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Integrative mRNA-miRNA interaction analysis associated with the immune response of *Epinephelus coioddes* to *Vibrio alginolyticus* infection

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ABSTRACT

MicroRNAs (miRNAs) are a kind of small non-coding RNAs that have been reported to play a vital role in mediating host-pathogen interactions. High-throughput sequencing technology was applied to identify and illuminate mRNAs and miRNAs from grouper infected with *Vibrio alginolyticus*. The KEGG pathway enrichment analysis showed that the most significate DEGs are associated with Toll-like receptor signaling pathway and NOD-like receptor signaling pathway. We obtained 374 known miRNAs and 116 novel miRNAs. During them, there are 31 up-regulated miRNAs and 93 down-regulated miRNAs. miRNA-mRNA GO and KEGG analysis show that there are 90 miRNAs associated with the immune system. The target genes of immune-related miRNAs (miR-142, miR-146, miR-150, miR-155, miR-203, miR-205, miR-24, miR-31) and genes (CD80, IL-2, AMPK, PI3K) in *Epinephelus coioddes* were predicted and validated. This study provides an opportunity to further understanding the molecular mechanisms especially the immune system of miRNA regulation in *Epinephelus coioddes* host-pathogen interactions.

1. Introduction

Orange-spotted grouper (*Epinephelus coioddes*) is one of the most cultivated marine fish in the tropical and subtropical, especially in China. While with the deterioration of the farming environment, more and more stress was added to groupers which can significantly impair the grouper immunity [1]. Various diseases caused by bacteria have caused catastrophic losses in cultivated groupers, becoming a major constraint on the sustainability of the industry. Vibrio is one of the most common causes of high mortality in marine animals [2]. A recent study showed that *Vibrio alginolyticus* (*V. alginolyticus*) has been associated with a few epidemics of vibrio is in marine animals, including fish, crustaceans, shellfish and coral reefs which is a ubiquitous organism in seawater [3,4]. *V. alginolyticus* plays a pivotal role in causative agents of vibriosis which endangered the aquaculture of marine animals in the coastal provinces of South China [5]. Studies have shown that vibrio density can grow up to about 1.3×10^7 CFU/g sediment during a high-intensity aquaculture process [6]. In addition, *Vibrio* strain has TonB/ExbB/ExbD complex, which can play a regulatory role in the iron absorption process, making the invading bacteria more resistant to microenvironmental conditions with limited iron availability, thus showing high toxicity to the host [7]. Therefore, studying the immune response of grouper to vibrio invasion may be beneficial to the sustainable development of grouper aquaculture.

MicroRNAs (miRNAs) are endogenous 21–23 nucleotides (nt) RNAs, that can regulate gene expression by base-pairing to partially complementary mRNAs, and are widely expressed by diverse viruses and eukaryotic organisms [8,9]. Regulation by miRNAs can play essential roles in the developmental, response to viral infection, stress and immune responses of various organisms [10–12]. In mammals, microRNAs as regulators of the immune response which indicated miRNAs play a major role in the outcome of infectious diseases [13]. Regulate miRNAs can either facilitate pathogen immune evasion or enhance the immune response during infection [14]. Groupers are defective in miRNA processing arrest in the immune response. Together, the study of immune-related miRNAs, which may represent a new therapeutic target for immune-related disease control, is essential to understand host and pathogen interactions [15,16].

High-throughput, deep sequencing transcriptome method, can be used to express the production of a large-scale library of sequence tags, increasing the speed of gene discovery. In addition to identifying and sequencing mRNA and small RNA transcribes, the Illumina RNA-seq

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platform also allows researchers to examine patterns of transcription expression in tissues of interest. This method has been applied to a variety of species, including shrimp [17], medaka [18], zebrafish [19], catfish [20] orange-spotted grouper [21] and Nile tilapia [22]. mRNA-miRNA integration has been widely used to research the interaction between mRNA and miRNA based on paired expression profiles. The liver is a central organ that controls many physiological functions and synthesizes plasma proteins, it needs to alter most of its transcriptional and translation mechanisms to synthesize high levels of acute phase reactants under acute infection conditions [23]. Thus, in this study, we used high-throughput sequencing to identify mRNA and miRNA levels in groupers' liver after *Vibrio* challenge to characterize the molecular mechanisms especially the immune system. Liu et al. reported that downregulation of miR-1423p in macrophages from aged mice thus results in increased IL-6 expression [24]. MiR-146 function in the innate immune response of zebrafish embryos to Salmonella typhimurium infection [25]. MiR-150 has been reported associated with B cell functions and regulate inflammatory cytokine production through targeting ARRB-2 and JAK1-STAT1/c-Fos pathway [26–28]. MiR-155, miR-203, miR-205, miR-24, miR-31 all has been discovered to be related to immune response [29–34]. Therefore, miR-142, miR-146, miR-150, miR-155, miR-203, miR-205, miR-24, miR-31were used to identify their putative target genes.

In the present study, *V. alginolyticus*, a well-documented pathogen associated with grouper hepatomegaly, was used to explore the potential mRNA-miRNA interactions to provide insights regarding the underlying molecular mechanism. This work provides many mRNA-miRNA interaction resources and will assist in understanding the molecular mechanism of gene regulation in the diseased *Epinephelus coioddes*.

