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MicroRNAs involved in innate immunity regulation in the sea cucumber: A review

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ABSTRACT

The sea cucumber is one of the most economically significant echinoderms. The immunity against exogenous stimulation of sea cucumber is of great academic and economic importance. MicroRNAs (miRNAs) are a class of short endogenous non-coding RNAs (ncRNAs) that are considered as vital regulators of both innate and adaptive immune responses in most eukaryotes. In sea cucumbers, some miRNAs (such as miR-133, miR-137, and miR-2008, among others) that participate in the regulation of innate immunity have been recently identified and characterized. This review focuses on those known miRNAs and their corresponding target genes that participate in the regulation of the complement system, Toll-like receptor (TLR) pathway, reactive oxygen species (ROS) production and apoptosis pathways in sea cucumbers. Moreover, we cover immune-related miRNA investigations in sea cucumbers that provide insights into developing more miRNA-based biomarkers and therapeutic strategies for sea cucumber diseases.

1. Introduction

Echinoderms, complex marine invertebrates with radially symmetrical bodies, are important components of the benthic fauna. For decades, echinoderms have been a special group of deuterostomes that have served as an excellent model organism for inferring the evolution of innate immunity [1]. The sea cucumber belongs to the class Holothuroidea of the phylum Echinodermata. More than 70 species of sea cucumbers are currently used in commercial aquaculture worldwide [2]. In Asia, the sea cucumber industry has boomed since the 1980s due to an increasing interest in their nutritional and medicinal value [3]. For example, as the primary commercial species in the Western Pacific region, the aquaculture yield of *Apostichopus japonicus* in China increased from 93 kilotons in 2008 [4] to 220 kilotons in 2017 [5].

In recent years, quality control of sea cucumber fishery has become more difficult due to the tremendous growth of sea cucumber production [6,7], as well as the more frequent outbreaks of pathogen-induced diseases [1]. These issues have severely restricted the sustainable growth of the sea cucumber industry [8]. Thus, studies on innate immune responses and mechanisms of sea cucumbers are not only of academic importance but also critical for the development of strategies to combat pathogenic infections in economically important sea cucumbers. The immunity of sea cucumbers is mediated by coelomocytes and immune-related humoral factors [1]. To date, a growing number of immune-related genes have been identified and characterized at the genomic, transcriptomic, and proteomic levels in echinoderms [9]. However, the regulatory mechanisms of the immunity of sea cucumbers remain elusive.

MicroRNAs (miRNAs) are a class of short, endogenous non-coding RNAs (ncRNAs) of about 20–25 nucleotides (nt) in length [10]. Typically, the biogenesis of miRNAs can be divided into four steps: (1) the generation of primary miRNAs (pri-miRNAs) in the nucleus, (2) the process of converting pri-miRNAs into precursor mRNAs (pre-miRNAs), (3) the transport of pre-miRNAs from the nucleus to the cytoplasm, and (4) the formation of mature miRNAs (or pre-miRNA modification) [11]. Since *lin-4* was first discovered in *Caenorhabditis elegans* in 1993 [12], it has been widely demonstrated that miRNAs can regulate the expression of their target genes at the post-transcriptional level by binding to specific "seed sequences" at the 3′-untranslated regions (UTRs) of their specific mRNA targets in most eukaryotes [13]. Research conducted on echinoderms on the roles of miRNAs in the normal developmental process and stress response has been increasing in recent years [14]. To date, 554 miRNAs have been identified in echinoderms, and only about 169 entries of echinoderm miRNAs have been submitted and annotated in miRBase (http://www.mirbase.org/) (Fig. 1); therefore, it is necessary to improve our knowledge of sea cucumber miRNAs and their

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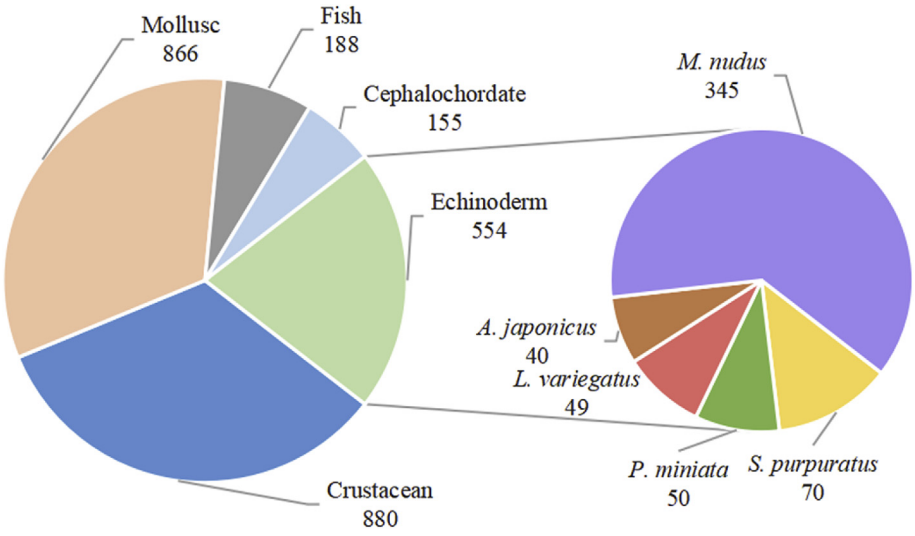

Fig. 1. Number of miRNAs identified in marine animals and echinoderms (as of April 2019). *S. purpuratus* represents *Strongylocentrotus purpuratus*, *P. miniatar* represents *Patiria miniata*, *A. japonicus* represents *Apostichopus japonicus*, *M. nudus* represents *Mesocentrotus nudus* and *L. variegatus* represents *Lytechinus variegatus*.

corresponding functions.

