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Sea cucumbers in a high temperature and low dissolved oxygen world: Roles of miRNAs in the regulation of environmental stresses☆

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ABSTRACT

The exacerbation of global warming has driven changes in environmental factors, including water temperature and oxygen concentration. The sea cucumber *Apostichopus japonicus*, an economically important aquatic animal, is constantly and directly challenged by heat and hypoxia. In this study, 12 small RNA libraries were constructed for this species, and a total of 21, 26 and 22 differentially expressed (DE) miRNAs were clarified in *A. japonicus* under thermal (26 °C), hypoxic (2 mg/L) and the combined stresses. Comparative miRNA sequencing analysis and real-time PCR were used to identify and validate the representative miRNAs, including Aja-miR-novel-299, Aja-let-7b-3p, Aja-miR-71b-5p, Aja-miR-novel-13218 and Aja-miR-2004 in response to high temperature, and Aja-miR-92b-3p, Aja-miR-210–5p and Aja-miR-novel-26331 in response to oxygen limitation. GO and KEGG pathway analysis revealed that the potential target genes of DE-miRNAs involved in biosynthesis, metabolism, immunity, cell growth and death, translation and signaling transduction. Key DE-miRNAs with potentially targeted genes associated with heat shock and hypoxia response were also determined. These results may help explaining the role of miRNA regulation in stress resistance, as well as the potential molecular regulation mechanism of the echinoderm *A. japonicus* in the context of global warming.

1. Introduction

The sea cucumber (*Apostichopus japonicus*) is one of the most commercially important aquatic species in Asia. However, as a result of global warming, deteriorating water conditions are increasingly challenging the survival of sea cucumbers and their development for the mariculture industry. For example, 174,340 tons of sea cucumbers were harvested in 2018—a reduction of approximately 20.72% compared with 2017 (Ministry of Agriculture, 2018). The main causes of the massive mortality were extremely high temperature and low dissolved oxygen concentration. In China, the mean surface temperature has shown an increase of 0.4–0.6 °C in the last 100 years (IPCC, 2001), and it is predicted to be continue to rise by 1.7 °C in the next 30 years and by 2.2 °C over the next 50 years (Qin, 2003). Based on the measured value of summertime bottom water DO from the 1980s–2010s, a remarkable decline was observed in the central Bohai Sea in China (Zhai, 2019). For example, the DO was at rather high levels of over 190 μmol O_2/L in 1982 (Tang, 1997). But in late summer 2015, the historically lowest measured Bohai Sea DO of 67 μmol O_2/L was observed (Zhai, 2019). Sea cucumbers were greatly impacted by thermal and hypoxic stress at the level of genes, proteins and metabolites (Huo et al., 2019a, 2019b, 2020). Sea cucumbers likely developed specific strategies in response to the environmental stress.

Organisms would like respond with cellular modifications to tolerate the adverse environment, including transcriptional, translational and post-translational modification. The action of

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non-coding RNA molecules is indispensable in post-transcriptional regulation. MicroRNAs (miRNAs) represent a class of small, non-coding RNA molecules with a length of 18–28 nucleotides (Tanase et al., 2012). Argonautes (Agos) first bind to endogenous miRNA, and guide strands are incorporated into Agos after discarding passenger strands. Finally, mature RNA-induced silencing complex (RISC) is guided to complementary target mRNAs. According to the complementarity, RISC would endonucleolytically cleave the target mRNA or reduced translation and/or stability of target mRNAs (Janas et al., 2012). MiRNAs may play roles in regulating gene expression and restoring homeostasis (Leung and Sharp, 2010), and they were considered as key modulators for the development of abiotic stress tolerance (Noman et al., 2017). In recent years, a multitude of reports have demonstrated that specific miRNAs are involved in the hypoxic response and thermal response, and contribute to the regulation of biologically important genes in low oxygen tension and high temperature, such as hypoxia-inducible factor-1α (HIF-1α) (Liang, 2017), vascular endothelial growth factor (VEGF) (Hua, 2006), heat shock protein 70 (Hsp70) (Yin, 2009), heat shock cognate protein 70 (Scott, 2012), Hsp72 (Beninson, 2014), Hsp60 (Shan, 2010) and *etc*. In previous studies of sea cucumbers, miRNAs changed in response to skin ulceration syndrome (Sun et al., 2016, 2018), intestine regeneration (Sun et al., 2017), aestivation (Chen et al., 2013), heat shock response (Li and Xu, 2018), salinity stress (Tian et al., 2019) and hypoxia stress (Huo et al., 2017). As shown previously, heat is usually accompanied by hypoxia (Huo et al., 2020). However, little attention has been paid to combined environmental stressors.

Therefore, we conducted experiments to determine the effects of three types of environmental stresses on the miRNA profiles in sea cucumber, including thermal stress, hypoxic stress and a combination of the two. Our main objectives were to identify and characterize the differentially expressed (DE) miRNAs, and to determine the biological processes with which they are mainly involved. Our results confirmed that miRNAs are involved in the regulation mechanisms of aquatic animals exposed to environmental stress. They will also help facilitate understanding on the molecular regulation mechanisms of sea cucumbers in the context of global warming.