2. Materials and methods

2.1. Ethics statement

All animal procedures were licensed under the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

2.2. Sample collection

Healthy juvenile Orange-spotted groupers with average length 10.70 ± 0.33 cm and weight 12.50 ± 0.25 g, were purchased from an aquaculture farm in Leizhou (Guangdong, China). Groupers were acclimatized in 50 × 75 × 65 cm plastic aquarium (25 fishes/aquarium) with diluted seawater (15‰ salinity, PH 8.0, 25 ± 1 °C) for two weeks before experimental. Groupers were fed with the commercial diet twice a day. For the challenge experiment, the pathogen bacteria *V. alginolyticus*, obtained from our laboratory, were cultured at 28 °C with shaking at 200 rpm overnight and were centrifuged at 4000 × rpm for 1 min to harvest the bacteria. 30 groupers in an aquarium were immersed in 100 μl of working solution bacteria diluted in sterilized phosphate-buffered saline (PBS, 0.1 mM, pH 7.4) at the concentration of 10^7 CFU/ml and injected with 100 μl of sterilized PBS were used as the control group in another aquarium. We harvested the livers from infected groupers on 0, 3, 6, 12, 24 and 48 h. Transcriptome analysis was performed at 6 h. Four infected groupers were used for biological replication. Harvested livers were stored at −80 °C until RNA extraction.

2.3. Histopathology

The liver samples were fixed in 4% paraformaldehyde solution for 48 h. Then washed with 70% ethanol, dehydrated and embedded in wax. The tissue wax was cut into 4 μm sections. According to standard procedure, fixed sections were then stained with hematoxylin and eosin (H&E). The cell morphology and structural changes of liver tissue were observed by a fluorescence microscope (Leica DM6).

2.4. Library construction, deep sequencing of small RNA

RNA samples were prepared using the DGE-Small RNA Sample Prep Kit (Illumina, San Diego, CA). Small RNAs of 18–30 nt in length were prepared from total RNA by gel fractionation. Then the 3′ adapters were added on the 36–44 nt RNAs were enriched. The 5'adapters were then ligated to the RNAs as well. The ligation products were reverse transcript by PCR amplification and the 140-160bp size PCR products were enriched to generate a cDNA library and sequencing on an Illumina HiSeq™2500 Analyzer at the Genedenovo Biotechnology Co., Ltd (Guangzhou, China).

2.5. Real-time quantification of miRNAs by Poly(A)-Tailed reverse transcription

The isolated RNA of individual samples were reverse transcribed into cDNA using a PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China) in a total volume of 10 μl containing 1 μg of total RNA, following the manufacturer's instructions. A total of 1 μg of miRNA was reverse-

Table 1
The sequences of primers used in this study.

Gene	Primer
qRCR-IL2-F	AGAGACCAGCGAGCGAAAAG
qRCR-IL2-R	CGGAGCATACAGCATAGCGT
qRCR-AMPK-F	AGACCTCTCCAGCTCTCCTC
qRCR-AMPK-R	GTTGGGCTCTGGGAGTAACG
qRCR-PI3K-F	GGTTCGCTCTATGGCTGTCA
qRCR-PI3K-R	CGATGCGCTTACTCCTCAGT
qRCR-CD80-F	GGAGGCAAAGACGACAGGAA
qRCR-CD80-R	GCAACAACAGCAACCCTACG
RT-PCR-miR150x	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACTGG
RT-PCR-miR155x	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCCT
RT-PCR-miR146x	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCATCT
RT-PCR-miR142x	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCATCCA
miRNA-qPCR-R	CAGTGCAGGGTCCGAGGTAT
qPCR-mir150x-F	GCTCTCCCAACCCTTGTACCA
qPCR-mir155x-F	TTAATGCTAATCGTGATAGGGGTGT
qPCR-mir146x-F	TGAGAACTGAATTCCATAGAT
qPCR-mir142x-F	CAGTGCAGGGTCCGAGGTAT
U6-F	GCTTCGGCAGCACATATACTAAAAT
U6-R	CGCTTCACGAATTTGCGTGTCAT

transcribed using 0.5 μl U6 antisense primers and 0.5 μl specific primers, with other conditions remaining unchanged. Primers were designed by Primer Premier 5 software and are shown in Table 1. The expression patterns of mRNA and miRNA were measured by qRT-PCR using the ABI 7500 Real-Time PCR system (Applied Biosystems, USA). U6 gene was used as a reference gene to normalize all experimental data. All samples were analyzed in triplicate.

2.6. Statistical analysis

Reads obtained from the sequencing machines included dirty reads containing adapters or low-quality bases which would affect the following assembly and analysis. Raw reads were filtered by removing low-quality reads, reads without 3′ adapters, containing 5' adapters, reads containing polyA, and reads shorter than 18 nt. All the clean tags were aligned with small RNAs in GeneBank database (release 209.0) and in Rfam database to identify and remove rRNA, scRNA, snoRNA, snRNA, and tRNA. Meanwhile, all of the clean tags were also aligned with the reference genome. The tags mapped to exons, introns and repeated sequences were also removed. All of the clean tags were then searched against the miRBase database to identify *E. coioides* miRNA. All of the unannotated tags were aligned with the reference genome. According to their genome positions and hairpin structures predicted by software Mireap v0.2, the novel miRNA candidates were identified.

