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Comprehensive transcriptome analysis reveal key molecular events in the pearl oyster after pre-grafting conditioning

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ABSTRACT

Pre-grafting conditioning is a crucial procedure before transplant surgery during pearl production. To investigate the molecular response of the pearl oyster *Pinctada fucata martensii* to conditioning, we constructed two hemocyte transcriptomes from pearl oysters with and without conditioning. A total of 134,222,686 raw reads were generated and assembled using the reference genome of the pearl oyster. Transcriptome analysis revealed 3,074 differentially expressed genes (DEGs). Gene ontology and pathway enrichment analyses revealed that these DEGs were mainly associated with "microtubule-based process", "regulation of actin cytoskeleton", and "cell cycle". All related genes were over-expressed in pearl oysters after conditioning. Some nucleotide-binding oligomerization domain-like receptors (NLR), toll-like receptor, myd88, proinflammatory cytokine interleukin-17 (IL-17), and apoptosis-related genes were highly expressed in pearl oysters after conditioning, indicating that conditioning induced the immune response of pearl oysters. "Fatty acid biosynthesis" (FA biosynthesis) was included in the enriched terms, and all eight FA synthase genes in this pathway were highly induced after conditioning. Four tandemly duplicated arginine kinase genes (PmAK) were found in the genome of *P. f. martensii*, gene structure and sequence analysis indicated PmAK genes were more diverse compared with that from human and zebra fish. The four tandemly duplicated PmAKs were highly up-regulated after conditioning. These findings will help to elucidate the responding molecular events after conditioning and explain the high pearl oyster survival rate with conditioning after transplantation, thereby providing useful information in perfecting the conditioning method to improve pearl oyster survival rate after transplantation.

1. Introduction

Pearl oyster *Pinctada fucata martensii* is an important economic shellfish species and mainly cultured for marine pearl production in the southern provinces of China and Japan. In producing cultured pearls, a mantle graft (approximately 4 mm^2) from a donor pearl oyster with a nucleus, which is a spherical bead of shell material, is transplanted into a host pearl oyster. Before transplantation, pearl oysters (approximately 2 years old) selected for transplantation should undergo a conditioning or weakening phase for 1–2 weeks; this process is called pre-grafting conditioning [1]. There are different methods of conditioning pearl oysters, such as high density, starvation, changing temperature, and salinity [2]. These methods are always performed by placing more pearl oysters than usual in a cage and raising the lines to the water surface during the heat of the day [1]. The purpose of conditioning was to stimulate pearl oysters and make them to spawn, thereby eliminate the gonad of most of the egg or sperm, which can interfere with grafting. Pre-grafting conditioning is considered a key step to reduce the immune response, neural activity, and other physiological activities in minimizing stress and damage caused by transplantation [2–5]. After conditioning, the pearl oyster survival rate after transplantation, bead retainable ratio, and high-quality pearl output ratio are all higher, compared with the group without conditioning [2,4,5].

During pearl production, the excess immune response after transplantation is a major factor that affects pearl quality and quantity because it leads to nucleus rejection, pearl sac formation failure, and host pearl oyster death [6]. Many studies have elucidated the mechanism underlying the immune response after transplantation and found many crucial genes and pathways involving in the immune response after transplantation [7–10]. However, the molecular responds to the conditioning and how those genetic mechanisms affect the transplantation are not well understood. Herein we present the transcriptomic analyses of the pearl oyster with and without conditioning toward a better understand the molecular responding mechanisms involved pearl

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production, which will help improve pearl quality and quantity.

2. Material and methods

2.1. Sample preparation

Pearl oysters (approximately 2 years old) were cultured at Liushagang, Zhanjiang, and Guangdong Province, China. Pre-grafting conditioning was conducted by placing 50 more than the usual 30 pearl oysters in a cage (30-cm diameter) and raising the lines to the water surface during the heat of the day for 8 days. After conditioning, hemolymphs from 30 individuals were extracted from the adductor muscle by using 1 mL syringes and then subjected to centrifugation at 3,500 r/min for 5 min, and the precipitant at the bottom was separated to collect hemocytes. The hemocytes were first placed in liquid nitrogen and then stored at 80 °C refrigerator. The control group included pearl oysters without conditioning and cultured normally, and 30 individuals from the control group were prepared similar to the pearl oysters with conditioning at the same day.

2.2. RNA extraction and library construction

RNA was individually extracted using TRIzol reagent (Invitrogen, USA) and mixed in equal amounts for RNA-seq. mRNA was enriched using oligo (dT) magnetic beads and fragmented into short fragments (200–500 bp) with the addition of fragment buffer. First-strand cDNA was synthesized via a random hexamer primer by using mRNA fragments as templates. The second strand was synthesized by adding the buffer, dNTPs, RNase H, and DNA polymerase I. Finally, we purified the double-strand cDNA, end repair, and base A addition by using a QiaQuick PCR extraction kit. Sequencing adapters were ligated to the fragments. The fragments were purified through agarose gel electrophoresis and enriched through PCR amplification. The library products were sequenced using Illumina HiSeq 2000.