2. Materials and methods

2.1. Animals

For sea cucumbers *A. japonicus* in the present study, we maintained the thermal environment at 26 °C by using a 2-kW heating rod, and the dissolved oxygen concentration at 2 mg/L to build hypoxic stress by using a dissolved oxygen control system (Huo et al., 2020). Following collection from the coast of Weihai, China, sea cucumbers (100 ± 20 g) were acclimated in tanks for 1 week prior to the formal experiment. During acclimation, the conditions of the aquatic environment were kept the same as those of the control group—salinity: ~30‰, pH: ~8.0, temperature: ~16 °C, and dissolved oxygen: ~8 mg/L. All healthy individuals were then randomly divided into four groups—HT: thermal stress, LO: hypoxic stress, HL: the combined stress of heat and hypoxia, or NC: normal control—and cultured separately. After 48 h, twelve sea cucumbers were promptly dissected in each group, and their respiratory trees were sampled, preserved in liquid nitrogen, and stored at −80 °C until subsequent analysis.

2.2. Small RNA library construction and sequencing

Total RNA from each sample was extracted using phenol/chloroform following the manufacturer's recommendations, and the quality was checked using 1% agarose gels and a kaiaoK5500® spectrophotometer (Kaiao, Beijing, China). The RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA) was used for RNA integrity (RIN) and concentration assessment. Nine qualified RNA samples (OD260/280 ≥ 1.8, OD260/230 ≥ 1.5, RIN ≥ 7) were selected from different sea cucumbers in each group and used for small RNA library construction. The 18- to 30-nt size range of RNA was purified from 15% agarose gels. Ethanol was used for precipitation, and then small RNA samples were centrifuged for enrichment. A Small RNA Sample Preparation Kit (RS-200-0048, Illumina, San Diego, CA, USA) was used to prepare the library according to protocol. The RNA concentration of the library was measured using the Qubit® RNA Assay Kit (Life Technologies, Grand Island, NY, USA) in Qubit® 2.0 for preliminary quantification and then diluted to 1 ng/μl. The qualified libraries (valid concentration > 2 nM) were sequenced by an Illumina Hiseq 2500 platform.

2.3. Sequence data analysis

After obtaining the raw reads, clean data were processed using ACGT101-miR (LC Sciences, Houston, Texas, USA) to remove adapter dimers, junk, low complexity, rRNA, tRNA, snRNA, snoRNA and repeats. Unique sequences (18–26 nt) were mapped to miRBase 21.0 by BLAST search to identify known and novel miRNAs, and at most one mismatch inside of the sequence was allowed in the alignment. Data normalization followed the procedures as described in a previous study (Li et al., 2016). If the normalized expression was zero, it was changed to 0.01. DE-miRNAs were calculated by comparing the miRNA expression between control and treatment samples. T-test was used for analysis, with $P < 0.05$ as the criterion to determine significant differences in miRNA expression. In the present study, valid expressed detections were identified as those miRNAs detected in all three groups with the same treatment conditions. A heatmap was constructed using TBtools and clustered by row scale (Chen, 2020). The Venn diagram was constructed using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html). Mfold was used to predict the secondary structure of miRNA. The criteria for secondary structure prediction were: (1) number of nucleotides in one bulge in stem ≤ 12 and in one bulge in mature region ≤ 8; (2) number of base pairs in the stem region of the predicted hairpin ≥16 and in the mature region of the predicted hairpin ≥12; (3) length of hairpin (up and down stems + terminal loop ≥ 50) and length of hairpin loop ≤ 20; (4) number of biased errors in one bulge in mature region ≤ 4, number of biased bulges in mature region ≤ 2, and number of errors in mature region ≤ 7; (5) cutoff of free energy (kCal/mol ≤ −15); and (6) percent of mature region in stem ≥ 80.

2.4. Real-time PCR (qPCR) validation

Total RNA was extracted using a kit (TRK1002, Norgen, NO.25700), and the quality was checked by Nanodrop. The TURE-script 1st Strand cDNA SYNTHESIS Kit (Aidlab, Beijing, China) was used for reverse transcription according to the experimental protocol. The reaction proceeded for 10 min at 25 °C, 50 min at 42 °C and 15 min at 65 °C, followed by a final hold at 4 °C. To normalize for technical variations, 5.8s rRNA was taken as an internal control. Primers designed by PRIMER 3 are shown in Table S1 (Untergasser, 2012). Three biological replicates for each group and three technical replicates for each biological replicate were performed. Following the manufacturer's recommendations, amplification was performed in a 10-μl reaction solution containing 1 μl of cDNA, 5 μl of SYBR Green Master Mix, 0.5 μl of each primer (200 nM), and 3 μl of ddH_2O. The PCR reaction conditions were as follows: 3 min at 95 °C

followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s and a final cooling step at 4 °C. Melting curves of products were obtained to confirm amplification specificity. The $2^{-\triangle\triangle CT}$ method was used to analyze the relative expression level of miRNA in the treatment and control groups. Statistical analyses were performed using SPSS 19 software (IBM Corp., Armonk, NY, USA). All data are shown as mean ± SD. A one-way ANOVA with Duncan's test was used to identify significant differences between different groups of each gene, and the threshold of statistical significance was $P < 0.05$.

2.5. miRNA target prediction, GO enrichment and KEGG pathway analysis

TargetScan 50, miRanda 3.3a and RNAhybrid software were used to predict the genes targeted by DE-miRNAs. The predicted target gene with max energy > −10 and context score percentile less than 50 were removed. The Gene Ontology (GO) database (http://www.geneontology.org/) and KEGG database (http://www.genome.jp/kegg/) were used for GO and KEGG analyses. The GO and pathway terms conforming to P-value < 0.05 through Fisher's exact test were defined as significantly enriched GO terms and pathways. The network of predicted genes of miRNAs was illustrated using Cytoscape 2.8.3 software (Shannon et al., 2003).

