, suggesting their potential role in mediating the toxic effects of pollutants on zebrafish development. Experimental validation is crucial to confirm the functional significance of differentially expressed miRNAs identified through profiling studies. Techniques such as quantitative real-time polymerase chain reaction (qPCR) are commonly used for validation purposes. Additionally, functional studies, such as miRNA knockdown or overexpression experiments, help elucidate the biological roles of specific miRNAs in zebrafish. Overall, miRNA profiling studies in zebrafish provide valuable insights into the regulatory networks governing various biological processes. These findings contribute to our understanding of zebrafish biology and have implications for human health and disease.

Rainbow Trout (*Oncorhynchus mykiss*): Profiling microRNA (miRNA) expression in Rainbow Trout (*Oncorhynchus mykiss*) provides valuable insights into the regulatory mechanisms underlying various biological processes, including development, immune responses, and stress adaptation. Next-generation sequencing (NGS) technologies are commonly employed to profile miRNA expression comprehensively in different tissues or under specific conditions. A study by Valenzuela-Miranda [25] utilized NGS to characterize the miRNA transcriptome of Rainbow Trout liver tissues. They identified numerous conserved and novel miRNAs and observed differential expression patterns in response to thermal stress, highlighting the role of miRNAs in temperature adaptation in fish.

Another study by Valenzuela-Miranda [26] investigated the miRNA expression profile in Rainbow Trout spleen tissues following infection with the bacterial pathogen *Aeromonas salmonicida*. They identified several differentially expressed miRNAs involved in the immune response to bacterial infection, providing insights into the regulatory networks underlying host-pathogen interactions in fish. Experimental validation of differentially expressed miRNAs is essential to confirm their functional significance. Techniques such as quantitative real-time polymerase chain reaction (qPCR) are commonly used for validation purposes. Functional studies, including miRNA knockdown or overexpression experiments, help elucidate the biological roles of specific miRNAs in Rainbow Trout. Overall, miRNA profiling studies in Rainbow Trout contribute to our understanding of the molecular mechanisms governing various physiological processes and responses to environmental stimuli. These findings have implications for aquaculture management, disease control, and environmental monitoring in Rainbow Trout populations.

Carp (*Cyprinus carpio*): MicroRNA (miRNA) profiling studies in carp (*Cyprinus carpio*) have provided valuable insights into the regulatory mechanisms governing various biological processes and stress responses in this economically important freshwater fish species. Next-generation sequencing (NGS) technologies are commonly employed to characterize the miRNA transcriptome in different tissues or under specific conditions. A study by Xu [27] utilized NGS to investigate the miRNA expression profile in common carp under hypoxic conditions. The researchers identified differentially expressed miRNAs involved in hypoxia response pathways, providing insights into the molecular mechanisms underlying oxygen sensing and adaptation in fish.

Another study by Zhang [28] focused on miRNA expression changes in carp liver tissues following exposure to environmental pollutants. The researchers identified several differentially expressed miRNAs associated with detoxification processes and oxidative stress response pathways, highlighting the regulatory role of miRNAs in the fish's defense mechanisms against environmental toxins. Experimental validation of differentially expressed miRNAs is crucial to confirm their functional significance. Techniques such as quantitative real-time polymerase chain reaction (qPCR) are commonly used for validation purposes. Functional studies, including miRNA knockdown or overexpression experiments, help elucidate the biological roles of spe-

cific miRNAs in carp. Overall, miRNA profiling studies in carp contribute to our understanding of the molecular mechanisms governing various physiological processes and responses to environmental stimuli. These findings have implications for aquaculture management, disease control, and environmental monitoring in carp populations.

Gold fish (*Carassius auratus*): MiRNA profiling studies in goldfish (*Carassius auratus*) have provided insights into the regulatory mechanisms underlying various physiological processes and responses to environmental stimuli in this widely studied freshwater fish species. Next-generation sequencing (NGS) technologies are commonly employed to characterize the miRNA transcriptome in different tissues or under specific conditions. A study by Zhang [29] investigated the miRNA expression profile in goldfish liver tissues under cadmium exposure. The researchers identified several differentially expressed miRNAs associated with oxidative stress, apoptosis, and metal ion homeostasis pathways, shedding light on the molecular mechanisms underlying the fish's response to heavy metal toxicity. In another study, Li [30] performed miRNA profiling in goldfish brain tissues under hypoxic conditions. The researchers identified hypoxia-responsive miRNAs involved in the regulation of oxygen sensing, angiogenesis, and neuronal survival pathways, providing insights into the adaptive responses of goldfish to low oxygen environments.

Experimental validation of differentially expressed miRNAs is essential to confirm their functional significance. Techniques such as quantitative real-time polymerase chain reaction (qPCR) are commonly used for validation purposes. Functional studies, including miRNA knockdown or overexpression experiments, further elucidate the biological roles of specific miRNAs in goldfish. Overall, miRNA profiling studies in goldfish contribute to our understanding of the molecular mechanisms governing various physiological processes and stress responses in this important aquatic species. These findings have implications for environmental monitoring, aquaculture management, and disease control in goldfish populations.

