Combined analysis of mRNA–miRNA reveals the regulatory roles of miRNAs in the metabolism of clam Cyclina sinensis hepatopancreas during acute ammonia nitrogen stress

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Abstract

Clam Cyclina sinensis behaves a stronger ammonia nitrogen tolerance, whereas themolecular regulation mechanism remains unknown. In the present study, C. sinensiswas exposed to ammonia nitrogen (32.90 mg/L) for 0 h (the control), 12 h (T1)and 24 h (T2). Integrated analysis of miRNA-mRNA was conducted to reveal theregulatory roles of miRNAs in the metabolism of C. sinensis exposed to acute ammonia nitrogen. Numerous genes involved in detoxification and ammonia excretion expressed differently indicate that nitrogen assimilation and utilization is adjusted to accommodate ammonia exposure. The clam has to alter their glucose and lipid metabolism to meet energy requirements to adapt acute ammonia exposure in the environment. 12, 948 genes were identified as target genes of the differently expressed miRNAs. Most of mRNA–miRNA pairs were involved in metabolisms of carbohydrate, lipid and amino acid. miRNA-mRNA integrated analysis revealed involvement of Purine metabolism, Wnt signalling pathway, Hippo signalling pathway, PI3K-Akt signalling pathway in ammonia-stress response of C. sinensis. Exposed to ammonia, clam may suffer hypoxia exposure. Overall, exposed to ammonia, clam has to alter their glucose and lipid metabolism to meet energy requirements. Numerous mRNA–miRNA pairs were involved in metabolisms of carbohydrate, lipid and amino acid and miRNA can regulate the metabolism of C. sinensis by regulating the expression of target genes during acute ammonia nitrogen stress. The net result is that nitrogen assimilation and utilization is adjusted to accommodate ammonia exposure. The current study is helpful for further investigation into the ammonia nitrogen response mechanisms in mollusks.

KEYWORDS

clam, metabolism, miRNA, molecular response, mRNA, NH_3

MATERIALS AND METHODS

Test C. sinensis with a body weight of 2.26 ± 0.02 g was obtained from an aquaculture farm as the experimental object. During the acclimatization and exposure periods, seawater was dechlorinated (temperature 22 ± 0.3°C, DO >5.0 mg/L, salinity 21‰ and pH 7.8).the level of 32.90 mg/L (about half of 96 h -LC50) of ammonia nitrogen was used as acute exposure concentration. During the exposure periods, the ammonia nitrogen was measured every 6 h, and the level of ammonia nitrogen was adjusted with ammonia stock solution (1 mol/L NH4Cl) in time. Under this condition, 24 h of acute ammonia nitrogen exposure was performed, and three sampling time points were set, that is, 0 h, 12 h and 24 h.

Initial reads quality of the RNA sequences was evaluated and the clean reads were obtained by filtering the adaptor sequences and ploy-N, and other low-qualityreads.Initial sequencing data were processed to obtain the sequence of miRNA. Only the unique sequences with nucleotides ranging from18 to 30 were kept and annotated.

RESULTS

Identification of different expression genes exposed to ammonia nitrogen treatment

Based on the above results of variance analysis, DEGs between two groups were identified. We identified 2537 DEGs (1614 up-regulated and 923 down-regulated) in T1 group (Figure 1) and1488 genes expressed differently in T2 group (778 up-regulated and 710 down-regulated) compared with the control group (Figure 1). When compared the ammonia nitrogen exposure groups, 2942 genes altered significantly (1079 up-regulated and 1863 down-regulated) (Figure 1). We selected six of these DEGs and validated the RNA-Seq results with qRT-PCR (Figure 2). We found that the expression levels of these selected DEGs (Figure 3) were mostly consistent with the RNA-Seq results (Figure 1).

Sequencing and expressionprofiling of miRNAs

We identified a total of 433 known miRNAs and 267 novel miRNAs.Edge R was applied to identify differently expressed miRNAs (DE miRNAs) between two groups and we found that 102 DE miRNAs(79 up-regulated and 23 down-regulated) were significantly differently expressed in T1 group and 61 miRNAs (21 up-regulated and 40 down-regulated) expressed differently in T2 group (Figure 4). We selected 6 DE miRNAs and validated the DE miRNAs sequence results with qRT-PCR. The results showed that the expression levels of these selected miRNAs were mostly consistent with the RNA-Seq results (Figure 5).