We used SPSS (version 20.0 for Windows) for all statistical analyses and considered $P < 0.05$ as significant for all statistical tests. For quantitation of gene expression, we used the comparative CT method ($2^{-\Delta\Delta CT}$). All results are expressed as means ± *SD*. Non-normally distributed data were transformed to natural logarithms. Different groups were analyzed with one-way *t-test*.

3. Results

3.1. Histopathology

Liver histopathology was characterized to analyze the influence of *V. alginolyticus* challenged groupers. As shown in Fig. 1, in the control group, the hepatocytes were oval nucleus with homogeneous cytoplasm. While infected groupers, the liver showed significant histology changes, such as vacuolation, swelling cells and nuclei shifted to the periphery of the hepatocytes.

3.2. Differential expression of the transcriptome in response to V. alginolyticus

cDNA libraries of liver tissues from the trial (T) and control (C) groups were sequenced. A total of 66,282,202 and 65,541,200 raw reads were obtained from high-throughput sequencing. After filtering the adaptors and low-quality reads, 65,056,516 (98.15%) and 64,820,486 (98.9%) clean reads were obtained in the T and C groups. All the clean reads were ranging from 201 to 18,172bp and total assembled genes were 56,748. Venn diagram showed that 31,582 unigenes were annotated by Nr, Swiss-port, KEGG and KOG database. Among them, 12,720 unigenes were both annotated by the four databases (Supplementary Fig. 1A). GO and KEGG pathway analyses were used to investigate the potential functions of the differentially expressed genes. The KEGG database annotated about five aspects: metabolism, cellular processes, organismal systems, environmental information processing and genetic information processing (Supplementary Fig. 1B). Among these, signal transduction is the most obvious, followed by carbohydrate metabolism, transport and catabolism, cellular community, endocrine systems. We used FDR and $\log_2$FC to select different expressed genes with FDR < 0.5 and $|\log_2 FC| > 2$. All differently expressed genes compared with control were 11,958, there are 3565 down-regulated genes and 8393 up-regulated genes (Supplementary Fig. 1C). Among all the DEGs, there are 192 significant genes associated with immune systems (Supplementary File S1). KEGG pathway enrichment analysis of the DEGs indicated that “Cell adhesion molecules” possessed the highest rich factor and “Endocytosis” had the most DEGs (Supplementary Fig. 1D).

3.3. Mapping and annotation of miRNA sequencing data

Small-RNA libraries of liver tissues from groupers were sequenced. A total of 11,441,578 reads were obtained after sequencing. After removing poly A sequences, reads without 3'adapters, reads containing 5'adapters sequences less than18 nt, sequences longer than 32 nt and low quality reads, 10,752,927 clean reads were obtained. The length of clean reads was ranging from 18 to 32 nt, most of the reads were between 21 and 23 nt and reads count of 22 nt were the highest (Fig. 2A).

3.4. Identification of known and novel miRNA

To characterized miRNAs in groupers, all miRNA sequences were compared to known miRNAs in miRbase 21.0. A total of 374 miRNAs were identified in the present study. We compared grouper miRNAs with those from human (*Homo sapiens*), mouse (*Mus musculus*), crap (*Cyprinus carpio*), zebrafish (*Danio rerio*), salmon (*Salmo salar*). A total of 70 miRNAs have homologs in the six species and 72 miRNAs were specific in grouper. There are 79 miRNAs both appeared in human, mouse and grouper (Supplement File S2). In detail, 10 miRNAs were both identified in crap, zebrafish, salmon and grouper including miR-454, miR-459, miR-460, miR-722, miR-724, miR-725, miR-727, miR-729, miR-730, miR-734. All the miRNAs have a special secondary structure, we used MIREAP_v 0.2 to predict secondary structure for identifying novel miRNA. A total of 116 novel miRNA were identified in the end (Supplement File S3).

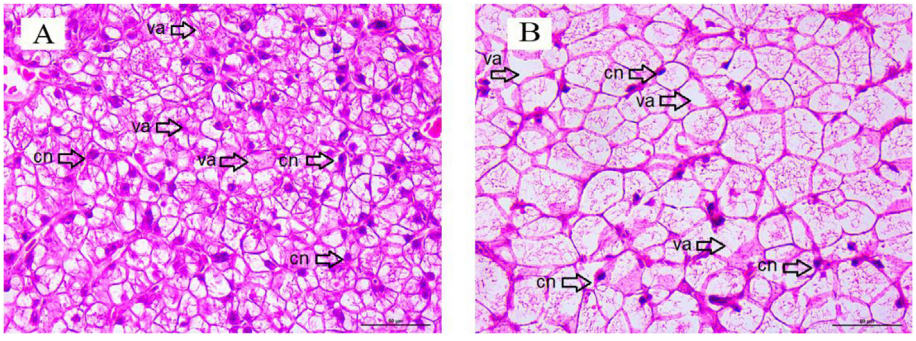

Fig. 1. Histological observation of control and trial livers. (A) Control: groupers injected with *V. alginolyticus* for 0h. (B) Trail: growers injected with *V. alginolyticus* for 6h. cn: cell nucleus; va: vacuolation.