3. Results

3.1. Small RNA library construction

In total, 12 small RNA libraries (high temperature treatments: HT1, HT2, HT3; low dissolved oxygen treatments: LO1, LO2, LO3; high temperature and low dissolved oxygen treatments: HL1, HL2, HL3; and normal controls: NC1, NC2, NC3) were constructed from sea cucumber in the present study, and all raw data have been submitted to the SRA database (accession numbers SRR11117653−SRR11117664). A total of 24, 115, 744 ± 1,074,106, 23, 249, 108 ± 1,736,316, 23,616,333 ± 585,151 and 23,280,200 ± 488,593 raw reads were generated from the HT, LO, HL and NC libraries, respectively. After removing low-quality reads, a total of 20,541,861 ± 1,286,576, 16,959,548 ± 4,734,984, 18,235,217 ± 1,283,308 and 16,800,149 ± 1,619,614 clean reads were obtained from the HT, LO, HL and NC libraries, respectively. Based on the clean reads, the length distribution showed that 22 nt was the most common type.

3.2. Different expression profiles of miRNAs and real-time PCR validation

In the present study, compared with the NC group, 21, 26 and 22 DE-miRNAs were identified in the HT, LO and HL groups, respectively (Fig. 1A, B and C). Compared with the NC group, five up-regulated miRNAs and 16 down-regulated miRNAs were shown in the HT group; 15 up-regulated miRNAs and 11 down-regulated miRNAs were shown in the LO group; and 10 up-regulated miRNAs and 12 down-regulated miRNAs were shown in the HL group. A total of 54 non-repetitive miRNAs were identified to be significantly differentially expressed in the pairwise comparison among the three treatments ($P < 0.05$), and the heatmap is shown in Fig. 1D.

Five significantly altered miRNAs were selected for real-time PCR validation (Fig. 2). According to the sequencing analysis, Aja-miR-novel-299 and Aja-miR-novel-13218 were significantly down-regulated in the HT and HL groups. Aja-miR-71b-5p was significantly up-regulated in the HT group. Aja-miR-210−5p was significantly up-regulated and Aja-miR-92b-3p was significantly down-regulated in the LO and HL groups. Real-time PCR results showed that four of them (Aja-miR-novel-299, Aja-miR-71b-5p, Aja-miR-novel-13218 and Aja-miR-210−5p) have same tendency in all the three treatment groups compared with sequencing results, and Aja-miR-92b-3p showed the same tendency in one treatment group, indicating that the sequencing analysis was accurate.

3.3. Gene ontology and pathway enrichment analysis for target genes of the miRNAs

Having identified a set of DE-miRNAs in sea cucumbers under environmental stress, we sought to define the role of these miRNAs in shaping the expression of target genes and their functions. The putative target genes of DE-miRNAs were predicted and used for GO analysis to identify enriched functional groups ($P < 0.05$). The top five significantly enriched GO terms (with lowest p-values) of three categories under three different stresses are shown (Fig. 3). Among the terms of biological processes (BP), the three most enriched categories with the lowest P-values were "peptidyl-tyrosine dephosphorylation", "positive regulation of GTPase activity" and "anatomical structure morphogenesis" in sea cucumbers under thermal, hypoxic and the combined stress, respectively. The three most highly represented cellular component (CC) categories with the lowest P-values were "integral component of membrane", "nucleus" and "membrane" in sea cucumbers under thermal, hypoxic and the combined stress, respectively. Finally, the three most abundant molecular function (MF) categories with the lowest P-values were "protein tyrosine phosphatase activity", "ATP binding" and "protein tyrosine phosphatase activity" in sea cucumbers under thermal, hypoxic and the combined stress, respectively.

Significantly enriched KEGG pathways were identified based on the mRNAs targeted by significantly changed miRNAs ($P < 0.05$; Fig. 4). "Insulin resistance", "Huntington's disease", "homologous recombination", "fanconi anemia pathway", "ABC transporters" and "tight junction" were the significantly enriched KEGG pathways co-identified in the three treatments. With P-values lower than 0.001, five pathways ("insulin resistance", "ABC transporters", "tight junction", "ubiquitin mediated proteolysis" and "ErbB signaling pathway") were identified in sea cucumbers under thermal stress; five pathways ("Huntington's disease", "notch signaling pathway", "fanconi anemia pathway", "intestinal immune network for IgA production" and "cell cycle") were identified in sea cucumbers under hypoxic stress; and three pathways ("dorso-ventral axis formation", "notch signaling pathway" and "tight junction") were identified in sea cucumbers under thermal combined with hypoxic stress.

3.3.1. Key DE-miRNAs response to environmental stress

A Venn diagram showed that five miRNAs (Aja-miR-novel-299, Aja-let-7b-3p, Aja-miR-71b-5p, Aja-miR-novel-13218 and Aja-miR-2004) may play important roles in sea cucumbers subject to high temperature condition (Fig. 5A). Meanwhile, Aja-miR-92b-3p, Aja-miR-210−5p and Aja-miR-novel-26331 may be important in sea cucumbers under oxygen-limited condition. The secondary structure of the eight key DE-miRNAs were shown in Fig. 5B. No common miRNA was identified in the three comparisons in the present study.