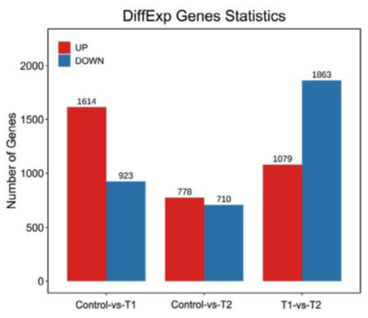

Fig.1

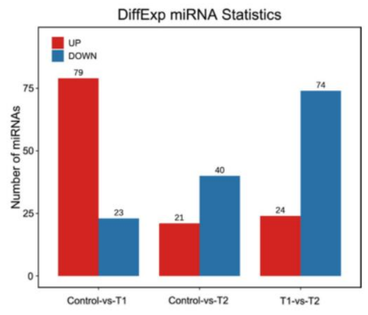

Fig.4

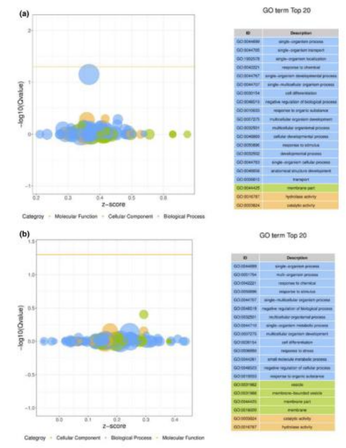

Fig.3

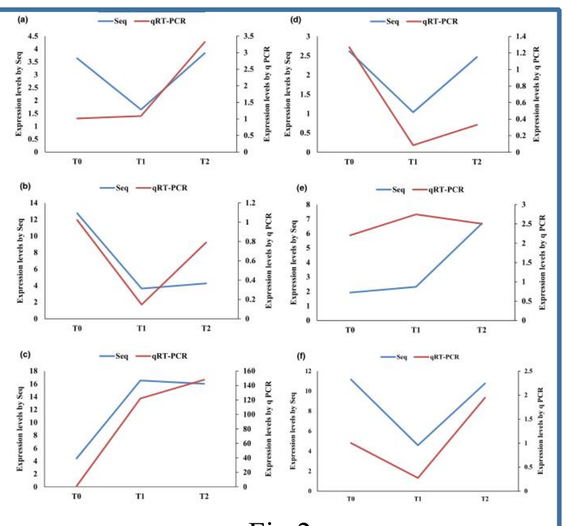

Fig.2

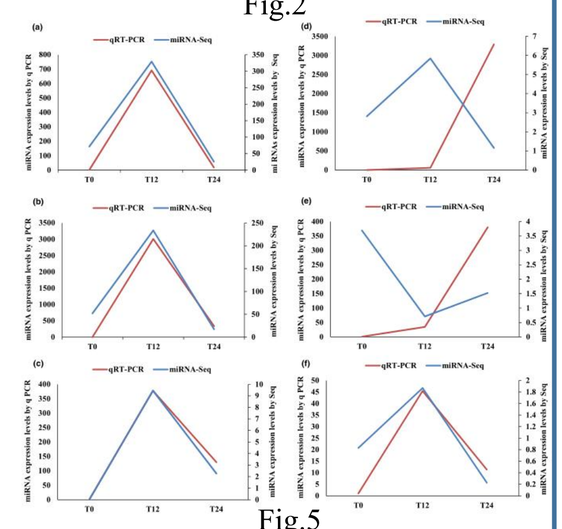

Fig.5

FIGURE1 DEGs statistic
FIGURE2 Validation of mRNAs expression level. (a) hiflα; (b) rorab; (c) Diap1; (d) ABCG2; (e) Npl; (f) Pde9a
FIGURE3 The enrichment of GO for DEGs and the top twenty of biological GO terms. (a) Control versus T1; (b) Control versus T2; (c) T1 versus T2
FIGURE4 Differently express miRNAs statistic
FIGURE5 Validation of miRNAs expression level. (a) miR-263-x; (b) miR-263-z; (c) miR-6751-x; (d) novel-m0073-3p; (e) novel-m0086-5p;(f) novel-m0148-5p