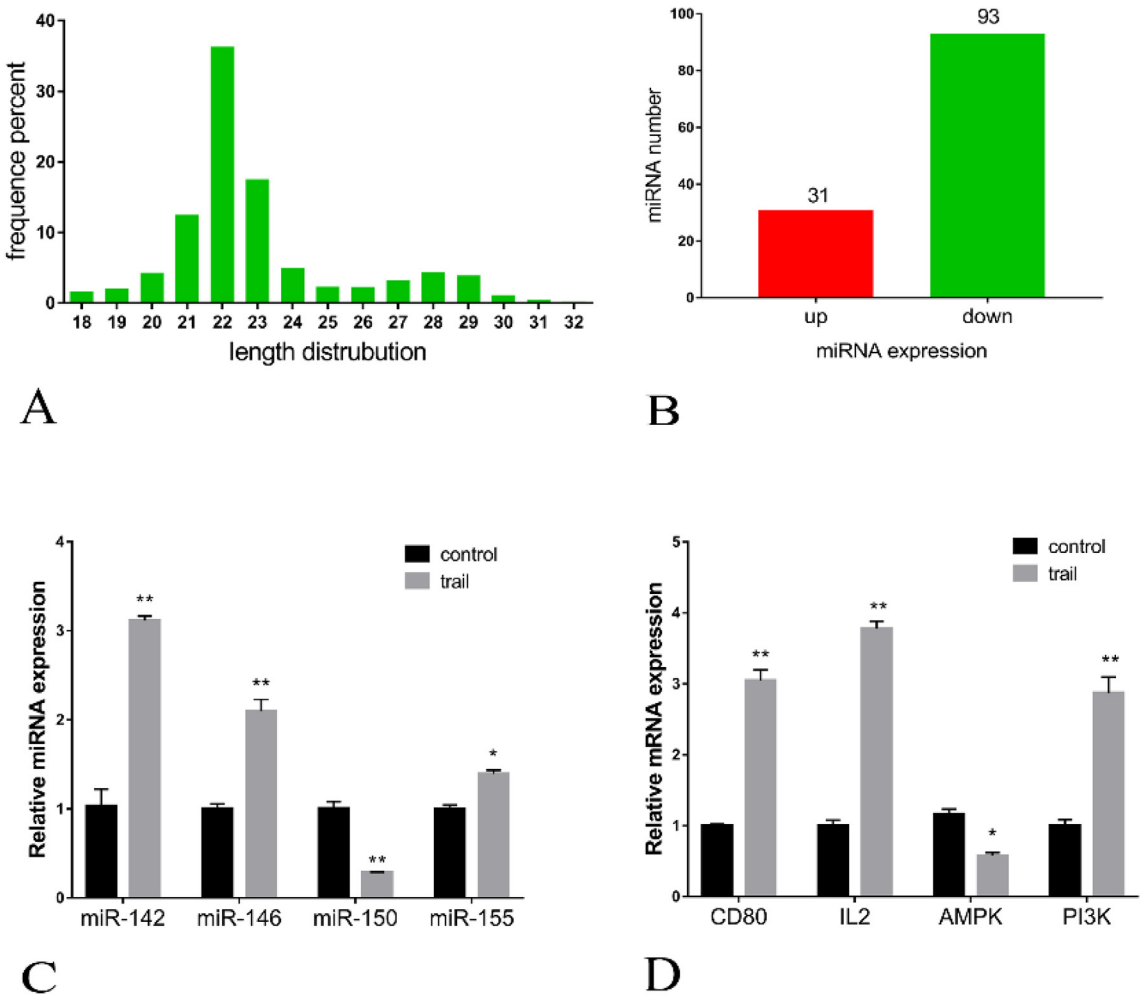

Fig. 2. Annotation and analysis of miRNA. (A): Analysis of the sequencing reads of orange-spotted grouper of *V. alginolyticus* infection; (B) All miRNAs compared with control. The red column means miRNAs were up-regulated while the green ones were down-regulated; (C) and (D) Expression of four groupers miRNA and mRNA during infection at the control and trail. Values are shown as menas ± SD. Significant difference was indicated by asterisks, *: $0.05 > p > 0.01$, **: $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5. Identification of differentially expressed miRNAs

A total of 124 miRNAs were differentially expressed, with the criteria of at least a 2-fold difference and a $p < 0.05$. Among them, 31 were up-regulated and 93 were down-regulated (Fig. 2B) including 4 novel up-regulated and 8 novel down-regulated miRNAs (Supplement File S4). Among these, miR-730-y, miR331-y, miR885-x, and miR328-y were the most down-regulated miRNAs while miR291-x, miR298-x and miR205-y were the most up-regulated miRNAs. To validate the expression patterns of the mRNA and miRNA, four mRNAs and miRNAs associated with immune systems were selected for RT-PCR analysis (Fig. 2C and D). The results of RT-PCR were in good agreement with our sequencing data, indicating the reliability of our RNA-seq and miRNA-seq data.

3.6. Bioinformatics analysis of miRNA-target genes

We used RNAhybrid (v2.1.2) + svm_light (v6.01), Miranda (v3.3a), TargetScan (Version:7.0) three method to predict target genes. And then chose the intersection of the three method results as miRNA-target genes. Finally, a total of 376 miRNA target 2659 genes were predicted. The 2659 target genes cover a wide range of functions, notably those related to immunity. Immune-related genes include interleukin (IL)-8, IL-18, Toll-like receptors, CD40, CD80, NOD1, NOD2 (Supplemental File S5).