3.3.2. Important molecular and miRNAs associated with thermal and hypoxic stress

In the present study, we selected some important miRNA-potentially-targeted genes related to heat shock (heat shock protein 70, heat shock protein 90 and *etc.*) and hypoxia response (hypoxia-inducible factor 1-alpha, hypoxia up-regulated protein 1 and *etc.*). The network is shown in Fig. 6. Among the 85 miRNAs potentially targeting the heat shock-related genes, 42 of them were

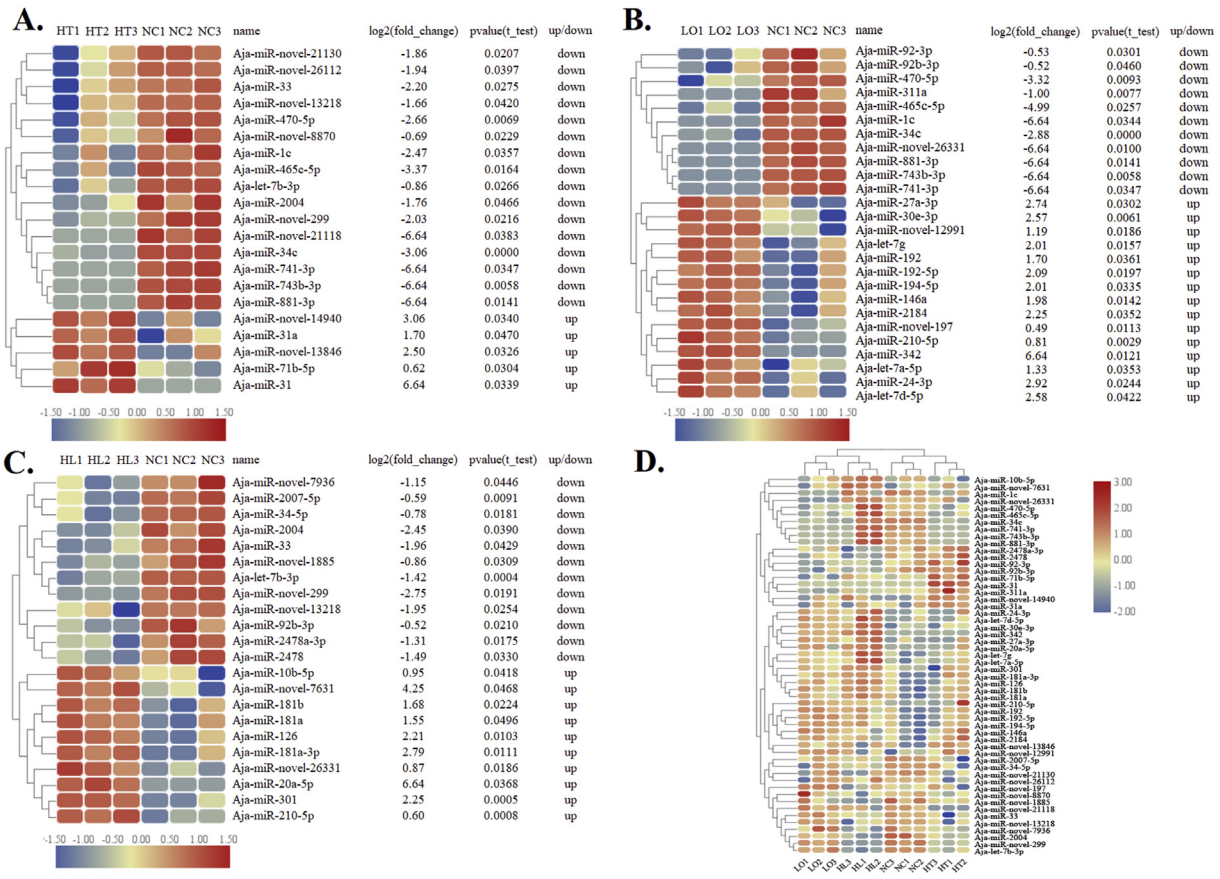

Fig. 1. Differentially expressed miRNA in the A) high temperature (HT) group; B) low dissolved oxygen (LO) group; C) high temperature and low dissolved oxygen (HL) group compared with the normal control (NC) group; and D) differentially expressed miRNAs (non-repetitive) in the pairwise comparison among the three treatments ($P < 0.05$). All red rectangles indicate higher levels of miRNAs, and blue rectangles indicate lower levels of miRNAs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