GO and KEGG classification analysis of miRNAs' target genes

With the GO term enrichment analysis, the target genes were classified into different gene ontologies. The GO classification analysis results of these target genes showed with Figure 6. The KEGG results classified 7040 of the target genes into 346 differ-ent pathways (Figure 7).

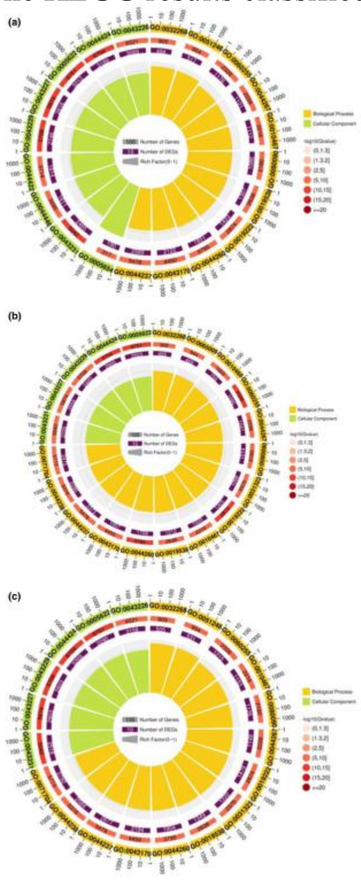

Fig.6

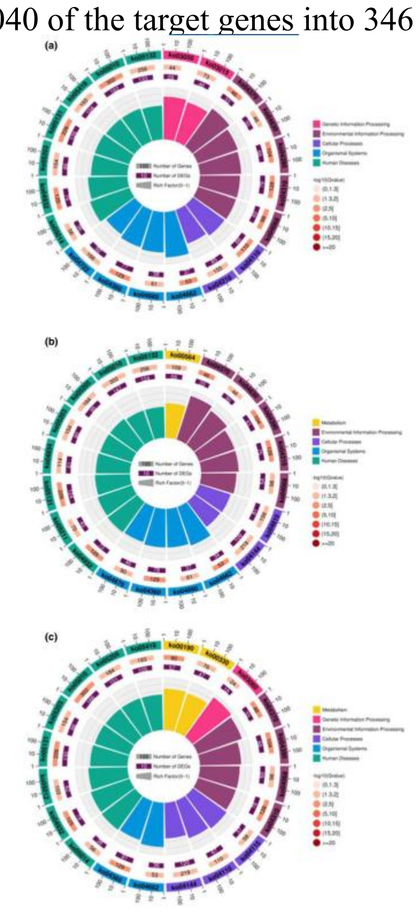

Fig.7

FIGURE6 GO enrichment for miRNA-target genes. (a) Control versus T1; (b)Control versus T2; (c) T1 versus T2
FIGURE7 KEGG enrichment analyses for DEGs for miRNA-target genes. (a)Control versus T1; (b) Control versus T2; (c) T1 versus T2

CONCLUSIONS

We constructed nine mRNA-seq and nine miRNA-seq libraries for C. sinensis hepatopancreas tissue exposed ammonia nitrogen. Further study, we reveal some miRNAs and DEG genes may play important roles in ammonia nitrogen adaption for the clam. Exposed to ammonia, clam may suffer hypoxia exposure and clam has to alter their glucose and lipid metabolism to meet energy requirements. The net result is that nitrogen assimilation and utilization is adjusted to accommodate ammonia exposure. Our results demonstrate that ammonia nitrogen could significantly affect the expression of mRNAs and miRNAs involved in metabolism and we can speculate that miRNAs play important regulatory roles in the transformation of metabolic patterns during acute ammonia nitrogen stress. The current study is helpful for further investigation into the ammonia nitrogen response mechanisms in mollusks.