3.7. Functional enrichment analysis of miRNA target genes

KEGG pathway was used to better illuminate the function of the DE miRNAs after infected with *V. alginolyticus*. KEGG enrichment analysis showed that the 14,599 predicted target genes of groupers were clustered into 195 KEGG terms (Supplemental File S6). It indicated that DE miRNAs were significantly enriched in the protein processing in the endoplasmic reticulum, SNARE interactions in vesicular transport, RIG-I-like receptor signaling pathway and apoptosis. The KEGG terms with $Q \leq 0.05$ and $p \leq 0.05$ are shown in Fig. 3. To get a deep understanding of the DE miRNAs, we performed hierarchical clustering of the DE miRNAs and their target pathways with $|\log_2| \geq 2$ and $p \leq 0.05$ (Fig. 4). Similar pathway functions or regulation patterns were clustered together. DE miRNAs were considerably enriched in pathways about folding, sorting and degradation, glycan biosynthesis and metabolism, immune system and signal transduction. Hierarchical clustering helps us better understand the novel miRNAs. For example, novel m0004.3p, m0008.3p were cluster with miR-411x, suggesting these novel miRNAs may have similar functions.

3.8. Network of predicted interactions between immune system miRNAs and target genes

miRNA-mRNA interaction networks were constructed based on the DEGs and DE miRNAs. According to KEGG and GO analysis, there are

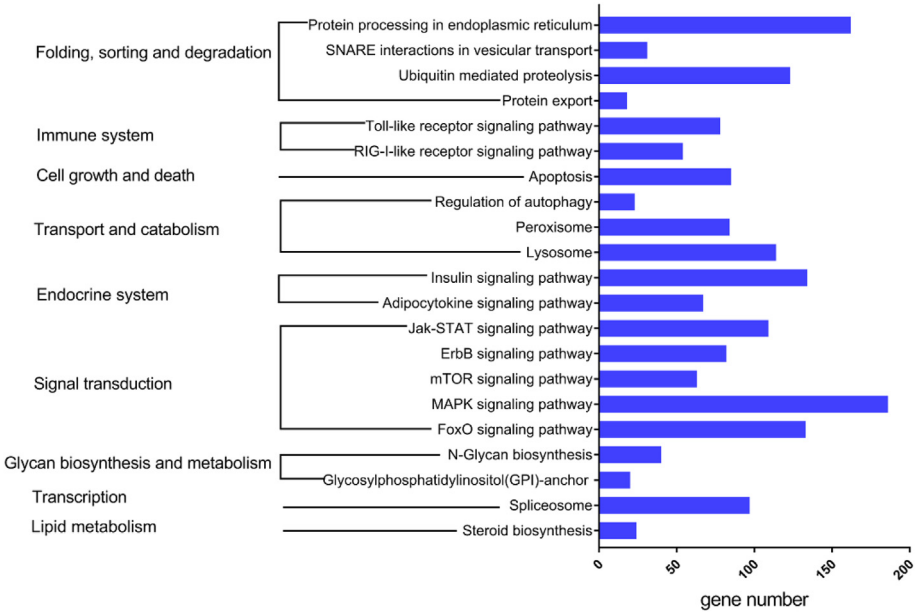

Fig. 3. The KEGG terms of miRNAs with Q ≤ 0.05 and $p \leq 0.05$.

90 DE miRNAs targeting 55 mRNAs related to immune response. All immune system pathway: RIG-I-like receptor signaling pathway, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway and Cytosolic DNA-sensing pathway (Supplemental File S7). From all the miRNAs, we have selected 8 miRNAs that have been reported to the immune system (Fig. 5). The 8 miRNAs are miR-142, miR-146, miR-150, miR-155, miR-203, miR-204, miR-24, miR-31. We found that miR-203 and miR-205 regulate the most target genes indicating miR-203 and miR-205 play a vital role in groupers after *V. alginolyticus* challenge. Among these target genes, some genes have two or more miRNA predictive binding sites. For example, GHR can be targeted by 6 miRNAs.

4. Discussion

With the ecological deterioration and environmental pollution pick up, the disease resistance of teleost fish is reduced and its susceptibility to pathogenic infection is significantly increased [35]. It is necessary to study the immunity of the orange-spotted grouper after the stress of *Vibrio alginolyticus*. In this study, we used a high-throughput sequencing method to identify and investigate the expression patterns of host mRNAs and miRNAs in *Epinephelus coioides* infected with *Vibrio alginolyticus*.