In this review, we summarize all reported miRNAs and their corresponding target genes that participate in innate immune responses in sea cucumbers. In addition, we discuss further directions of immune-related miRNA investigations in sea cucumbers.

2. miRNAs involved in the regulation of the complement system

The complement system is an essential component of innate immunity in sea cucumbers that plays an important role in eliminating invading pathogens and maintaining the stability of the internal environment [15]. The sea cucumber complement system mainly operates via an alternative pathway similar to that of vertebrates [16]. The activation of C3, the central molecule of the complement system, and the initiation of the terminal or lytic pathway are considered important functional mechanisms of the complement system.

MiR-133 is a highly conserved miRNA of 23 nt in length, which includes a 7-nt "seed region" (-UUGGUCC-). Sequence alignment indicates that mature miR-133a and miR-133b sequences are highly conserved in eukaryotes [17]. In marine animals, miR-133 family members have been identified and characterized in zebrafish (*Danio rerio*) [17] and sea cucumber (*A. japonicus)* [18,19]. MiRNA-binding site prediction showed that the seed region of miR-133 is in the 3′-UTR of *C3* gene in *A. japonicus* (designated as *AjC3*) (Fig. 3). Recent emerging evidence has indicated that the up-regulation of miR-133 occurs 6–12 h after lipopolysaccharide (LPS) stimulation in *A. japonicus*, and reaches its expression peak 9 h after LPS stimulation. Meanwhile, the down-regulation of *AjC3* expression occurs 9–12 h after LPS stimulation in *A. japonicus* [20]. This indicated a negative correlation of expression between miR-133 and *AjC3* at 9–12 h after LPS stimulation in *A. japonicus*. Considering these observations, we hypothesized that miR-133 might regulate the complement system by inhibiting the transcription of the *AjC3* in *A. japonicus*.

MiR-137 is a miRNA that is involved in regulating the development of various diseases in mammals by controlling the cell cycle and cell differentiation [21]. In miRBase (http://www.mirbase.org/), 95 mature sequences of miR-137 from 57 species have been identified. There is an obvious discrepancy between invertebrates and vertebrates. The first and ninth nucleotides of miR-137 in invertebrates are offset compared to those of miR-137 in vertebrates (Fig. 2). In the sea cucumber *A. japonicus*, miR-137 is 22 nt in length and includes a 7-nt "seed region" (-AUUGCUU-). A high-throughput sequencing study has shown the down-regulation of miR-137 in *A. japonicus* after 9 h of LPS stimulation [18],

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spu-miR-137        -UAUUGCUUGAGAA UACACGUAG
lva-miR-137-3p     -UAUUGCUUGAGAAUACACGUAG
pmi-miR-137-3p     -UAUUGCUUGAGAAUACACGUAG
dme-miR-137-3p     -UAUUGCUUGAGAA UACACGUAG
sko-miR-137        -UAUUGCUUGAGAA UACACGUAG
bbe-miR-137-3p     -UAUUGCUUGAGAA UACACGUAG
hsa-miR-137        UUAUUGCUUAAGAAUACACGUAG
mmu-miR-137-3p     UUAUUGCUUAAGAAUACACGUAG
dre-miR-137-3p     UUAUUGCUUAAGAAUACACGUAG
pma-miR-137-3p     UUAUUGCUUAAGAAUACACGUAG
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Fig. 2. Alignment of the mature miR-137 (Zhong et al.). Gray shadows represent the different bases between invertebrate miR-137 and vertebrate miR-137 at the first and ninth positions; Spu-miR-137, lva-miR-137–3p, pmi-miR-137–3p, dme-miR-137–3p, sko-miR-137, bbe-miR-137–3p, hsa-miR-137, mmu-miR-137–3p, dre-miR-137–3p, and pma-miR-137–3p represent the miR-137 of *Strongylocentrotus purpuratus*, *Lytechinus variegatus*, *Patiria miniata*, *Drosophila melanogaster*, *Saccoglossus kowalevskii*, *Branchiostoma belcheri*, *Homo sapiens*, *Mus musculus*, *Danio rerio*, and *Petromyzon marinus*, respectively.

and further quantitative real-time PCR (qRT-PCR) analysis indicated that miR-137 inhibits the translation of *AjC3* mRNA in coelomocytes, which suggests that miR-137 participates in the immune regulation in sea cucumbers by targeting *AjC3* [18].

MiR-2004 and miR-2006 are also two miRNAs that are associated with immune regulation by targeting *AjC3* in *A. japonicus* [18,20]. Both miR-2004 and miR-2006 are 22 nt in length [20,22]. By miRBase data mining with a "seed region" of "-CACACAC-", it has been demonstrated that miR-2004 is one of the miRNAs that only occur in echinoderms [23]. The specificity of miR-2004 therefore provides more clues for our understanding of the evolution of miRNAs. qRT-PCR analysis has shown that both miR-2004 and miR-2006 exhibit a regulatory relationship with the expression level of *AjC3* in coelomocytes of *A. japonicus* after LPS challenge [18,20].