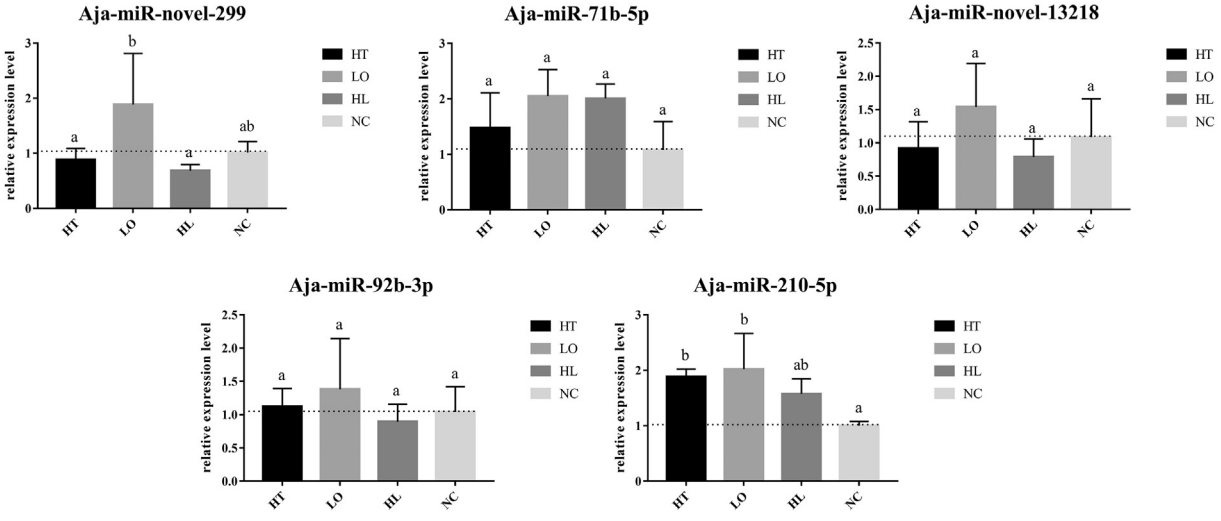

Fig. 2. Validation of high throughput sequencing results using real-time PCR.

significantly altered under the environment stress. Moreover, among the 54 miRNAs potentially targeting the hypoxia related genes, 25 of them were significantly changed under the environment stress. Both percentages exceeded 45%.

4. Discussion

In the present study, we provide the miRNA profiles of the sea cucumber *A. japonicus* under thermal and hypoxic stress individually and in combination. A total of 54 non-repetitive miRNAs were

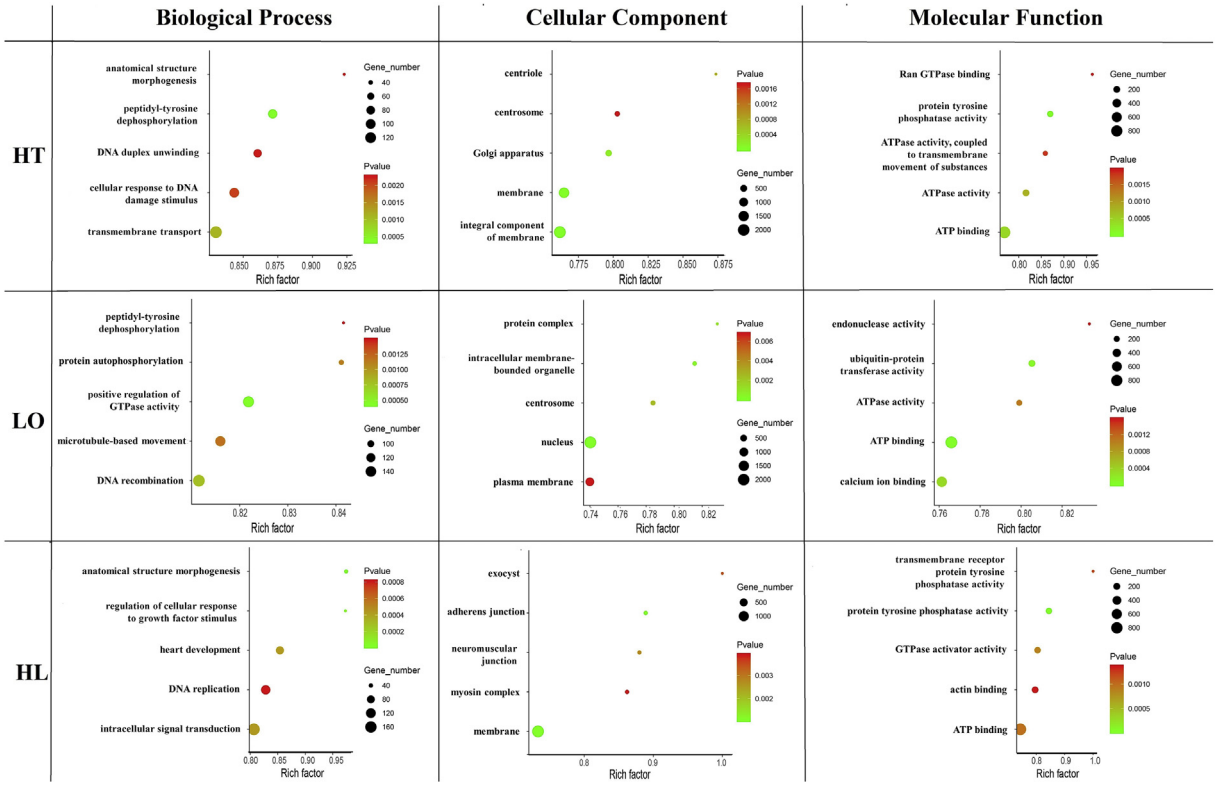

Fig. 3. Top 5 GO terms enrichment (biological processes, cellular component and molecular function) based on the predicted target genes of differentially expressed miRNAs under three treatments ($P < 0.05$).

determined in the three treatments compared with the control. Based on the comparative omics analysis, we characterized the representative miRNAs in sea cucumber in response to extremely high temperature and oxygen limitation, and real-time PCR was used to validate the accuracy. The identified DE-miRNAs are involved in multiple key biological processes related to biosynthesis, metabolism, immunity, cell growth and death, translation and signaling transduction, thus affecting the body state of sea cucumber.

miR-210-5p is one of the "hypoxamirs", which include a specific set of miRNAs that would be up-regulated by hypoxia (Chan and Loscalzo, 2010). We found that Aja-miR-210-5p is significantly up-regulated under hypoxic stress, with or without thermal stress, which is in agreement with the results of previous studies examining different cell types. The main targeted gene involved in the hypoxia response was hypoxia-inducible factor (HIF)-1α. HIF-1α can also upregulate expression of miR-210 (Li et al., 2019). miR-210-5p can negatively affect iron-sulfur cluster assembly pathways and also isocitrate production, thus affecting tricarboxylic acid cycle (TCA cycle) metabolism (Geiger and Dalgaard, 2017). The miR-210-modulated genes were also associated with some other key biological process, including nucleic acid processing, cell cycle control, apoptosis and cell survival (Fasanaro et al., 2009; Zhang et al., 2009).