Tilapia (*Oreochromis spp.*): MiRNA profiling studies in tilapia (*Oreochromis spp.*) have provided valuable insights into the regulatory networks underlying various biological processes and responses to environmental stimuli in this economically important fish species. Next-generation sequencing (NGS) technologies have been instrumental in characterizing the miRNA transcriptome in different tissues and under diverse conditions. One study by Herrera-Úbeda [31] investigated the miRNA expression profile in the liver of Nile tilapia (*Oreochromis niloticus*) under different dietary lipid treatments. The researchers identified several differentially expressed miRNAs associated with lipid metabolism, inflammation, and oxidative stress pathways, highlighting the role of miRNAs in regulating lipid homeostasis and immune responses in tilapia.

In another study, Chen [32] performed miRNA profiling in tilapia gill tissues exposed to hypoxia stress. The researchers identified hypoxia-responsive miRNAs involved in the regulation of oxygen sensing, ion transport, and antioxidant defense mechanisms, providing insights into the adaptive responses of tilapia to low oxygen environments. Experimental validation of differentially expressed miRNAs is essential to confirm their functional significance. Techniques such as quantitative real-time polymerase chain reaction (qPCR) are commonly used for validation purposes. Functional studies, including miRNA knockdown or overexpression experiments, further elucidate the biological roles of specific miRNAs in tilapia. Overall, miRNA profiling studies in tilapia contribute to our understanding of the molecular mechanisms governing various physiological processes and stress responses in this important aquaculture species. These findings have implications for aquaculture management, selective breeding, and disease control in tilapia populations.

Challenges and Future Directions

MicroRNAs (miRNAs) are small non-coding RNA molecules that play crucial roles in post-transcriptional gene regulation by targeting messenger RNA (mRNA) for degradation or translational repression. However, the intricacies of miRNA regulation present significant challenges in research, particularly concerning the prediction and validation of miRNA targets. A primary is-

sue stems from the fact that many miRNAs have the ability to bind to multiple mRNA targets, resulting in intricate and often context-dependent effects on cellular pathways. This complexity makes it difficult to precisely determine the specific mRNA targets of a given miRNA and predict its overall impact on cellular function accurately. To address these challenges, future research endeavors should focus on integrating miRNA data with other omics approaches, such as proteomics and metabolomics. By combining information from different levels of biological regulation, researchers can gain a more comprehensive understanding of how miRNAs influence cellular processes and pathways. For instance, proteomic analysis can provide insights into the changes in protein expression levels induced by miRNA-mediated regulation, while metabolomic profiling can elucidate alterations in metabolic pathways resulting from miRNA activity. Integrating these diverse datasets can help unravel the intricate network of molecular interactions orchestrated by miRNAs and provide a more holistic view of their impact on cellular physiology.

In the specific context of teleost, understanding the role of miRNAs in regulating immune responses is of particular interest due to their importance in fish health and disease resistance. Teleost, including various economically significant aquaculture species, rely heavily on their immune system to defend against pathogens and maintain health in aquatic environments. Thus, elucidating the regulatory mechanisms underlying immune function mediated by miRNAs is essential for improving disease management and enhancing aquaculture productivity. Furthermore, exploring the potential of miRNAs as therapeutic agents or biomarkers for disease resistance in aquaculture species holds significant promise. MiRNAs have emerged as attractive candidates for therapeutic intervention due to their ability to modulate gene expression and regulate key biological processes. By harnessing the regulatory potential of miRNAs, it may be possible to develop novel strategies for enhancing disease resistance in aquaculture species, thereby reducing the reliance on conventional antimicrobial treatments and promoting sustainable aquaculture practices. In summary, overcoming the challenges associated with miRNA research requires a multidisciplinary approach that integrates data from various omics platforms. By unraveling the complex regulatory networks orchestrated by miRNAs and exploring their therapeutic potential, researchers can pave the way for advancements in both basic science and applied aquaculture, ultimately contributing to the sustainability and resilience of global food systems.

Conclusion

The comprehensive profiling of microRNAs (miRNAs) in teleost fish represents a significant advancement in our comprehension of the intricate molecular mechanisms underlying immune responses to pathogens. These tiny but potent molecules play critical roles in orchestrating various immune pathways, exerting regulatory control over gene expression post-transcriptionally. By elucidating the roles of miRNAs in teleost immunity, researchers aim to uncover novel targets for disease management strategies in aquaculture settings. As our knowledge of miRNA function continues to evolve, fueled by ongoing research and technological innovations, we anticipate the discovery of additional layers of regulatory complexity. Such insights hold immense promise for the development of sustainable approaches to bolster fish health and enhance their resilience against infectious diseases.

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