From this study, we obtained 11,958 different expressed genes with 3565 up-regulated genes and 8393 down-regulated genes. Among these, there are 192 different expressed genes associated with immune

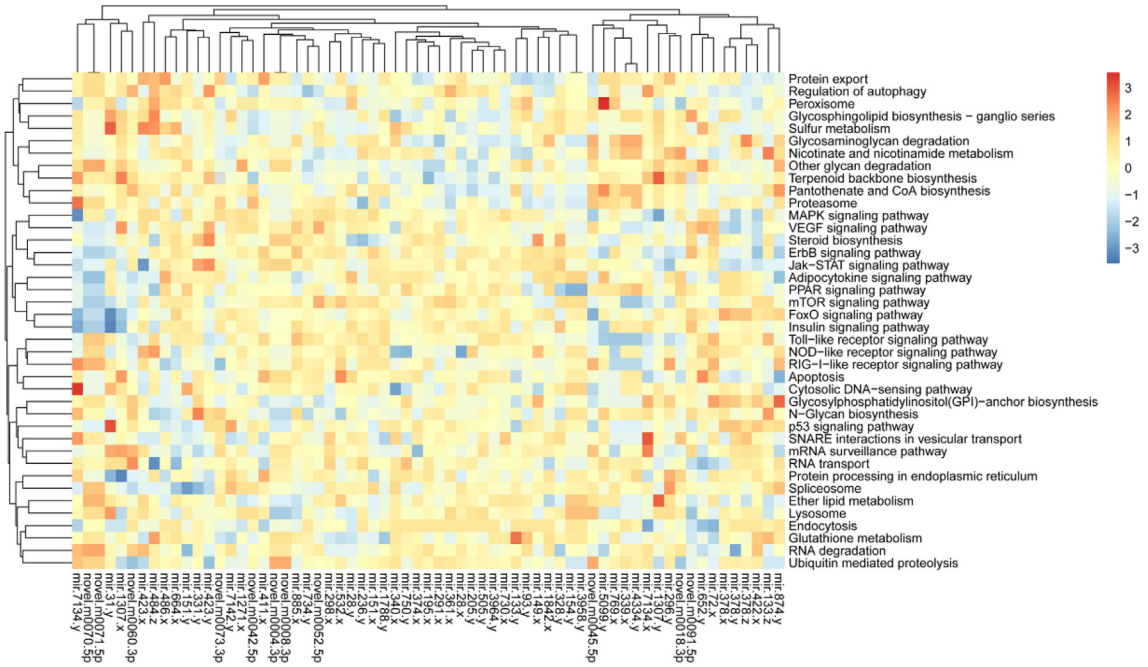

Fig. 4. Heatmap and cluster patterns of DE miRNAs and target-gene related pathways. MiRNAs are clustered together by exhibiting similar pathway targeting patterns and pathways are clustered together by related miRNAs.

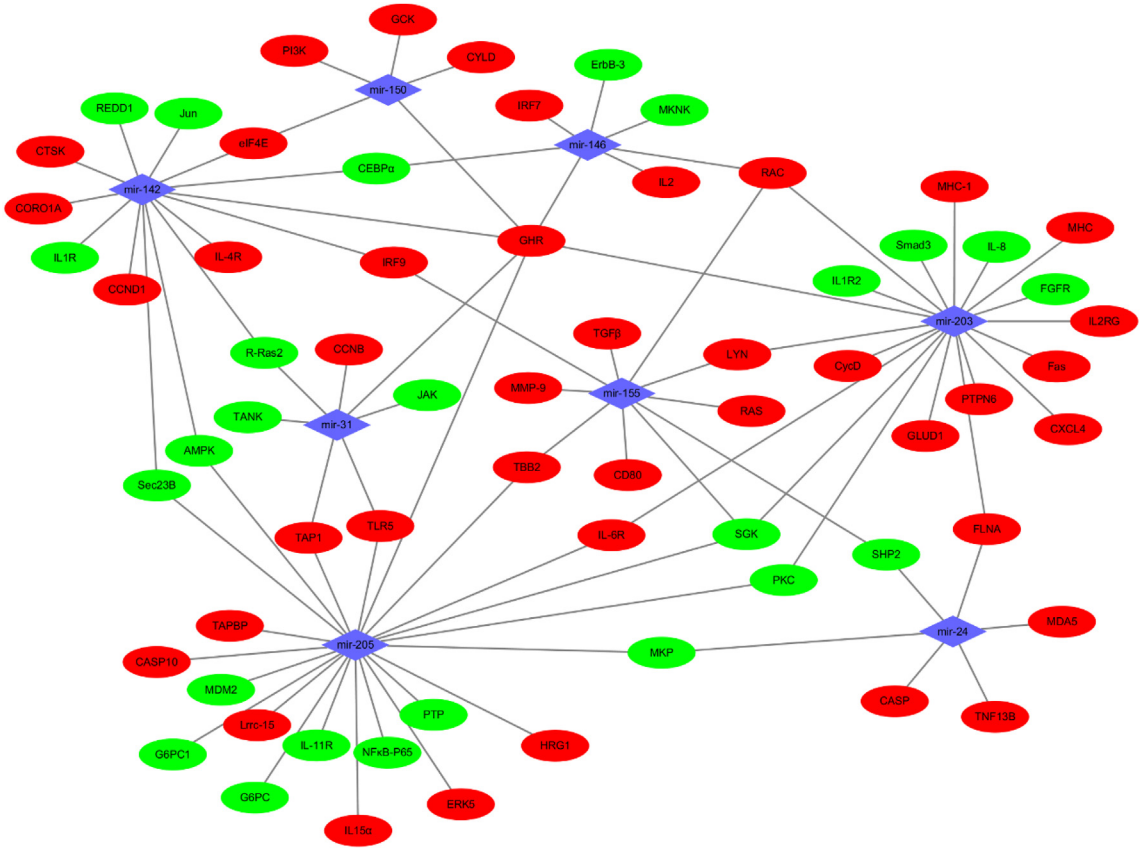

Fig. 5. The integrating biomolecular interaction network of target genes predicted to be regulated by miR-142, miR-146, miR-150, miR-155, miR-203, miR-205, miR-24, miR-31. The rhombus are miRNAs and the red oval are up-regulated genes while the green oval are down-regulated genes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

