Fish and Shellfish Immunology 90 (2019) 109–117

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Genome and transcriptome analyses providing insight into the immune response of pearl oysters after allograft and xenograft transplantations

Yu Jiao, Shuai Yang, Yanfei Cao, Zhe Zheng, Yuewen Deng, Qingheng Wang, Ronglian Huang, Xiaodong Du*

Fishery College, Guangdong Ocean University, Zhanjiang, 524025, China

ARTICLE INFO

Keywords:
Pearl formation
Xenograft transplantation
Transcriptome
Genomic analysis
Pearl oyster

ABSTRACT

The immune response after allograft or xenograft transplantation in the pearl oyster is a major factor that cause its nucleus rejection and death. To determine the mechanism underlying the immune response after allograft and xenograft transplantations in the pearl oyster *Pinctada fucata martensii*, we constructed two sets of transcriptomes of hemocytes at different times (6 and 12 h; 1, 3, 6, 12, and 30 d) after allograft and xenograft transplantations, in which the xenografted mantle tissue was from *Pinctada maxima*. The transcriptomic analysis reveals many genes are involved in the immune response to transplantation, such as transient receptor potential cation channel (TRP), calmodulin (CaM), DNA replication-related genes, and sugar and lipid metabolism-related genes. The expression of these identified genes was higher in the host pearl oyster transplanted with xenograft than that by allograft. The histological analysis of the pearl sac also confirmed that many hemocytes were still gathered around the transplanted nucleus, and no pearl sac was formed in the host pearl oysters at 30 d after xenograft transplantation. The genomic analysis indicated that pearl oysters evolved many copies of genes, such as TRP, CaM, and GST, to sense and cope with the immune response after transplantation. “Ribosome” and “Cytosolic DNA-sensing pathway” were specifically induced in the xenograft group, whereas “Notch signaling pathway” specifically responded to the allograft transplantation. These results can improve our understanding of the mechanism underlying the immune response of pearl oysters after allograft and xenograft transplantations.

1. Introduction

Most bivalves of genus *Pinctada* are well known for producing pearls, which have high commercial value. To produce 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. This process is called “transplantation”, “grafting”, “pearl implantation”, or “seeding” [1]. After transplantation, the seeded mantle tissue, nucleus, and infected pathogens during surgery can cause physiological response in the host pearl oyster. A strong response can lead to nucleus rejection, failure of pearl sac formation, and the death of host pearl oysters [1,2]. The immune response of the hemocyte in *Pinctada margaritifera* to the transplanted mantle pieces were examined by histological analysis, the level of haemocyte accumulation present from the 2–4 day, and reduced at 6 day after transplantation [3]. In *Pinctada fucata martensii*, the hemocyte gathered around the nucleus at about 1–3 day after transplantation, at day 15–20, the immune response gradually disappears, and a layer of secretory epithelium (pearl sac) develops [4]. Then, the pearl is formed from the deposition of nacre around the nucleus.

Generally, the transplanted mantle piece is from the same species. Xenograft transplantation (transplant across species), in which the donor and the host pearl oysters are different species, is recently used in pearl production to improve pearl quality [5,6]. Nacre deposition and weight were found to be higher in xenografts comprising of a *Pinctada maxima* donor and *Pinctada margaritifera* host, than in allograft [5]. Whereas, in the mantle xenografts, the rate of pearl sac formation appeared to be markedly different, depending on the immune reactions between grafts and recipients. Somsak [7] investigated mantle transplantations in three species of freshwater pearl mussels, *Hyriopsis* (*Limnoscapha*) *myersiana*, *H.* (*L.*) *desowitzi*, and *Chamberlainia hainesiana* using allograft and xenograft methods. When the recipient was *H.* (*L.*) *desowitzi*, no pearl sac was observed after xenografts from either of the two other mussel species. The successful rate of pearling for *P. fucata* transplanted with *Pteria penguin* and *P. margaritifera* mantle piece was found to be zero [6]. We also found a xenograft failure of pearl sac formation in which *P. f. martensii* is the host and *P. maxima* is the donor, the mantle piece from *P. maxima* was totally rejected and eliminated by

---

* Corresponding author.
E-mail address: gdhddxd@Hotmail.com (X. Du).

https://doi.org/10.1016/j.fsi.2019.04.061
Received 7 March 2019; Received in revised form 19 April 2019; Accepted 24 April 2019

the defense response of *P. f. martensii*, compared with 100% successful rate of pearl sac formation for allograft transplantation between *P. f. martensii* in this study, suggesting different immune responses to allogeneic and xenogeneic mantle pieces.