2.3. RNA-seq data analysis

After sequencing, “dirty” raw reads, which contain adapter sequence, high N content, and low-quality reads, were removed. After filtering, the remaining high-quality reads were called “clean reads.” Using SOAP2, all clean reads were mapped to the assembly genes and the completed pearl oyster genome [11]. We used Reads Per Kilobase of Transcript per Million Mapped Reads (RPKM) method to calculate the gene expression level. Then, gene ontology (GO) and pathway enrichment analyses were performed on differentially expressed genes (DEGs). The p-value corresponds to the results of differential gene expression tests. False discovery rate (FDR) is a method used to determine the p-value threshold in multiple tests. Thresholds (i.e., FDR_0.001 and log2 Ratio_1 absolute value) were used to assess the significance of the gene expression differences.

2.4. Identification of arginine kinases (AK)

Protein sequences of *Danio rerio* and *Homo sapiens* were downloaded from the National Center for Biotechnology Information (NCBI) database. For annotation, we queried all proteins against the functional databases Nr and KEGG by using the basic local alignment search tool (BLAST) (E-value ≤ 1-e5) and accepted the results with best scores for each query protein. We also used InterProScan to predict gene function on the basis of domain information. The annotation information of *P. f. martensii* and *Crassostrea gigas* was obtained from the previous research [11,12]. The protein domain of all of the identified AK genes was re-analyzed and confirmed by Simple Modular Architecture Research Tool (SMART) version 5.1 (http://smart.Embl-heidelberg.de/). To determine whether these genes are physically close enough to conclude that they probably arose through tandem duplications, we extracted their genome location and considered them as tandem duplication if they located in the same scaffold and no more than one genes were identified between them.

2.5. Confirmation by using quantitative real-time RT-PCR

To validate the RNA-seq data, we selected eight DEGs randomly for quantitative RT-PCR (qRT-PCR) analysis. The primer design was completed using Primer Premier 5 software. The primer sequence is shown in Supple https://www.sciencedirect.com/science/article/pii/S1050464818302341?via%3Dihub Table S1. The reaction system consisted of 0.4 μL of cDNA, 0.4 μL of upstream and downstream primers, and 5 μL of SYBR master mix, with a total volume of 10 μL. The qRT-PCR conditions are as follows: 95 °C for 2 min for 1 cycle, 95 °C for 15 s, 60 °C for 1 min, and 40 cycles. β-Actin was used as the internal reference. Gene expression multiples were calculated using a delta CT method ($2^{\wedge (Ct\ \beta\text{-}actin - Ct\ Target\ gene)}$). The relative expression levels of the target genes were compared through a *t*-test. The significance level in the analyses was considered at $p < 0.05$.

3. Results

3.1. Transcriptome sequence assembly

Approximately 66,663,090 and 67,737,418 raw reads from pearl oysters with conditioning and control pearl oysters without conditioning were generated using Illumina/Hiseq-2000 RNA-seq, respectively. After filtering, 60.62% and 59.4% clean reads were successfully matched to pearl oyster genome. And, 33.27% and 30.64% of the clean reads were mapped to the reference genes (Table 1). Among the reported 32,937 protein-coding genes in pearl oyster genome, 21,526 and 19,493 genes were detected in the hemocyte transcriptomes of pearl oysters with and without conditioning, respectively. A total of 3,074 genes were identified as DEGs (fold change > 2, FDR < 0.001) between the two transcriptomes. Among these DEGs, 794 and 2,280 genes were significantly down- and up-regulated after conditioning, respectively.

3.2. DEG functional annotation and classification

All 3,074 DEGs were classified into three functional categories, namely, cellular component, molecular function, and biological processes. Within the cellular component category, “microtubule cytoskeleton”, “microtubule associated complex” were the dominant annotated DEGs. In the molecular function category, many DEGs were in observed in “motor activity.” The majority of DEGs in the category of biological processes were involved in “microtubule-based process” (Fig. 1a). The related genes in these categories, such as tubulin and dynein, were highly expressed after conditioning treatment (Fig. 1b). We further mapped all DEGs to the terms in the KEGG database to search for the pathways involved in the cellular response to conditioning. Among the

Table 1
Statistical analysis of transcriptome sequencing data.

	total reads	total Mapped Reads to Genes	total Mapped Reads to genome	Number of the Mapped genes	DGE number
Pearl Oyster with conditioning	67559596	0.3327	0.594	21526	3074
Pearl Oyster without conditioning	66663090	0.3064	0.606	19493	