The lethal-7 (let-7) family consists of over 10 members that regulate cell cycling, proliferation, differentiation and apoptosis (Chu et al., 2014; He et al., 2018; Huang et al., 2017; Mäki-Jouppila et al., 2015; Shimizu et al., 2010). Among them, Aja-let-7b-3p showed significantly down-regulated expression in sea cucumber under thermal stress and thermal combined with hypoxic stress. Moreover, Aja-let-7g, Aja-let-7a-5p and Aja-let-7d-5p showed significantly up-regulated expression in sea cucumber under hypoxic stress. Based on the comparative omics analysis, Aja-let-7b-3p was supposed to be one of the most representative DE-miRNAs in sea cucumber when threatened by heat. Aja-let-7b-3p might regulate the potentially targeted genes, including arf-GAP with GTPase, leucine-rich repeat kinase 2, cathepsin O and kinesin heavy chain. Thus, the GO terms like lysosome (CC), GTPase activator activity (MF), ATPase activity (MF), and NDA replication (BP) were significantly enriched in the present study, and these processes might be important in sea cucumbers coping with environmental stresses.

In the present study, Aja-miR-92b-3p was significantly down-regulated in sea cucumber under hypoxic and thermal combined with hypoxic stress, and based on the comparative omics analysis, it was one of the most representative DE-miRNAs in sea cucumber when threatened by oxygen limitation. MiR-92b-3p was shown as a regulator of cell growth, cell cycle, proliferation, apoptosis, differentiation, migration, invasion, intravasation, and metastasis (Gong et al., 2018; Li et al., 2013; Wang et al., 2013). A previous study found it to be down-expressed under hypoxic conditions in tissues and cells (Hao et al., 2018). It was also identified to be an oncogenic miRNA and involved in the pathogenesis of diabetic nephropathy (Wang et al., 2020). In the present study, we found that Aja-miR-92b-3p may regulate the potentially targeted genes involved in lysosome, histone binding, and DNA duplex unwinding in sea cucumbers under environmental stresses.

Some other not yet well-known miRNAs responded to environmental stresses were also identified in the present study. Aja-miR-33 showed significantly down-regulated expression in sea cucumbers under thermal stress and thermal combined with hypoxic stress. Many recent publications of miR-33 focus on its role in regulating cholesterol homeostasis, fatty acid and glucose metabolism (Cirera-Salinas et al., 2012; Rayner et al., 2010). It may