It is well established that an individual miRNA might have more than one mRNA target, and that one mRNA can be regulated by multiple miRNAs [24]. Combining the studies mentioned above and our miRNA-binding site prediction data (Fig. 3), we therefore propose in this review that the expression of *AjC3* in coelomocytes can be regulated by multiple miRNAs (such as miR-133, miR-137, miR-2004, and miR-2006). AjC3 is considered as the central hub in the sea cucumber complement system [15]. The observation that more than one miRNA

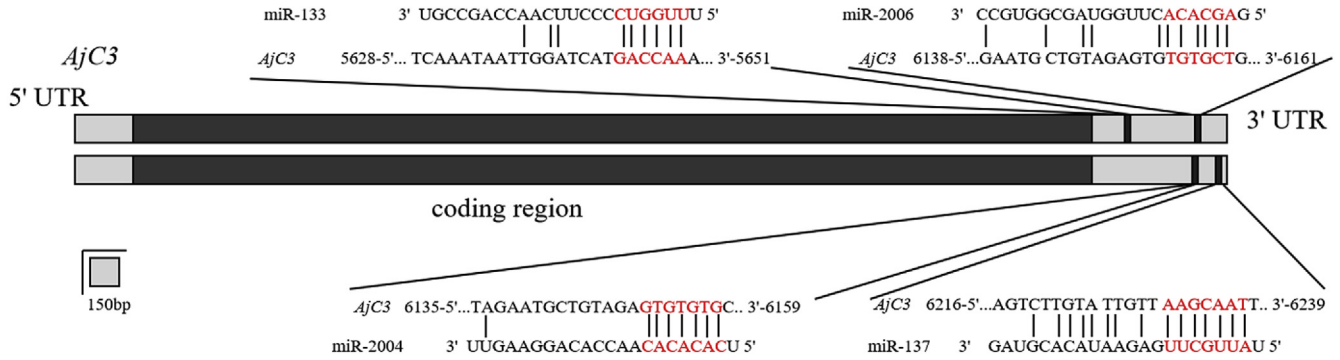

Fig. 3. Predicted miRNA binding sites in the 3′UTR of the *AjC3*. The red marking represented "seed regions". (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

controls the expression of *AjC3* on the one hand indicates the regulation mechanisms of innate immunity is further complicated than it was thought in sea cucumbers (even in echinoderms), and, on the other hand, provides evidence that different miRNAs can perform similar functions for maximization of the genome functionality [24]. Thus, further studies are necessary to clarify the origin of miR-133, miR-137, miR-2004, and miR-2006, and their cooperation in regulating *AjC3* in sea cucumbers.

3. miRNAs involved in TLR pathway regulation

The TLR pathway belongs to a class of germline-encoded pattern recognition receptor cascades and plays a crucial role in the innate immunity of eukaryotes [25]. In general, TLRs can be categorized into two main types based on their localization: one type includes those that are cell surface receptors, whereas the other type comprises those present in intracellular compartments (e.g., lysosomes and endosomes). It has been demonstrated that TLR signals can activate the expression of innate immune-related molecules (e.g., nuclear factor-kappa B, NF-κB, interferon-regulatory factors, IRFs, and tumor necrosis factors, TNFs) through specific adaptor recruitment [26,27]. Since TLR signals were first identified in *Drosophila melanogaster* and their ability to recognize and inhibit fungal reproduction has been confirmed, relevant molecules in the TLR pathway and their downstream targets have been identified and characterized in other invertebrate species, suggesting the existence of TLR pathway-mediated innate immunity in these lower marine animals [28–30].

Given the important roles of the TLR signaling pathway in innate immunity, several molecules associated with the TLR signal pathway in sea cucumbers have been recently identified and characterized such as *MyD88* and *TRAF6* [31], *IRAK-1* [19], *p105* [32], *Tollip* [33], *Toll* [30], NF-κB/Rel [34], and *TLR3* [30]. Most recently, several studies have validated that miR-133, miR-31, miR-200, miR-210, and miR-2008 modulate the TLR cascade activation by targeting *IRAK-1* [19], *p105* [32], *Tollip* [35], *Toll* [36], and *TLR3* [23], respectively (Fig. 4).

In vivo and *in vitro* experiments have shown that a potential target of miR-133 in TLR pathway in sea cucumber is interleukin-1 receptor-associated kinase-1 (*IRAK-1*) gene [19]. IRAK-1 is one of the core components of TLR pathway that can recruit TRAF6 (tumor necrosis factor receptor-associated factor 6) and p105 (nuclear factor-kappa B p105). Co-infection with miR-133 mimics (agomirs) or a specific siRNA targeting *IRAK-1* transcript significantly represses the mRNA and protein expression levels of *IRAK-1* and its downstream molecules (*TRAF6* and *p105*) in primary coelomocytes after *Vibrio splendidus* (the main pathogen associated with skin ulceration disease in sea cucumbers) infection and LPS challenges in *A. japonicus*. These observations prove that miR-133 can modulate the TLR cascade by targeting *IRAK-1* and suggest that a single miRNA might be an innate immunity regulator via multiple pathways in sea cucumbers.