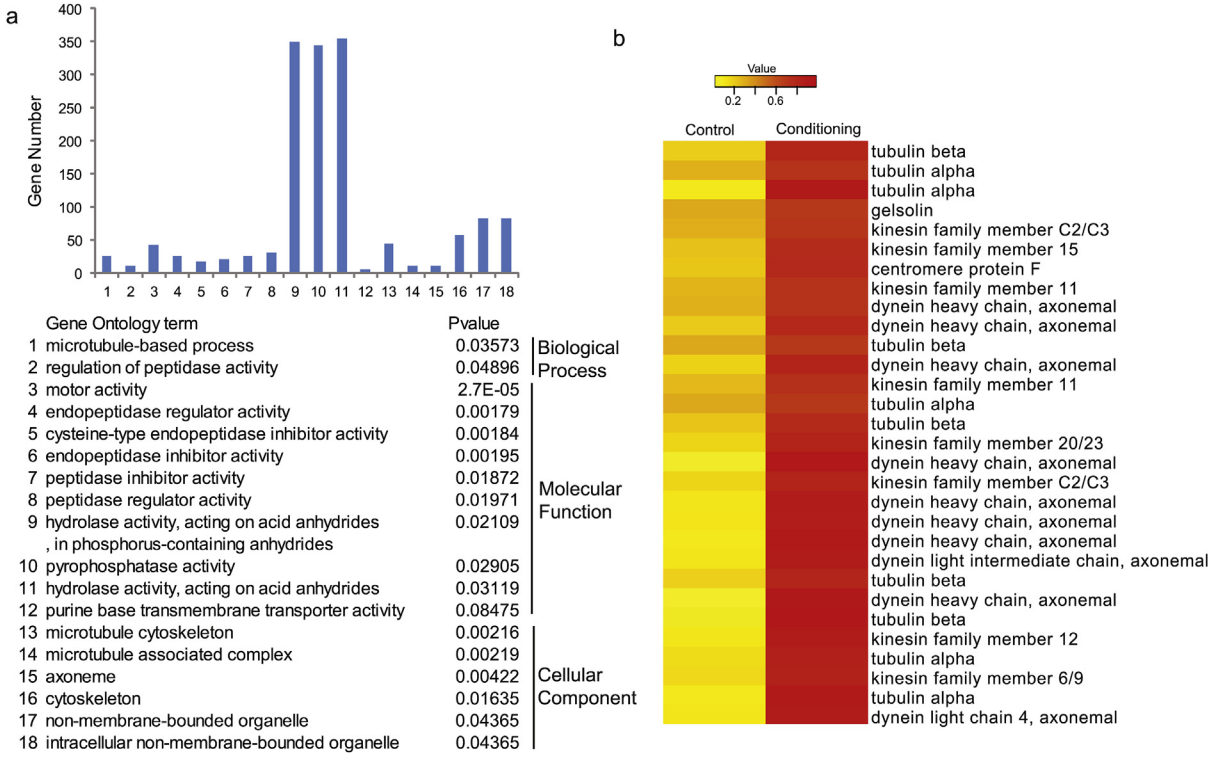

Fig. 1. GO classifications of the DEGs. a. All of the DEGs were assigned to three categories: cellular component, molecular function, and biological process. b. Expression of the genes in the "microtubule-based process".

Table 2
Enriched KEGG pathways of the DGE.

Pathway	Pvalue	Pathway ID
Hypertrophic cardiomyopathy (HCM)	8.760122E-07	ko05410
Dilated cardiomyopathy	5.002806E-06	ko05414
Cardiac muscle contraction	5.261634E-06	ko04260
Progesterone-mediated oocyte maturation	3.141385E-05	ko04914
Viral myocarditis	4.249099E-05	ko05416
Bile secretion	4.267898E-05	ko04976
Antigen processing and presentation	8.896736E-05	ko04612
Vasopressin-regulated water reabsorption	0.0001165124	ko04962
Tight junction	0.0001227575	ko04530
Cell cycle	0.0001459579	ko04110
Protein digestion and absorption	0.0002363219	ko04974
Glycosaminoglycan biosynthesis - heparan sulfate	0.001066242	ko00534
Pyrimidine metabolism	0.004521999	ko00240
Pathways in cancer	0.005371169	ko05200
Huntington's disease	0.006743648	ko05016
Pathogenic Escherichia coli infection	0.007009676	ko05130
Regulation of actin cytoskeleton	0.008948377	ko04810
Cell adhesion molecules (CAMs)	0.00968103	ko04514
Vitamin digestion and absorption	0.009820982	ko04977
Gap junction	0.01116916	ko04540
Purine metabolism	0.01120606	ko00230
NOD-like receptor signaling pathway	0.01184574	ko04621
Basal cell carcinoma	0.01213374	ko05217
RNA polymerase	0.01457425	ko03020
Carbohydrate digestion and absorption	0.01467559	ko04973
African trypanosomiasis	0.01543085	ko05143
Proximal tubule bicarbonate reclamation	0.01818687	ko04964
Ubiquitin mediated proteolysis	0.01896525	ko04120
Fatty acid biosynthesis	0.02185754	ko00061
Toxoplasmosis	0.02202604	ko05145
Small cell lung cancer	0.02690584	ko05222
Primary immunodeficiency	0.0345914	ko05340
Arginine and proline metabolism	0.03731459	ko00330
ABC transporters	0.03814498	ko02010
Hematopoietic cell lineage	0.03895221	ko04640
Starch and sucrose metabolism	0.04656937	ko00500

3,074 DEGs, 1,445 genes were assigned to 36 KEGG pathways with the $p < 0.05$ (Table 2). "Regulation of actin cytoskeleton" pathway was significantly enriched ($p = 0.0078$). In this pathway, the expression levels of actin and actin regulatory genes, such as myosin and gelsolin, were significantly up-regulated after conditioning (Fig. 2a). Meanwhile, the "cell cycle" pathway was significantly enriched ($p < 0.0002$), and the expression levels of the related genes, such as cyclin B, cyclin A, and cell division cycle 25B, were all highly induced after conditioning (Fig. 2b).