systems. The KEGG pathway enrichment analysis showed that the most significate DEGs are associated with Fc gamma R-mediated phagocytosis, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, Cytosolic DNA-sensing pathway and Intestinal immune network for IgA production. And from this, Toll-like receptor signaling pathway and NOD-like receptor signaling pathway has the most DEGs (Fig. 6). The innate immune system is an effective defense against microbial pathogen [36]. Toll-like receptors(TLRs) are the best illumination of the innate immune receptors against infections of mammals [37]. Up to now, there are at least 16 TLR types were identified in fish [38]. From this experiment, we have obtained five Toll-like receptors, including TLR1, TLR2, TLR21, TLR21B and TLR5S and both of them were up-regulated. Different from TLRs, nucleotide-binding oligomerization domain-like receptors(NLRs) are a recently discovered family of intracellular PRRS [39]. NLRs have characterized the group of bacteria recognition receptors which associate with innate immunity [40]. Previous studies have characterized a function for NLRs, as cytoplasmic mediators of innate invasive bacterial infection [41]. We have obtained two NLRs, NOD1 and NOD2, both of them were up-regulated. This showed that genes belonged to pathogen-associated pathways may probably upregulate in response to bacterial infection.

MicroRNAs are 20–24 nt noncoding RNAs. Most miRNAs execute their functions by complementarily binding to target genes 3' untranslated sequence [42]. While most animal miRNAs are imprecisely complementary to their target mRNAs [43]. MicroRNAs inhibit and destabilize mRNAs to execute their functions [44]. From this experiment, we have obtained 374 miRNAs targeting 2659 mRNAs. As we can see from Fig. 4, miRNAs are associated with diverse biological processes including fundamental cellular operations and stress response.

miRNA-mRNA GO and KEGG analysis show that there are 90 miRNAs associated with the immune system which indicated miRNAs play a pivotal role in the immune system. Some miRNAs including miR-142, miR-146, miR-155 and miR-150 are affected during bacterial infection and associated with immune response. Among these, miR-142 has been reported to directly regulate IL-6 expression upon LPS stimulation of macrophages thus to be associated with the regulation of immune responses [24]. Forced expression of miR-142 inhibited inflammatory cytokines (TNF-α, IL-12, IL-6) release upon LPS stimulation [45]. The previous study has shown a role for miR-142 in hematopoietic-derived cell functions, including myeloid cell differentiation [46–48], T cell function [49,50] and megakaryopoiesis [51]. The induction of miR-146 genes of IL-1, LPS, TNF-α was mediated by NFκB transcription factors through promoter analysis [52]. The previous study indicated that miR-146 regulates TRAF6 and IRAK1 for down-regulation to function as a negative regulator of cytokine and TLR signaling [53]. MiR-146 also functions in a negative feedback pathway of interferon (IFN) signaling by targeting the STAT-1 and IRF5 transcription factors [54]. MiR-155 was also known as a key regulator of the immune system [55,56]. IKK, TAB2 and c-Jun are target genes of miR-155 that are associated with TLR-mediated immune response [57,58]. Previous research showed that miR-155 is upregulated by the LPS/NFκB/AP-1 pathway in trophoblasts and conduces to preeclampsia by down-regulating cyclin D1 and cysteine-rich 61 [59–61]. And recent research indicated that miR-155 inhibits NKIRAS1 and IRAKM expression to play a positive feedback role in AP-1/NFκB pathway whereas PTEN 3′UTR compete for miR-155 binding in trophoblasts to block the activation of AP-1/NFκB pathway [62]. MiR-150 is preferentially expressed in NK cells as well as mature T and B cells and other cell types of the hematopoietic, therefore it has been identified as an important regulator of immune cell activation and differentiation [63–65]. Recently a study has reported miR-150 inhibits pancreatic cancer cells' growth and survival [66]. MiR-150 has also been reported in the host