The miR-200 family is one of the best-known miRNA families in mammals. It has been discovered to be involved in pathogen-induced host immune responses by downregulating target gene expression [37–39]. The fourth and ninth nucleotides in the miR-200 family in *Homo sapiens* interchange between has-miR-200a and has-miR-200b. In sea cucumber, miR-200 is 23 nt within a 7 nt "seed sequence" located at positions 2–8 at the 5′ end of this molecule, which is different from has-miR-200a at the fourth nucleotide and has-miR-200 b at the ninth nucleotide in *H. sapiens*. Increasing evidence shows that miR-200 is involved in TLR cascade regulation by indirectly or directly targeting different members of this pathway [40]. For example, miR-200 was demonstrated to indirectly enhance the expression of cytokine interleukin-6 (IL-6) in *H. sapiens* via the TLR pathway by inhibiting Forkhead box a2 (Foxa2) expression in the cholangiocytes [41]. In sea cucumber, positive expression correlations were detected in all examined time points between miR-200 and Toll-interacting protein (designated as *AjTollip*) both in *V. splendidus*-challenged *A. japonicus* individuals and LPS-challenged *A. japonicus* primary coelomocytes, indicating that miR-200 has the dual function of enhancing coelomocytes antibacterial activities and suppressing LPS priming [35]. Co-infection with miR-200 mimics (agomirs) significantly elevated the expression of *AjTollip* and its downstream molecules [35]. Based on these observations, we infer that some miRNAs promote target gene expression in sea cucumbers in addition to negatively modulating the expression of target genes.

MiR-210 is generally considered as a major hypoxia-response factor that is involved in various disease-related cellular activities [42]. A previous report suggested that miR-210 can be potentially utilized in the development of novel products against bacterial infections and plays a role in the immunity of echinoderms [43]. Recent research has demonstrated that the 3′UTR of *A. japonicus Toll-like receptor* gene (designated as *AjToll*) has a binding site that completely matches the miR-210 "seed sequence" (-UGUGCG-). Relative expression determination showed an up-regulation of *AjToll* but a down-regulation of miR-210 in coelomocytes of *A. japonicus* after *V. splendidus* challenge. Transfection of miR-210 mimics (agomirs) in coelomocytes significantly depressed the expression of *AjToll* in cells [36]. This result suggests that the host defense of *A. japonica* against *V. splendidus* infection can be regulated by miR-210 through the *TLR* gene in sea cucumbers.

MiR-2008 is 21 nt in length, with a "seed sequence" (-UCAGCCU-), and has been identified by deep sequencing as a regulatory factor in sea cucumber skin ulceration syndrome (SUS) outbreaks [22]. MiRanda software (MiRanda v3.01) prediction and RNA-seq data mining showed that the potential target gene of miR-2008 in sea cucumbers is *TLR3*. Verification experiments demonstrate that the increase in miR-2008 and decrease in TLR3 expression can be detected in sea cucumbers at 12 h after *V. splendidus* challenging [23]. As an important member of the TLR family, TLR3 has been widely used in studying immune responses that are related to bacterial infections [44]. The opposite