To elucidate the mechanism underlying the immune and physiological reactions of *P. f. martensii* to xenograft from *P. maxima*, Wei et al. [8] constructed the transcriptomes of *P. f. martensii* at early time points (0, 6, 12, 24, 48, 72, and 96 h) after the transplantation of allograft or xenograft from *P. maxima* and revealed crucial pathways and genes were involved in response to transplantation, such as apoptosis, hippo signaling pathway, ribosome and NF-kappa B signaling pathway. Many genes related to oxidation-reduction reactions, the MAPK signaling pathway, and apoptosis were also identified by comparison of the allograft group and the xenograft group. Whereas, previous research showed there were also many hemocytes around the inserted nucleus between 7 and 15 day after transplantation [4], indicating the immune response remained after 7 day, thus the transcriptomes analysis of the pearl oyster after 7 day was important to understand the defense mechanism of pearl oyster after transplantation.

In this study, we constructed 15 transcriptomes of hemocytes from the pearl oyster *P. f. martensii* including not only in the early stage but also after pearl sac formation, and compared the similarities and differences in responses to allograft and xenograft transplantations in the transcriptomic level. In addition, we performed a histological analysis before and after pearl sac formation around the transplanted nucleus. Considering the results of our comprehensive and genomic analyses, we provided a detailed discussion on the immune response in *P. f. martensii* after allograft and xenograft transplantations.

2. Material and methods

2.1. Experimental design and sample preparation

To investigate the genome-wide response of the host pearl oyster after the surgical transplantation in the process of pearl formation, we built two sets of transcriptomes of hemocytes in *P. f. martensii* in October 2012. The difference of the two sets was the grafted mantle tissue, one from allogenic donor pearl oysters (*P. f. martensii*), and the other from xenogenic donor pearl oysters (*P. maxima*). The donor pearl oysters *P. f. martensii* were sampled from the fifth generation line selected for fast growing in the base stock and the procedure to develop the selected line was detailed by Du et al. [9]. The donor pearl oysters *P. maxima* were produced in the hatchery by the breeders introduced from Indonesia [10]. In each group, 200 pearl oyster were prepared and processed with the surgical implantation. After surgical implantation, the host pearl oysters were cultured on rafts in off shore waters in Liushagang, Zhanjiang, Guangdong Province, China. Then, at different times (6 and 12 h; 1, 3, 6, 12, and 30 d) after the surgical implication, hemolymph from at least 10 host pearl oysters of each group were collected using 1 mL syringes from adductor muscles. The hemolymph was subjected to centrifugation at 3500 r/min for 5 min and the precipitant at the bottom was separated to collect hemocytes. Then, the hemocytes immersed in TRIzol reagent (Invitrogen, USA), and stored at − 80 °C. (Invitrogen, USA). The 10 pearl sacs were removed from the host pearl oysters at 3, 6, 12, 18, and 30 d after transplantation. The sacs were fixed with 10% neutral buffered formalin for 24 h. After standard paraffin methods, 6 μm-thick sections were cut and stained with H&E for light microscopy.

The control group included the pearl oysters processed with pregrafting conditioning but no grafting surgery. During this month, the temperature of the seawater had no enormous changes and keep at about 29 °C between 1 and 12 d, and drop to 25 °C at 30 d. No contamination occurred during this time.

2.2. RNA extraction and library construction

RNA was individually extracted using TRIzol reagent (Invitrogen, USA) and then mixed in equal amounts for RNA-seq. mRNA was enriched using the oligo (dT) magnetic beads and fragmented into short fragments (200–500 bp) with the addition of fragment buffer. The first-strand cDNA was synthesized by random hexamer primer using the 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 and end repair and base A addition using the QiaQuick PCR extraction kit. Sequencing adapters were ligated to the fragments. The fragments were purified by Agarose gel electrophoresis and enriched by PCR amplification. The library products were sequenced using IlluminaHiseq 2000.

2.3. RNA-seq data analysis

Raw reads were filtered using FastQC-V0.10.1, using the settings –N 0.05 -Q 10,0.5. After filtering, the remaining reads were considered as high-quality “clean reads”. Using SOAP2 with settings (-m 0 -x 1000 -s 40 -l 32 -v 5 -r 2 -p 3), all clean reads were mapped to the assembly genes and the completed genome of *P. f. martensii* [11]. Mapping was filtered for MAPQ > 60. We used reads per kilobase of transcript per million mapped reads (RPKM) method to calculate the gene expression level. The differentially expressed genes were taken out by DEGseq. The threshold ($FDR \leq 0.001$, $\log2 \text{ Ratio} \geq 1$) were used to assess the significance of gene expression differences. Then, DGEs were performed by gene ontology (GO) and pathway enrichment analyses. For the GOs, Blast2GO (*P*-value < 0.05) were used to extract the enrichments.