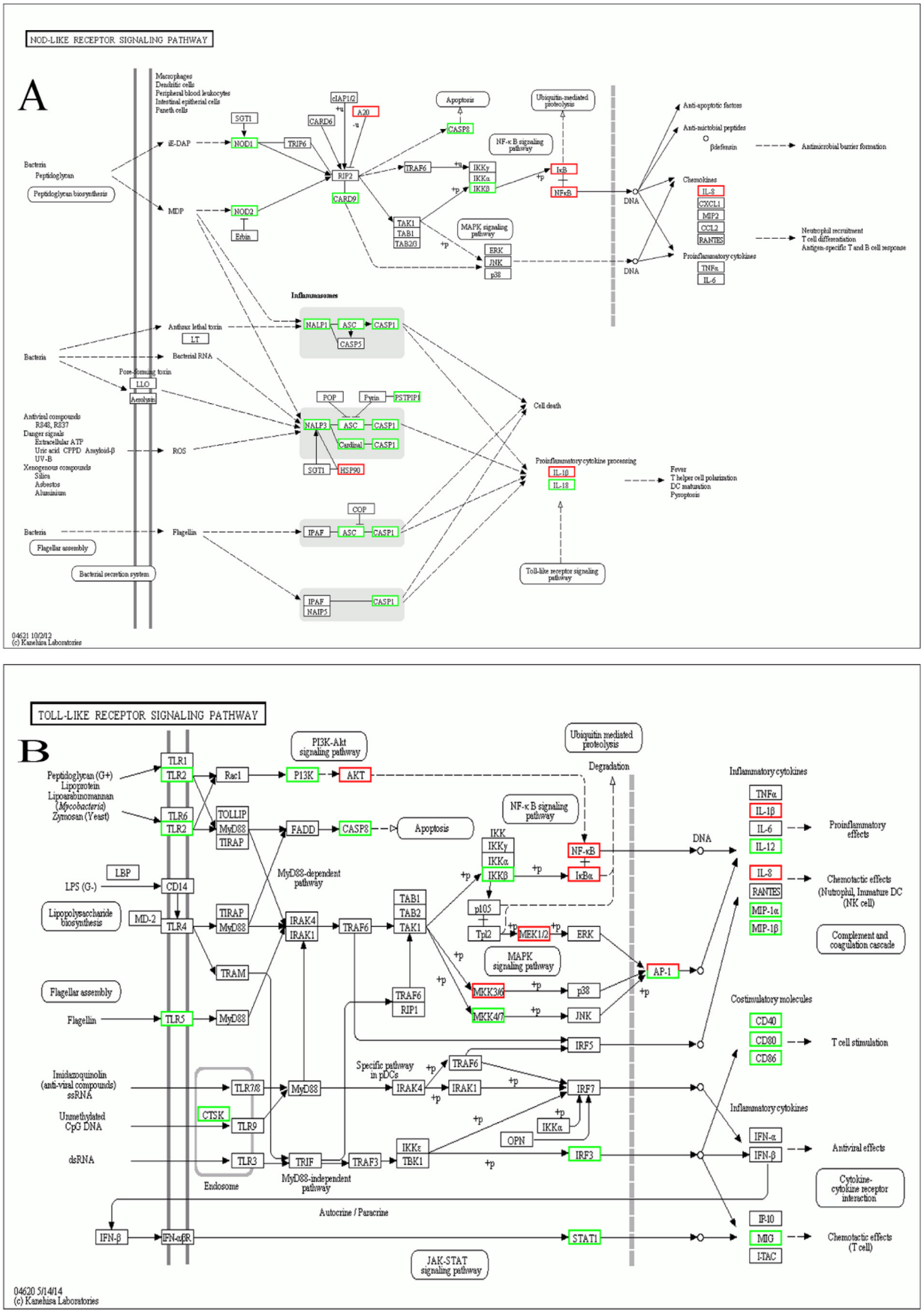

Fig. 6. Pathway expression patterns. The red orthogon were the down-regulated genes and the green orthogon were the up-regulated genes. (A) Toll-like receptor signaling pathway; (B) NOD-like receptor signaling pathway. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

response to the endotoxin lipopolysaccharide [67,68].

Among all the different expressed miRNAs, we have selected eight different expressed miRNAs that regulate genes associated with the immune system and signal transduction. As shown in the miRNA-mRNA network, miR-142, miR-146, miR-150, miR-155, miR-203, miR-205, miR-24 and miR-31 regulate 67 target genes associated with the immune system and signal transduction. From our study, miR-150 was down-regulated after *V. alginolyticus* challenge. MiR-150 target genes CYLD, GCK, PI3K and eIF4E were both up-regulated. One of its target genes, PI3K, is involved in a multiple of cellular behaviors, such as survival, proliferation, metabolism, immunity and trafficking [69]. The inhibition of PI3K signal can reduce the immune response by inhibiting the secretion of proinflammatory cytokines [70]. MiR-150 suppression activated the PI3K-AKT pathway. Thus, miR-150 is a key regulator of PI3K [27]. Interestingly, miR-142, miR-146, miR-155, miR-203, miR-205, miR-24 and miR-31 were all up-regulated after *V. alginolyticus* challenge, and their target genes including up and down-regulated genes. For example, the current miR-146 model proposes a negative feedback mechanism for bacterial products, which reduces LPS sensitivity and prevents excessive inflammation. Therefore, microRNAs fine-tuning of the innate immune response may lead to inflammatory diseases [71]. The previous study of miR-142 has shown LPS treatment of control cells increased IL-6 mRNA levels 80-fold, while miR-142 overexpression reduced this to 40-fold. This indicates that miR-142 truly down-regulated IL-6. Recently, a new theory that miRNAs also function to induce gene expression has been reported. MiR-373 and pre-miR-373 induce cold-shock domain-containing protein C2 (CSDC2) expression through targeting gene promoters' specific sites [72].

In conclusions, our current prediction work demonstrated a new pattern of *V. alginolyticus* infected groupers based on miRNA-mRNA interaction network. More than 100 miRNAs showed altered expression upon *V. alginolyticus* challenge, indicating grouper miRNAs may play an important role in the host response to viral infection. The targets of host miRNAs are classified into a wide range of functional categories, especially those related to immune defense. These results suggest that, like higher vertebrates, miRNAs in teleost significantly promote immune response and protect it from excessive inflammation in response to infection. The transcriptomics data may also provide important resources for future studies.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.05.006.

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