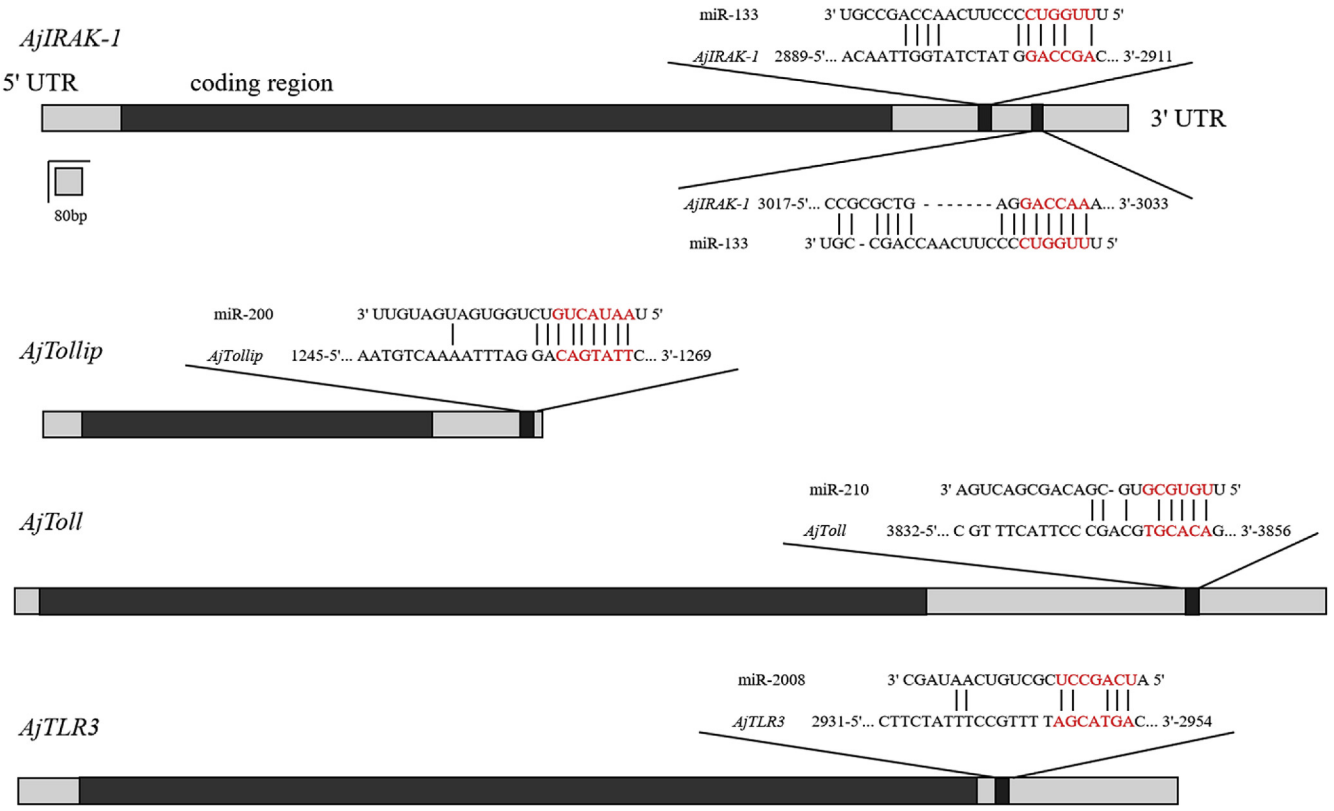

Fig. 4. Predicted miRNA binding sites in the 3′UTR of genes of the TLR pathways. miR-133 binding sites in the *AjIRAK-1* 3′UTR, miR-200 binding sites in the *AjTollip* 3′UTR, and miR-210 binding sites in the *AjToll* 3′UTR. The red markings represent "seed regions.". (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

expression correlation between miR-2008 and *TLR3* in sea cucumbers after a bacterial infection is indicative of the interaction between miR-2008 and genes related to the TLR pathway in sea cucumbers.

MiR-31 is a critical regulator of gene expression in various pathogenic processes in vertebrates [45]. In *A. japonicus*, miR-31, which is 21 nt in length, is significantly upregulated in SUS-samples, indicating that this molecule is involved in SUS-induced host pathogen interactions [22]. Sequence analysis showed that the 3′UTR of *A. japonicus p105* (designated as *Ajp105*) has a completely matching binding site to the miR-31 "seed sequence" (-GGCAAGA-) [32]. *p105* is a vital member of the TLR family that regulates NF-κB dependent immune responses [46]. The negative expression profiles between miR-31 and *Ajp105* have been detected in both LPS-exposed primary coelomocytes and *V. splendidus*-challenged sea cucumbers [32]. Additionally, co-infection with miR-31 mimics (agomirs) induces a significant down-regulation of *Ajp105 in vitro* [32], indicating that miR-31 might be involved in the regulation of innate immune responses in sea cucumbers.

Notably, many studies have found that in mammals (such as mice and humans) TLR4 and myeloid differentiation 2 (MD2) serve as specific receptors that recognize LPS and then activate innate immune defense NF-κB signal cascades [47,48]. However, these genes, which recognize LPS, have not been identified in most aquatic animals, except for the grass carp (*Ctenopharyngodon idellus*) [49]. This led to the speculation that cytokines associated with LPS stimulation in sea cucumbers are possibly produced via signal pathways different from those of mammals. Although some downstream signal molecules associated with the TLR signal pathway in sea cucumbers have been identified [19,30–34], the mechanisms of LPS recognition and cytokine generation remain unclear in sea cucumbers, and further efforts should be developed.

4. miRNAs involved in the regulation of reactive oxygen species (ROS) production

Reactive oxygen species (ROS) are byproducts of the reduction of molecular oxygen and include the superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH·), hydroperoxyl radical (HOO·), singlet oxygen ($^1O_2$), and peroxyl radical (ROO·) [50]. ROS are generated by membrane-associated NAD(P)H-oxidases through univalent reduction of molecular oxygen to O_2^- and cause oxidative damage to cellular membranes and macromolecules [51,52]. Microbial infections induce the release of a large amount of intracellular ROS [53]. In both vertebrates and invertebrates, ROS can eliminate foreign microorganisms or particles by causing DNA damage, lipid peroxidation and enzymatic activity disruption intra- or extracellularly [54]. However, ROS are not always deleterious and can also act as intracellular second messengers. Previous studies have indicated that ROS can mediate the production of inflammatory cytokines (e.g., tumor necrosis factor-alpha, TNF-α, and interleukin-1 beta, IL-1β) [55] and modulate sea cucumber defense [56].

Target screening and dual-luciferase reporter assays have revealed that the 3′UTR of *A. japonicus* betaine homocysteine S-methyltransferase (designated as *AjBHMT*) is targeted by miR-137 and miR-2008 (Fig. 5), which alters homocysteine (Hcy) accumulation in coelomocytes and modulate respiratory bursts as a defense against invasive microorganisms [57]. The *BHMT* gene was first identified as a facilitator of methyl group donation for the remethylation of Hcy into methionine [58], and reduced BHMT functions can elevate Hcy levels [59]. After *V. splendidus* infection or LPS stimulation, miR-137 expression is slightly attenuated, and miR-2008 is significantly enhanced. Further analysis using qRT-PCR and western blotting *in vitro* and *in vivo* showed that the inhibition of miR-137 increases the mRNA and protein expression levels of *AjBHMT*, whereas the overexpression of miR-2008