3.3. Expression of immune-related genes after conditioning

Among the enriched 36 KEGG pathways of 3074 DEGs, "NOD-like receptor signaling pathway" ($p = 0.012$) was included. Two NLRs were induced by > 40-fold after conditioning (Fig. 3a). A total of 8 toll-like receptors (TLRs) were identified and over-expressed (2–340-fold) after conditioning (Fig. 3a). Two myeloid differentiation primary response proteins (MyD88) were also found in hemocyte transcriptomes, both of which were highly induced (8- and 46-fold, Fig. 3a), thereby indicating that the TLR signal was active after conditioning. Meanwhile, IL-17 was highly induced (> 5000-fold) after conditioning (Fig. 3a). Nuclear factor kappa-B (NF-kB) is a prototypical pro-inflammatory signaling pathway due to its activation by pro-inflammatory cytokines. Four NF-kB and three NF-kB kinase (IKK) inhibitors were found in the hemocyte transcriptomes (RPKM > 1), whereas the expression of all these genes remained constant after conditioning (Fig. 3a). Meanwhile, six SODs, one CAT, four ACPs, and seven AKPs were found in hemocyte transcriptomes, and only one SOD and one AKP were significantly down-regulated after conditioning (Fig. 3b).

3.4. Highly induced apoptosis-related genes after conditioning

Among the DEGs, 24 apoptosis-related genes, such as Fas ligand (FasL), caspase 8 (Casp8), caspase 9 (Casp9), BCL-2, and inhibitor of apoptosis protein (IAP), were found. FasL belongs to the tumor necrosis

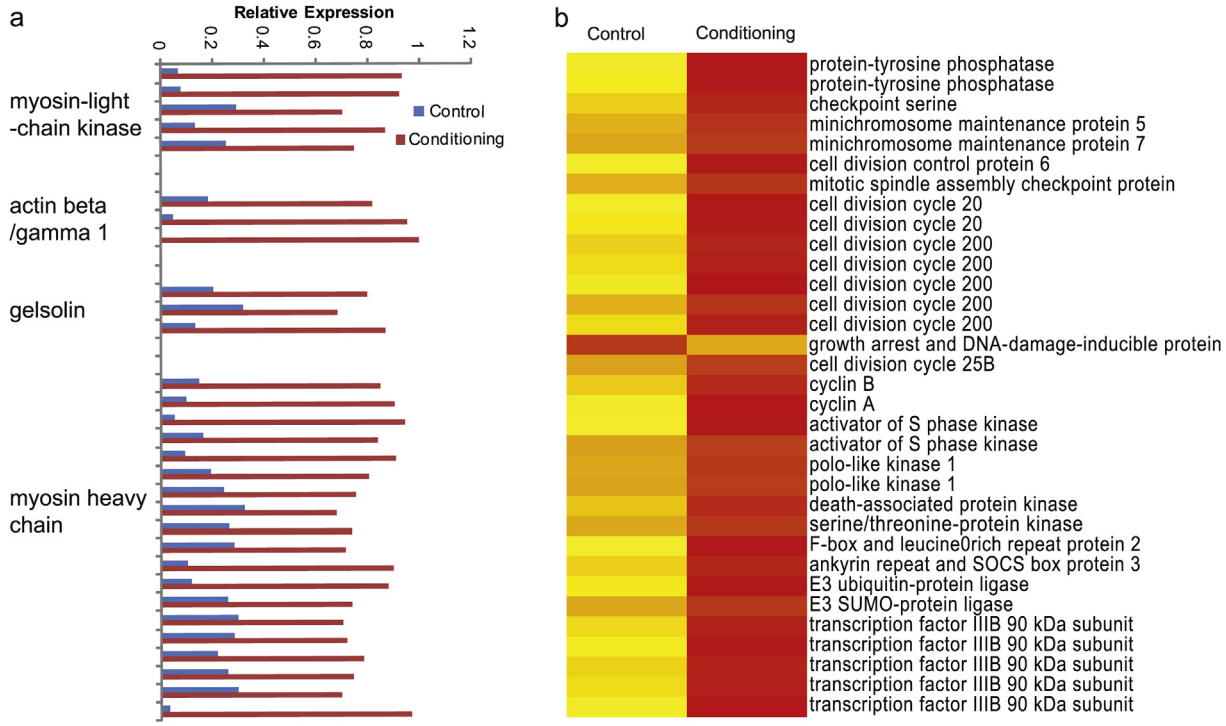

Fig. 2. Expression of the genes in "Regulation of actin cytoskeleton" and "cell cycle" pathway.