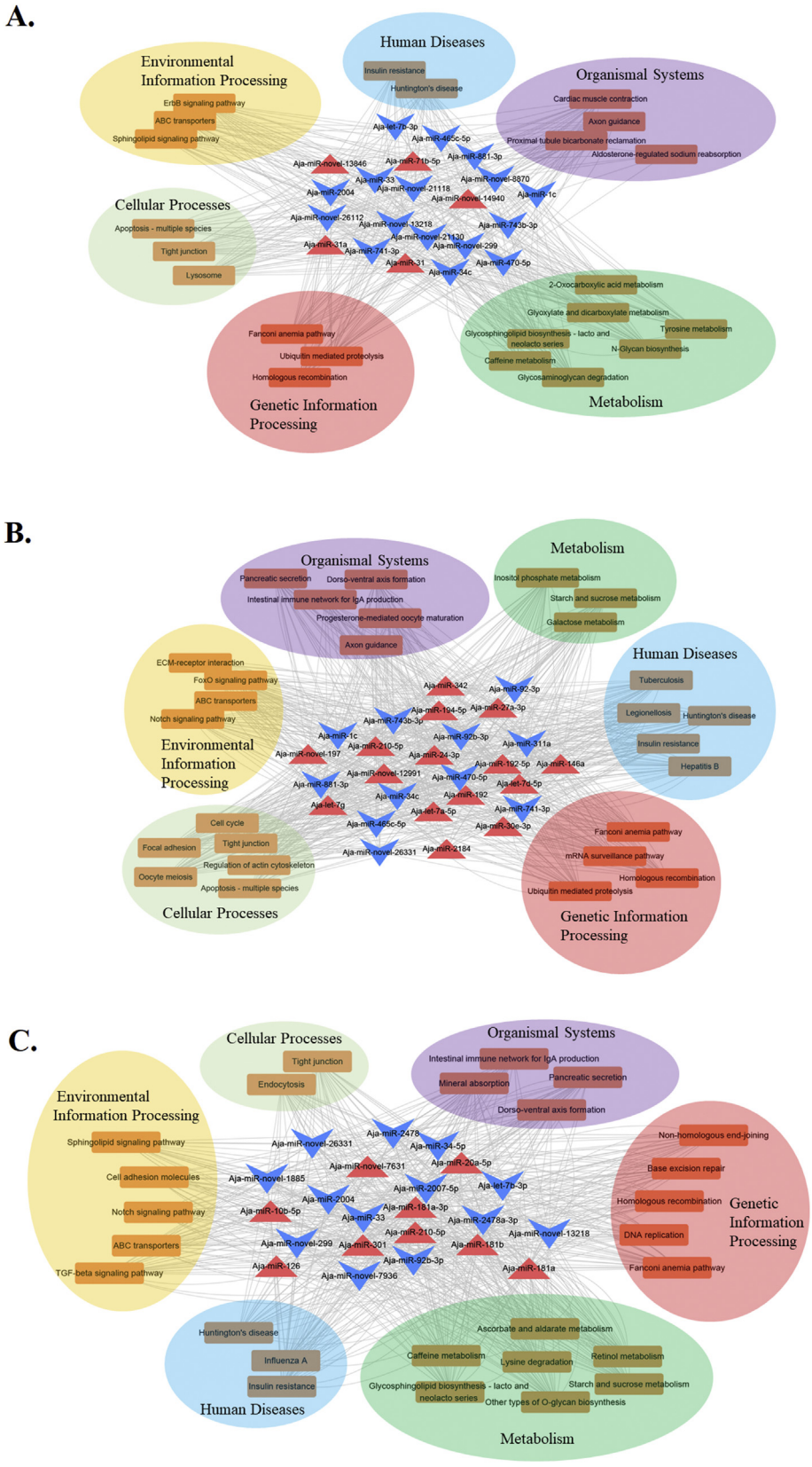

Fig. 4. Pathway enrichment based on the predicted target genes of differentially expressed miRNAs in sea cucumbers under A) thermal stress; B) hypoxic stress and C) the combined stress.

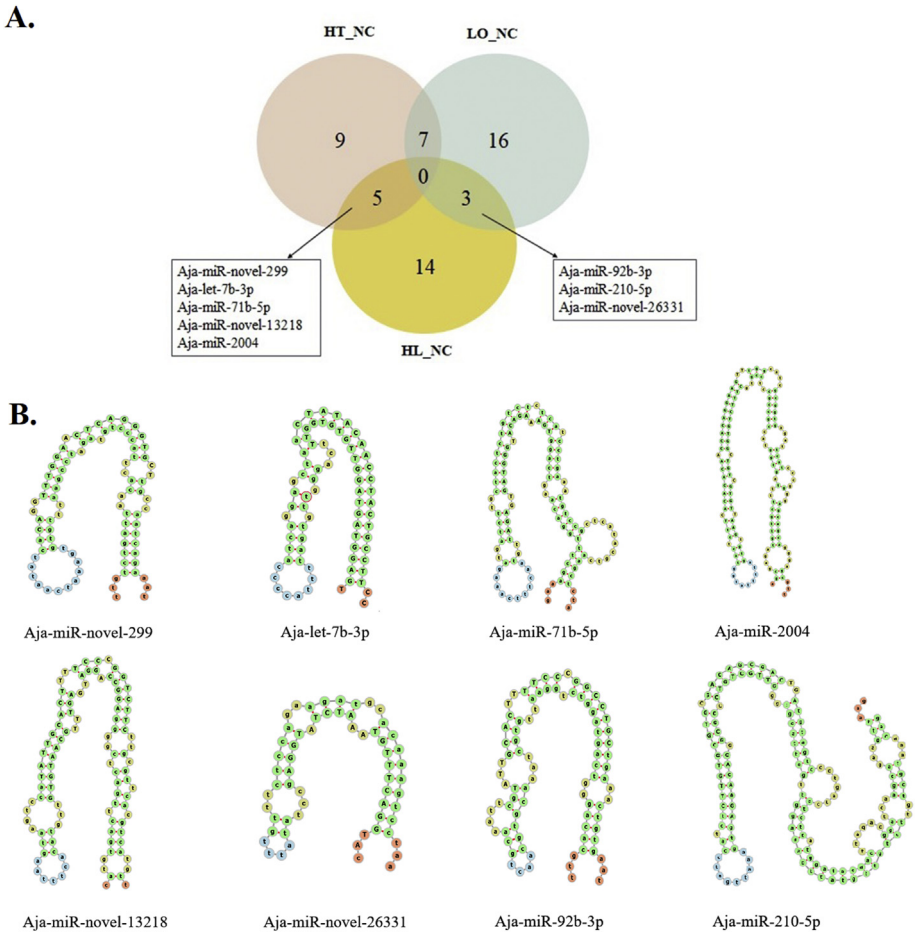

Fig. 5. Key differentially expressed miRNAs response to environmental stress. A) Venn diagram of differentially expressed miRNAs in the comparisons. B) Secondary structure prediction of the key differentially expressed miRNAs.