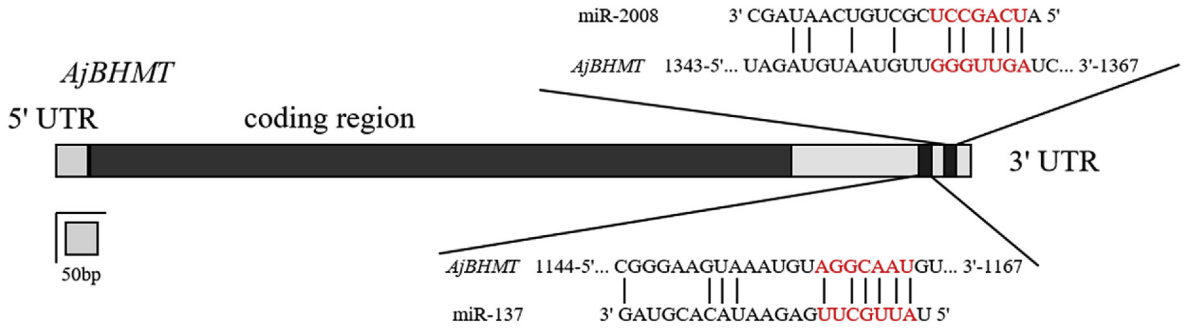

Fig. 5. Predicted miRNA binding sites in the 3′UTR of the *AjBHMT*. The red markings represent "seed regions.". (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

decreases AjBHMT protein expression without affecting *AjBHMT* mRNA expression [57]. These results suggest the differential regulatory levels and roles of sea cucumber miR-137 and miR-2008 in controlling the expression of the common target *AjBHMT* in sea cucumbers. In the analysis of abnormal expression of these two kinds of miRNAs, the content of Hcy also changed accordingly. Inhibiting the expression of *AjBHMT* significantly increased the production of ROS and reduced the number of invasive pathogens that survived in the coelomocytes of sea cucumber [57], which confirmed that miR-137 and miR-2008 play pivotal roles in host-pathogen interactions by targeting the *AjBHMT* gene to regulate Hcy metabolism, thereby directly affecting cellular oxidative-redox stress and improving bacterial elimination.

MiR-210 can indirectly control the production of ROS by targeting *AjToll* in *A. japonicus* [36]. The production of ROS can be successfully diminished by introducing miR-210 mimics (agomirs), which induces the optimization of the expression levels of miR-210 in *A. japonicus* host cells to control the balance of immune responses against pathogenic particles. Another miRNA regulating the ROS signals in an indirect manner is miR-31 [32]. In a study on the regulation of ROS production stimulated by *V. splendidus in vivo* and *in vitro*, the expression levels of both miR-31 and *Ajp105* were downregulated after pathogen infection [32]. In addition, the overexpression of miR-31 or silencing *Ajp105* significantly increased the oxidative stress response of organisms [32]. Together, these results suggest that miR-31 indirectly modulates the production of ROS by regulating *p105* in sea cucumbers.

It should be kept in mind that ROS production and its mediated signal pathways in sea cucumbers are different from those of mammals. In sea cucumbers, due to the lack of acquired immunity, ROS is produced mainly by phagocytosis [51], while in mammals, ROS is produced mainly by neutrophils mediating the inflammatory response [60]. It has been demonstrated that ROS can regulate the expression and activities of signal transducers and activators of transcription (STAT) via activating and regulating protein tyrosine kinase (PTK) and protein tyrosine phosphatase (PTP) in human cancer cells (such as melanoma cells and pancreatic cancer cells) [61]. However, ROS-mediated signal pathways are still obscure in sea cucumbers although some information fragments of miRNAs associated with ROS generation and its possible downstream targets have been preliminary identified, further studies should be carried out to clarify the common and the specific features of ROS production and miRNA regulation mechanisms in sea cucumbers and mammals.

5. miRNAs involved in the regulation of apoptosis pathways

Apoptosis (or programmed cell death) is a complicated physiological process that controls cell proliferation and is essential to homeostasis maintenance [62]. To date, several miRNAs such as miR-212, miR-34, and miR-200c have been shown to regulate the complex network of apoptotic pathways, and alterations in their expression levels in cancer could play a relevant role in apoptosis escape [63,64]. In aquatic invertebrates, the activation of apoptosis is considered as one of the host defense strategies for pathogen elimination [65]. There is growing evidence showing that miRNAs play an important role in regulating apoptosis-related genes in metazoans via the innate immune system [66].

The 14-3-3 intracellular phosphoserine/threonine-binding proteins are adapter molecules with various functions that are related to the regulation of cell metabolism, division, differentiation, autophagy, and apoptosis [67–69]. Recent evidence indicates that this protein is also an important regulator of innate immunity [70]. 14-3-3ζ is one of seven 14-3-3 protein isoforms (ζ, γ, η, ε, β, θ, and σ) with a full cDNA length of 2,106 bp in the sea cucumber *A. japonicus* [71]. Recent studies have identified the *14-3-3ζ* as a new target of miR-92a and miR-137 in *A. japonicus* [66,71].