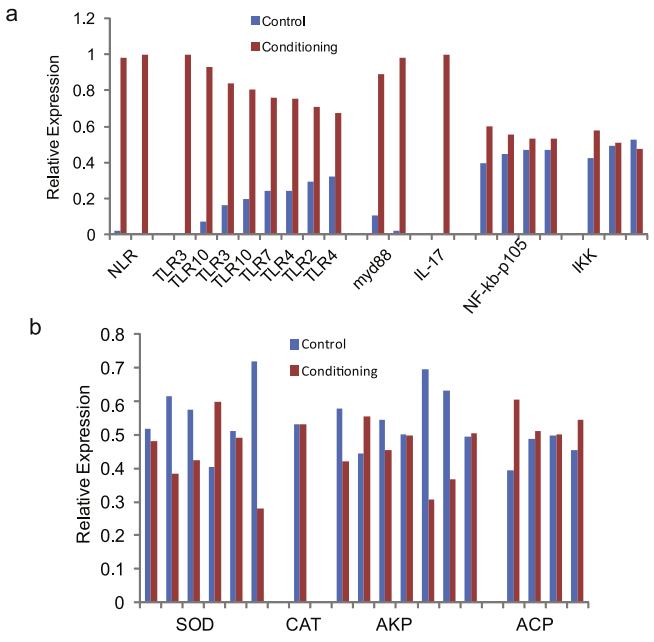

Fig. 3. Expression of immune related genes. NLR, nucleotide-binding oligomerization domain-like receptor; TLR, Toll like receptor; Myd88, Myeloid differentiation primary response 88; IL-17, interleukin-17; IKK, inhibitor of nuclear factor kappa-B kinase.

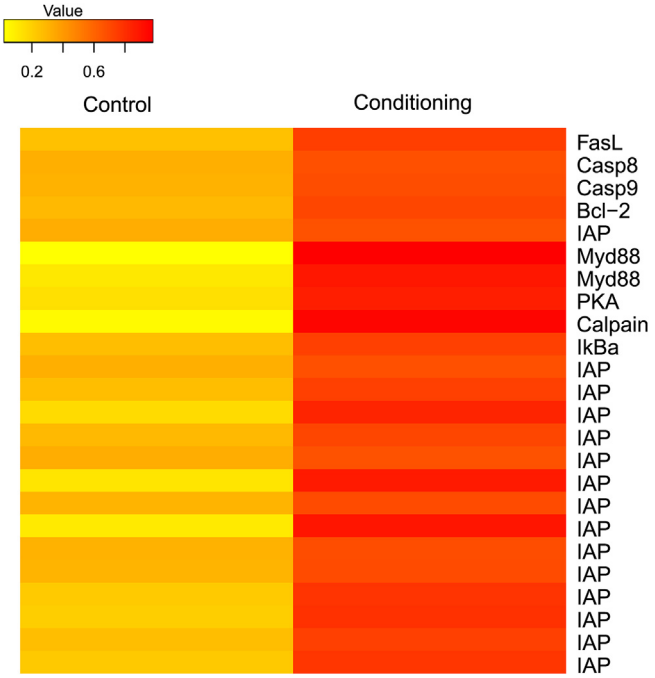

Fig. 4. Expression of apoptosis related genes. FasL, Fas-Ligand; Casp8, caspase 8; Casp9, caspase 9; BCL-2, B-cell lymphoma-2; IAP, inhibitor of apoptosis protein; PKA, protein kinase.

factor family. FasL binding with its receptor induces apoptosis. After pre-grafting conditioning, the FasL expression was up-regulated by 2.9-fold (Fig. 4). Casp8 and Casp9 are the members of the caspase family of cysteine proteases that are induced in apoptosis. Both Casp8 and Casp9 were up-regulated by approximately twofold after conditioning (Fig. 4). Corresponding to the highly increased proapoptosis-related genes, some antiapoptosis-related genes, such as Bcl2 and IAP, were up-regulated (two-to nine-fold, Fig. 4), thereby indicating that pearl oysters activated an anti-apoptosis system to cope with apoptosis induced by pre-grafting conditioning.

3.5. Energy production related genes

"Fatty acid (FA) biosynthesis" pathway ($p = 0.023$) was included in the enriched KEGG pathway. In this pathway, 8 FA synthase (FASN) genes were found, all of which were up-regulated by 2- to 32-fold after