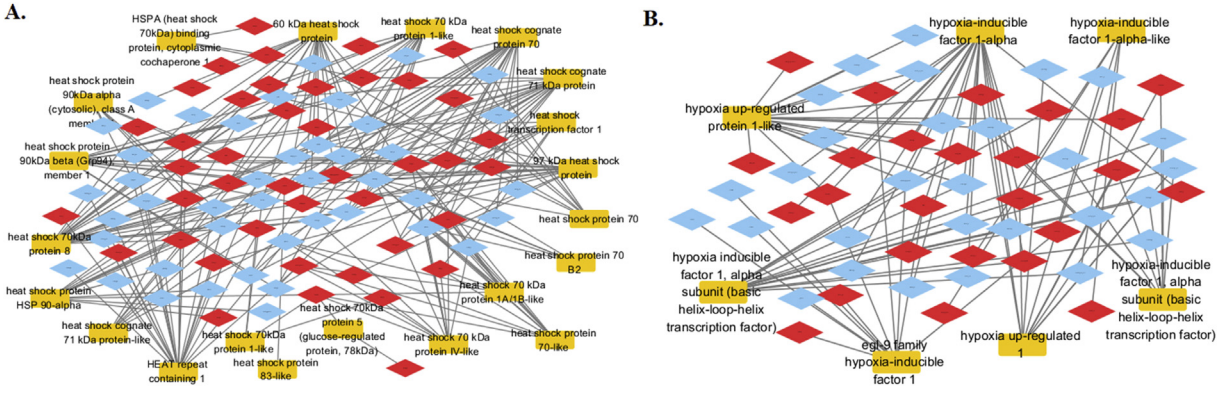

Fig. 6. The identified miRNAs and their targeted genes mainly involved in A) heat shock and B) hypoxia response. (The rectangles represent genes; the diamonds denote identified miRNAs; the red diamonds represent the miRNAs which were significantly changed under environmental stress in the present study). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

also modulate the cell cycle and proliferation by target genes involved in the process (Cirera-Salinas et al., 2012). In previous studies on miRNAs in sea cucumbers, miR-1c was identified to be the most abundantly expressed miRNA in the tube foot, longitudinal muscle and respiratory tree (Wang et al., 2014, 2015). It may also control proliferation and the cell cycle as do the other miRNAs mentioned above (Novello et al., 2013). Moreover, it could regulate angiogenesis by modulating the expression of VegfA as well as a set of actin-related and actin-binding proteins (Mishima et al., 2009). In the present study, Aja-miR-1c was significantly down-regulated in sea cucumbers under thermal and hypoxic stress. Further validation is required for the specific function and regulatory pathways

of the significantly altered novel miRNAs—including Aja-miR-novel-299 and Aja-miR-novel-13218—in environmentally stressed sea cucumbers.

Having discussed the identified DE-miRNAs, the mainly changed biological processes based on their potential target genes were also illustrated. At the protein level, a change in the lysosome pathway was previously found under the same conditions as those of our study (Huo et al., 2019b). Together with the changed "Intestinal immune network for IgA production" pathway and "Apoptosis - multiple species" pathway, we thought that the immune system was affected and apoptosis occurred in sea cucumbers under environmental stress. The "Insulin resistance" pathway was co-identified in all three treatment groups in the present study. Insulin resistance is caused by altered metabolic states, including persistent elevation of glucose, insulin, fatty acids and cytokines (Pessin and Saltiel, 2000). Insulin was involved in glucose homeostasis regulation, including gluconeogenesis and glycogenolysis. These two processes were also validated to be impacted in sea cucumber under environmental stress at the protein level in our previous study (Huo et al., 2019b). Besides, insulin resistance is a defect in signal transduction (Pessin and Saltiel, 2000). As for signal transduction, the "ErbB signaling pathway" and "sphingolipid signaling pathway" were enriched based on the DE-miRNA in the HT group; "FoxO signaling pathway" and "notch signaling pathway" were enriched based on the DE-miRNA in the LO group; "notch signaling pathway", "sphingolipid signaling pathway" and "TGF-beta signaling pathway" were enriched based on the DE-miRNA in the HL group. Thus, it seems that multiple signal transduction pathways could collectively activate the same defense response. The eight key DE-miRNAs (identified in part 3.3.1) were all significantly enriched in "p53 signaling pathway" and "mRNA surveillance pathway", indicating that translation and cell growth and death processes may be significantly impacted by environmental stresses. Furthermore, biosynthesis and metabolism related pathways, such as "starch and sucrose metabolism", "caffeine metabolism", "3-Oxocarboxylic acid metabolism" and "galactose metabolism", were also shown to be changed based on the DE-miRNAs. That may indicate the occurrence of an energy-altered event in response to environmental stress.

5. Conclusion

The present study reports the first miRNA expression profiles of the sea cucumber *A. japonicus* under thermal and hypoxic stress, separately and collectively. Comparative omics analysis was used to identify the representative miRNAs responding to elevated temperature and oxygen deficiency, including Aja-miR-novel-299, Aja-miR-71b-5p, Aja-miR-novel-13218, Aja-miR-92b-3p and Aja-miR-210−5p. Some novel DE-miRNAs need to be further studied to elucidate their function and regulatory pathways. DE-miRNAs identified in this study were mainly enriched in pathways related to biosynthesis, metabolism, immunity, cell growth and death, translation and signaling transduction, suggesting that these processes are involved in regulating the response to thermal and hypoxic stresses in this species of sea cucumber. More genes, proteins and metabolites associated with these key biological processes are expected to be analyzed correlatively. Our results indicate that *A. japonicus* may be developed to serve as a model for examining responses to environmental stress, and provide information for further stress resistance studies.

Credit author statement

Da Huo, Investigation, Data curation, Methodology, Validation, Writing - original draft, Writing - review & editing. Lina Sun, Formal analysis, Funding acquisition, Writing - review & editing. Jingchun Sun: Writing - review & editing. Libin Zhang, Project administration, Writing - review & editing. Shilin Liu, Resources, Writing - review & editing. Fang Su, Project administration, Writing - review & editing. Hongsheng Yang, Supervision, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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