The miR-92a family, which include the first discovered oncogene, belongs to the miR-17-92 cluster that can regulate various pivotal cell processes such as cell proliferation and apoptosis and is related to the formation of vascular endothelial cells [72–74]. Target analysis confirmed that a potential binding site is located from 357 nt to 378 nt in the 3′UTR region of *14-3-3ζ*, which matched the miR-92a "seed region" (-AUUGCAC-) with the highest single-residue pair score and lowest free energy (Fig. 6). Further expression analysis and functional assays

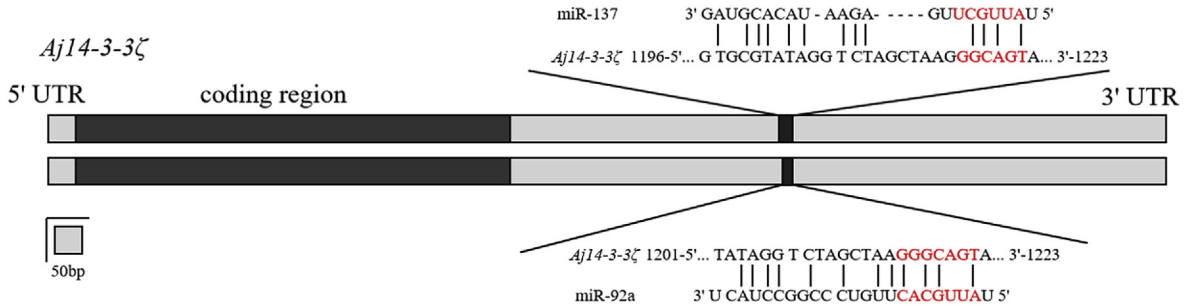

Fig. 6. Predicted miRNA binding sites in the 3′UTR of the *Aj14-3-3ζ*. The red markings represent "seed regions.". (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

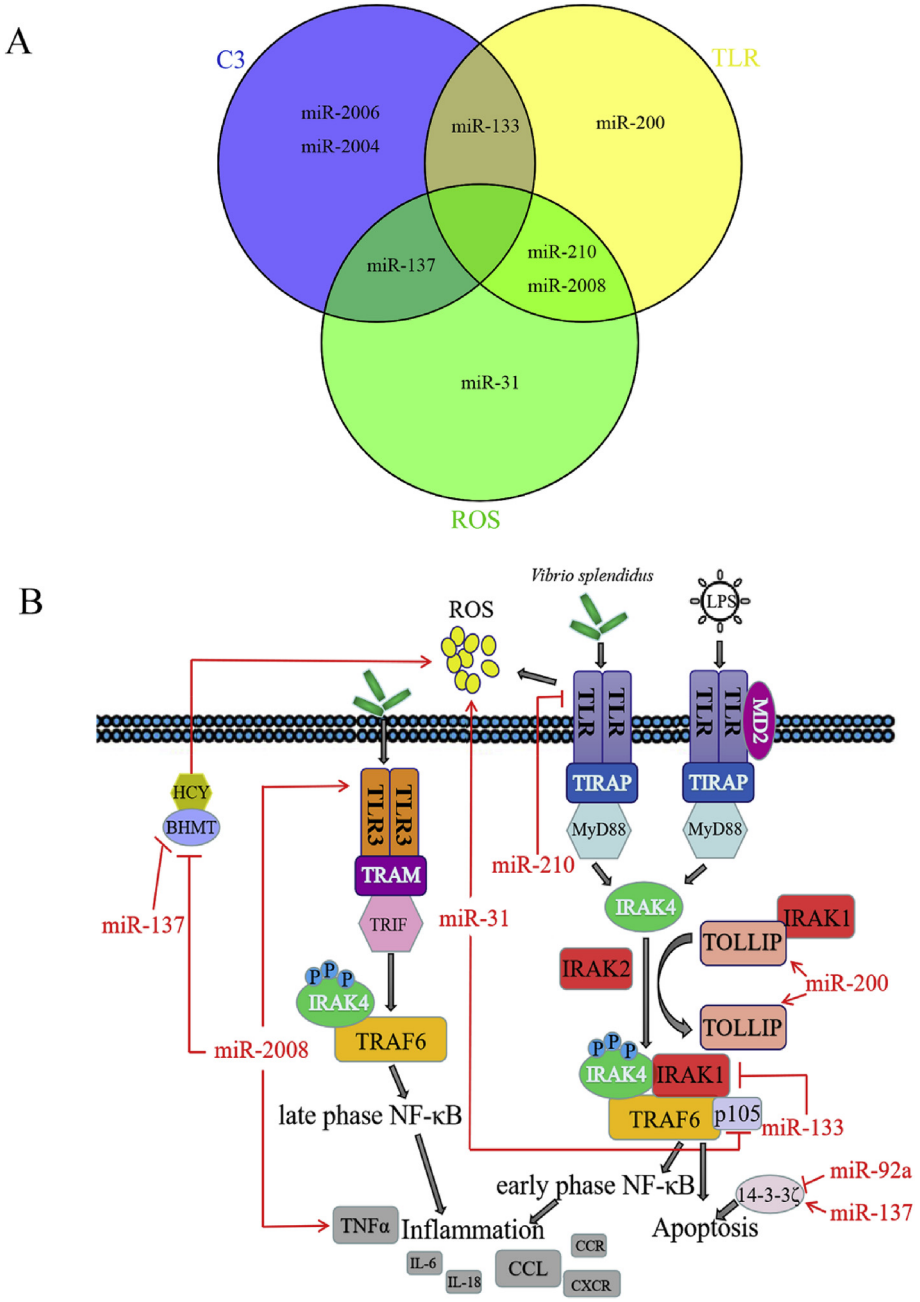

Fig. 7. Interactions between miRNAs and immune-related pathways in *Apostichopus japonicus*. A: miRNAs involved in immune regulation in different ways in *A. japonicus*, including regulation of the complement system, the Toll-like receptor pathways, and the ROS signals. B: Interaction between miRNAs and immune-related pathways in *A. japonicus*. Arrowhead lines, promotion; flat-end lines, repression.