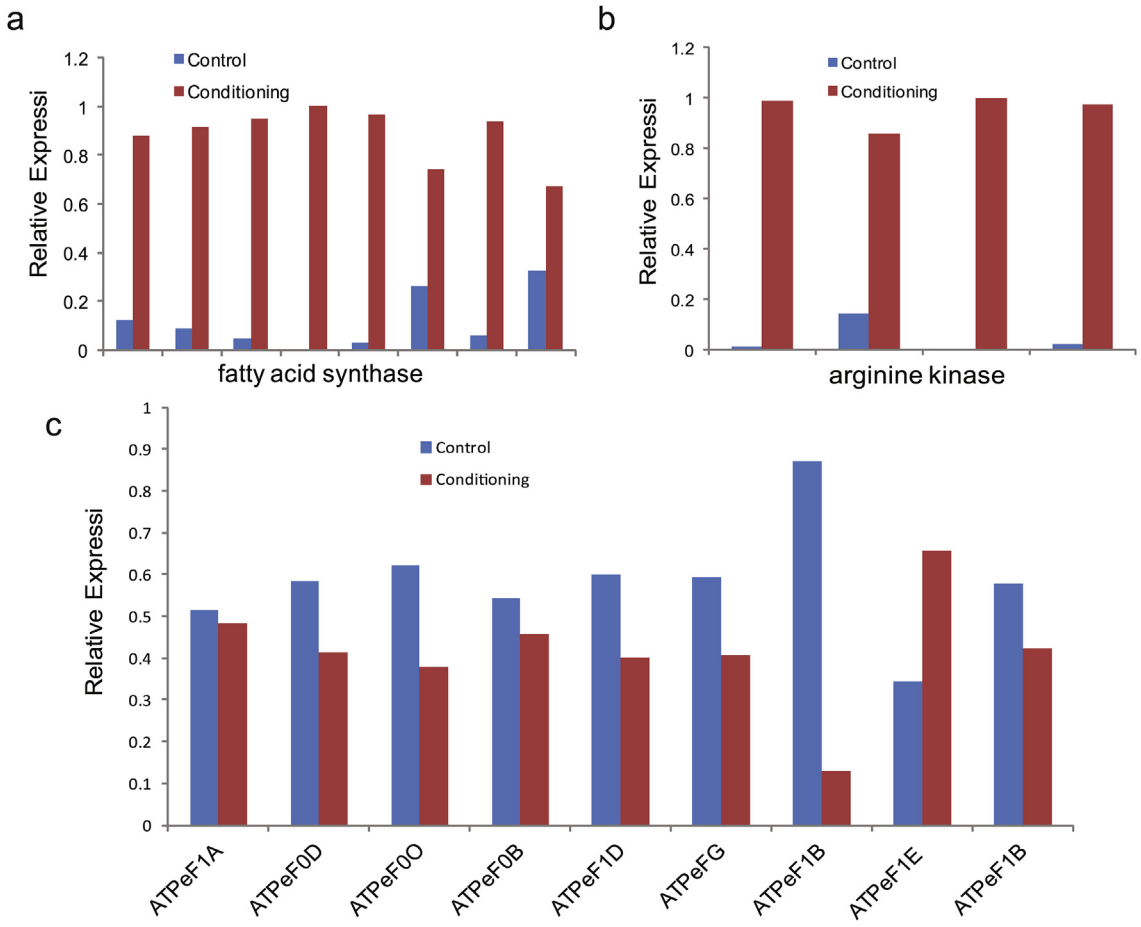

Fig. 5. Expression of energy production related genes.

conditioning (Fig. 5a). Whereas, among the 9 F-ATPase related genes found in the hemocytes transcriptomes, only one was down-regulated, and the others changed insignificantly (Fig. 5c). Interestingly, 4 arginine kinases (AKs) were identified in the hemocyts transcriptomes, all of which were over-expressed by 6- to 1000-fold after conditioning (Fig. 5b).

Homology-based annotation with InterProScan, KEGG, Nr and manual corrections showed there were only 4 AK genes from the genome of *P. f. martensii* (PmAK), and we also found 5 AK genes from *C. gigas* (CgAK), 5 analogous creatine kinase (CK) from human, 6 from zebra fish (Supple Table 2). Conserved domain analysis with SMART revealed all of the CK from human and zebra fish shared two conserved domains including ATP-gua PtransN domain which was described as a guanidine substrate specificity domain (GS domain) and an ATP-gua Ptrans domian which was responsible for ATP binding (APT domian). PmAK gene-Pm10029858 contained only GS domain, PmAK gene-Pm10029861 contained 3 repeat of GS-APT, PmAK gene-Pm10029861 included two GS and one short APT domain (Supple Table 2). Two CgAK genes (OYG10021482 and OYG10021483) have only GS domain, indicating the AK from pearl oyster and oyster are more diverse in domain structure. Sequence alignment indicated these genes are very conservative in APT domain compared with GS domain (Supple Fig. 1), indicating the substrates of AK in mollusk may be diverse, in agreement with the observations of Stein [13] and Kouji [14]. Gene location analysis indicated the 4 PmAK and 4 of 5 CgAK are located in the same scaffold from *P. f. martensii* and *C. gigas*, respectively (Fig. 6a), indicating one ancient gene duplication event. Between Pm10029858 and Pm10029860, there was inserted one gene Pm10029859. Extron-intron analysis indicated the intron number of PmAK ranged from 4 (Pm10029858) to 15 (Pm10029861), while Pm10029859 has no intron (Fig. 6b). Analysis of the developmental and tissue transcriptomes of *P. f. martensii* indicated that Pm10029859 was not transcribed (data not shown), indicating this gene was one retrogene. Interestingly, the expression of Pm10029858 and Pm10029860 seemly not affected by the insertion of Pm10029859. Both of Pm10029858 and Pm10029860 are highly expressed in the detected tissues and developmental stages with the same expression pattern and highest expression in the adductor (Fig. 6c/d). The other two AK genes (Pm10029857 and Pm10029861) showed different expression pattern in developmental stages and tissues, suggesting the function of AK genes are divergent after duplication.

3.6. Validation by quantitative real-time RT-PCR

To validate the expression profiles of genes in the DEG sequencing analyses further, we selected eight DEGs randomly to quantify their expression by using qRT-PCR (Fig. 7). The results showed that the expression patterns of the selected DEGs determined by qRT-PCR were identical to those determined by RNA-Seq, thereby indicating that the expression profiling of DEGs determined by RNA-seq was reliable and accurate.

4. Discussion

During pearl production, pre-grafting conditioning is a standard procedure before transplant surgery [1]. Conditioned pearl oysters always have high survival rate, bead retainable ratio, and high-quality output ratio [2,4,5]. The aim of this study was to investigate the molecular responses of pearl oyster after conditioning.