revealed a regulatory relationship between miR-92a and 14-3-3ζ protein in both LPS-exposed primary coelomocytes and *V. splendidus*-challenged individuals in *A. japonicus*. Moreover, inhibition of miR-92a decreased cellular apoptosis by increasing 14-3-3ζ protein expression *in vivo* [66], collectively supporting that miR-92a is possibly involved in the apoptosis signaling pathway by targeting *14-3-3ζ* in sea cucumbers. Interestingly, it was reported that inhibition of miR-92a can induce apoptosis in human acute promyelocytic leukemia through targeting tumor protein 63 (p63) [75]. Comparing the miR-92a targets in sea cucumbers and humans, we therefore speculate that apoptosis signal transduction might be different between sea cucumbers and mammals, and the same miRNA might regulate different target genes related to apoptosis in these different species.

The interaction between miR-137 and the 14-3-3ζ protein in sea cucumbers was established based on isobaric tags for relative and absolute quantification (iTRAQ) and transcriptome screening analysis (Fig. 6) [71]. Expression analysis indicated the downregulation of miR-137 and 14-3-3ζ consistently in both LPS-exposed primary coelomocytes and *V. splendidus*-challenged individuals in *A. japonicus*, similar to studies on miR-92a. However, miR-137 inhibition decreased both mRNA and protein expression levels of 14-3-3ζ, and significantly promoted coelomocyte apoptosis, which is in contrast to the regulatory role of miR-92a. These results confirm that miR-92a and miR-137 may be involved in the regulation of the apoptosis signaling pathway by targeting *14-3-3ζ* in the coelomocytes of sea cucumbers. These findings also indicate that although the target gene of miR-92a and miR-137 is the same *14-3-3ζ*, their regulatory effects are different, which illustrates the complexity of the regulatory roles of miRNAs.

6. Conclusions and perspectives

In this review, we describe the recent progress on some known miRNAs and how these nucleic acids contribute to the innate immunity of sea cucumbers (Fig. 7). With the development of sequencing and bioinformatics analysis techniques, our understanding of the interactions between known miRNAs and immune-related genes in sea cucumbers has improved, namely the miR-2008-*Apaf-1* and miR-2008-*CNTN4* associations in *A. japonicus* [23]. Moreover, new perspectives in the characterization of miRNAs and their target genes in echinoderm immunity have emerged from the comparative genomics and an integrated analysis of miRNA-mRNA transcriptomes. For example, Zhou et al. explored the miRNA-mRNA interaction network in *A. japonicus* after pathogen infection based on a transcriptomic-integrated approach [23].

In summary, to further enrich our understanding of the contribution of miRNAs to echinoderm innate immunity, (1) it is essential to continuously identify and characterize novel miRNAs that are associated with echinoderm immune responses; (2) given that a particular miRNA might have various multiple mRNA targets and could regulate different physiological and pathogenic processes, the identification of novel target genes of a given miRNA is of importance; (3) the model of "Transcription factor (TF)-miRNA-lncRNA" (TML) regulatory network motifs in invertebrate immunity warrants additional investigations on the spatial and temporal specificities of the "TML" interaction network motifs in the sea cucumber immune response [76]; and (4) the development of more miRNA-based biomarkers and therapeutic strategies for sea cucumber diseases is a priority.

Authors' contributions

Y.Q.C. and Y.Y.Z. conceived the manuscript. The reference collection and data analysis were performed by L.L., T.J.Z., J.X.S., D.Y.C., and Y.Y.L.. The paper was written by Y.Y.Z. and L.L.. All authors read and approved the manuscript.

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Abbreviations

miRNAs	microRNAs
ncRNAs	non-coding RNAs
TLR	Toll-like receptor
ROS	reactive oxygen species
nt	nucleotides
pri-miRNAs	primary miRNAs
pre-miRNAs	precursor mRNAs
UTRs	untranslated regions
C3	Complement component 3
LPS	lipopolysaccharide
qRT-PCR	quantitative real-time-PCR
NF-κB	nuclear factor-kappa B
IRFs	interferon-regulatory factors
TNFs	tumor necrosis factors
MyD88	myeloid differentiation factor 88
TRAF6	tumor necrosis factor receptor-associated factor 6
IRAK-1	interleukin-1 receptor-associated kinase-1
p105	nuclear factor-kappa B p105
Tollip	Toll-interacting protein
AjToll	*Apostichopus japonicus* Toll-like receptor
TLR3	Toll-like receptor 3
IL-6	interleukin-6
Foxa2	Forkhead box a2
RNA-seq	RNA-sequencing
SUS	skin ulceration syndrome
MD2	myeloid differentiation 2
O^2	superoxide radical anion
H_2O_2	hydrogen peroxide
OH·	hydroxyl radical
HOO·	hydroperoxyl radical
$1O_2$	singlet oxygen
ROO·	peroxyl radical
TNF-α	tumor necrosis factor-alpha
IL-1β	interleukin-1 beta
BHMT	betaine homocysteine S-methyltransferase
Hcy	homocysteine
STAT	signal transducers and activators of transcription
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
p63	protein 63
iTRAQ	isobaric tags for relative and absolute quantification
Apaf-1	Apoptotic protease activating factor-1
CNTN4	Contactin 4
TF	Transcription factor
TML	Transcription factor-miRNA-lncRNA

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