We obtained 3074 DEGs between the pearl oysters with and without

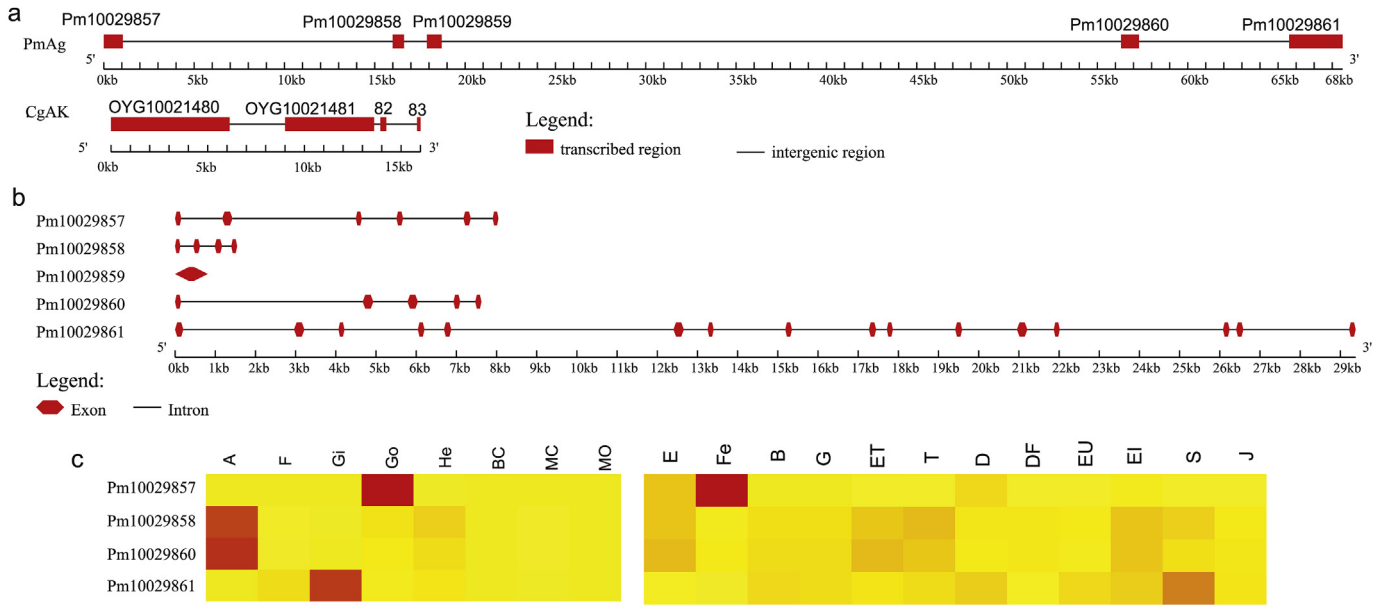

Fig. 6. Identification of AK genes. a. arrangement of AKs in two tandem duplicated arrays from *P. f. martensii* and *C. gigas*. b. Gene structure of the AK genes. Go:gonad, ME: mantle edge, He: hepatopancreas, F: foot, A: adductor muscle, Gi: gill, MC: mantle central, BC:haemocytes, E: egg; Fe: fertilized egg; B: blastula; G: gastrula; ET: early trochophore; T: trochophore; D: D-stage larvae; DF: D-stage larvae before feeding; EU: early umbo larvae; EL: eyed larvae; S: spat; J: juveniles.

conditioning through transcriptome analysis. GO and KEGG pathway enrichment analysis indicated that the GO term related to "microtubule-based process" and KO term related to "regulation of actin cytoskeleton" and "cell cycle" were significantly enriched among the 3,074 DEGs. The related genes were over-expressed in pearl oysters after conditioning. Some NLR, TLR, myd88, and one pro-inflammatory cytokine IL-17 were induced and highly expressed in pearl oysters after conditioning. Both NLRs and TLRs are the cellular sensor families of microbial- or danger-associated molecular patterns [15–17]. The intracellular signaling cascades triggered by NLRs and TLRs lead to transcriptional expression of inflammatory mediators that coordinate pathogens and infected cell elimination. IL-17 is classified as a proinflammatory cytokine due to its ability to induce the expression of numerous inflammation mediators, which leads to the proliferation, maturation, and chemotaxis of the immune-related cells [18–20]. The over-expression of these genes suggested that conditioning induced immune response in the body of pearl oysters. In the present study, conditioning was conducted by placing more pearl oysters than usual in a cage and raising the lines to the water surface during the heat of the day. High density, high temperature, ovulation, and pathogens from the environment may challenge pearl oysters and induce the immune response in their body. Meanwhile, this induction may be independent upon the increased NF-kB and IKK expression levels. For other mollusks, NF-kB activation is not shown at the transcription level against different immune challenges or induction [21–23]. For the hemocytes of abalone *Haliotis diversicolor supertexta*, NF-kB expression against LPS stimulation remained constant from 0 h to 9 h. However, the DNA-binding capacity of NF-kB increases [23]. Hence, the present study showed that conditioning may affect the DNA-binding capacity of NF-kB.

The induction of immune response after conditioning was different with the previous understanding. As elucidated in the introduction, some researchers believed that conditioning can reduce the immune response and neural activity to minimize the stress and damage caused by grafting, as the pearl oysters seems weakness after conditioning [2–5]. The results of the present study indicated that the immune system was activated. Proper immune activation may be good and crucial for damage recovery after conditioning. In humans, studies have shown that the pre-activation of neutrophils (polymorphonuclear leukocytes) and phagocyte can enhance their functional response [24–26]. For example, neutrophils from infected patients are hyper-responsive to stimuli [27], and the in vitro treatment of neutrophils with activation agents can cause the heightened responses of cells to subsequent stimuli [28]. The pre-activation of endothelial progenitor cells before transplantation can increase their pro-angiogenic potential and enhance the efficiency of cell therapy for ischemic vascular diseases [29]. The cell proliferation and movement of related genes were further up-regulated after transplantation (unpublished data). Thus, the pre-activation of cell

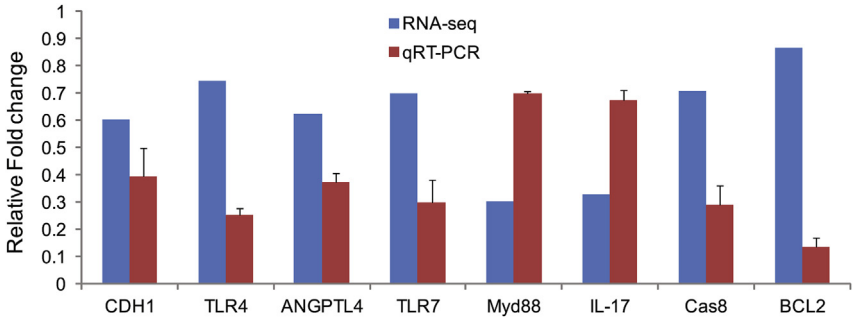

Fig. 7. Verification of the selected DEGs by qRT-PCR. CDH1: cell division cycle 20-like protein 1; ANGPTL4: angiopoietin-like 4; Supplement Fig. 1. Sequence multialignment of GS and APN domain from AK and CK genes. GS: ATP-gua PtransN domain, APT, ATP-gua Ptrans domian which was responsible for ATP binding.

proliferation and movement may be helpful for pearl oysters to cope with the damage and infection induced by transplantation. The unaffected SOD, CAT, ACP, and AKP indicated that conditioning did not change the redox environment in the body of pearl oysters, thereby indicating that the immune activity of pearl oysters was not impaired, which was different from long-time starvation that may cause serious stress in Li [3]'s research. Apoptosis-related genes were highly upregulated after conditioning. The activated apoptosis may be valuable for clearing virally-infected, activated, and stressed cells. During pearl production, there are several methods of conditioning pearl oysters, such as high density, starvation, changing temperature, and salinity. All of these methods could make them to spawn, and could be seen as stressors. In this paper, we showed high density, high temperature could induced the immune response of the pearl oysters. We proposed that other conditioning methods could also stimulate the immune response of the pearl oyster, but it may be from different mechanism, which need further elucidation.

Immune response and ovulation are energy-demanding processes. Whereas, the expression of F-ATPase-related genes in the hemocytes remained constant. This result suggested that the energy for immune response may have other sources. AK is a member of the guanido kinase family that plays an important role in buffering ATP concentration in cells with high and fluctuating energy demands [30–32]. This enzyme is present in some invertebrates and an analogous system to vertebrate CK [30]. Four tandemly duplicated PmAK were found in the genome of *P. f. martensii* and *C. gigas*, and gene structure and multialignment of these AK genes indicated PmAK genes is more diverse than that found from human and zebra fish. Combing the expression pattern of AK genes in developmental stages and tissues, we proposed that AK genes from pearl oyster may undergone substrates and function divergent during evolution. The highly induction after conditioning indicated these genes were functional in stress response and the tandem array in location may contribute to their frequent regulation and response after stimulation. AK over-expression increased the survival capability of *Trypanosoma cruzi* under pH and nutritional stress conditions [33]. Conditioning for pearl oysters can be regarded as nutritional stress condition, and AK over-expression after transplantation suggested AK may provide the major energy source when pearl oysters are under stress conditions.

In addition, FA metabolism was active in pearl oysters with conditioning because FASN genes were significantly upregulated. FASN is a key lipogenic enzyme catalyzing the terminal steps in the de novo biogenesis of FAs, which are the major substrates for energy production and storage in animals [34,35]. FAs are also involved in the modulation of structural and functional properties at the cellular level. Many animal studies have shown that FAs can have considerable anti-inflammatory and immunomodulatory activities in a wide array of diseases (e.g., autoimmunity, and infection) [35,36]. FASN upregulation indicated that pearl oysters may need additional FAs during conditioning, and the addition of some FA-containing feeds may be valuable for their recovery after conditioning.

In conclusion, pre-grafting conditioning activated the expression of cell cycle and movement-related genes and induced the over-expression of many immune and apoptosis-related genes, such as NRL, TLR, myd88, IL-17, FasL, and IAP, on the basis of the detailed transcriptome analyses. “FA biosynthesis” was also involved in this response. Four tandemly duplicated AK genes were found in the genome of pearl oysters, all of which were highly induced after conditioning. These findings will help elucidate the molecular mechanism underlying the immune response to conditioning and provide useful information to understand the molecular response of pearl oysters to other stress conditions.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fsi.2019.06.013.

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