2.4. Selection of gene responsive to surgical implantation

Two methods were used to identify the responsive genes to surgical implantation according to the method in oyster. First, at least 40% sample time points indicated the same trend (up- or down-regulation) compared with the control group. For example, genes that showed identical regulation patterns (up or down) in at least three of the seven sampling time (6 and 12 h; 1, 3, 6, 12, and 30 d) were considered responsive genes. Second, to include genes that differentially expressed only at a particular time, we identified genes with those that were differentially expressed by five-fold in any sample time compared with the control group. Finally, genes identified with these two methods were combined as the set of gene responses to surgical implantation. Then, the GO and pathway enrichment analyses were performed among the obtained responsive genes.

3. Results

3.1. Histological analysis in allograft and xenograft groups

The pearl sac tissues around the transplanted paraffin nucleus were removed together with the paraffin nucleus at 3, 6, 12, 18, and 30 d after transplantation. After standard hematoxylin and eosin (H&E) staining, many hemocytes appeared around the transplanted mantle piece in the allograft (Supple Fig. 1) and xenograft (Fig. 1a, Supple Fig. 2) groups. After 18 d, these hemocytes disappeared, and pearl sacs formed in each of the eight samples in the allograft group (Fig. 1b). In the xenograft group, many hemocytes appeared in the pearl sac cavity (PC), but no formed pearl sac was found in the eight samples after 18 and 30 d (Fig. 1c, Supple Fig. 2).

3.2. Transcriptome sequence assembly and DEG analysis

After allograft and xenograft transplantations at different times (6, 12, and 24 h; 3, 6, 12, and 30 d), two sets of transcriptomes of hemocytes and one control group were successfully constructed and

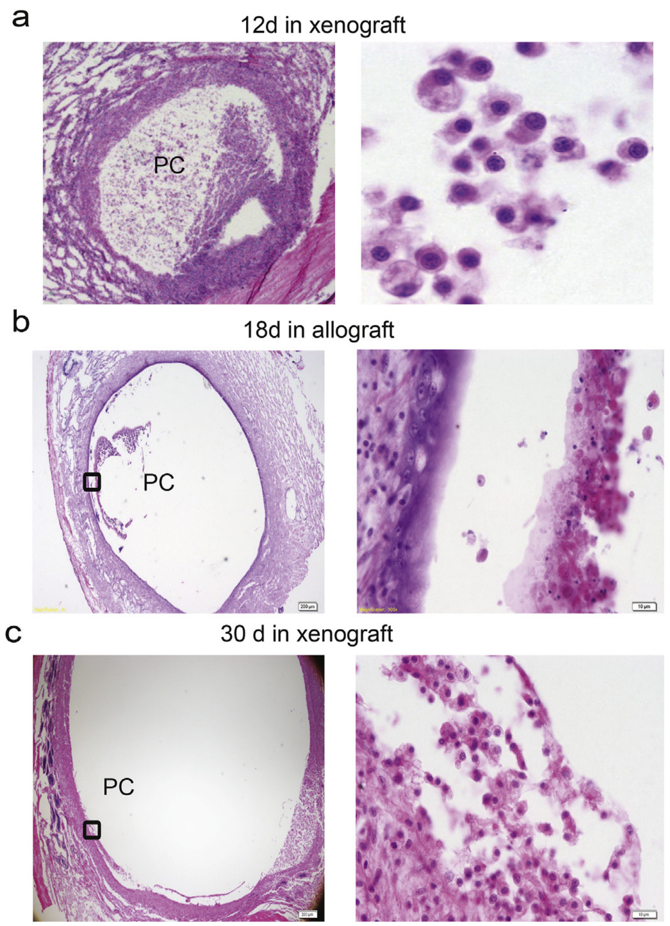

Fig. 1. Difference in histology between allograft and xenograft groups, a\c. Hemocytes around the transplanted nucleus at 12 and 30 d after xenograft transplantation, b. Pearl sac at 18 d after allograft transplantation. The right one was the amplification of part of the left one. PC: pearl sac cavity.

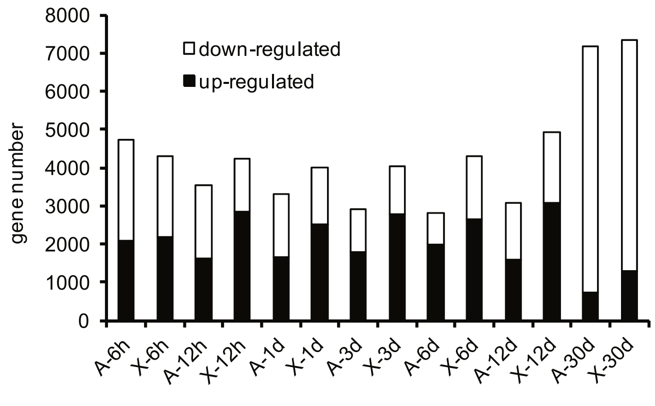

Fig. 2. DEG number at different times (6 and 12 h; 1, 3, 6, 12, and 30 d) compared with the control group in allograft (A) and xenograft (X) groups.

sequenced. A total of 981,948,054 reads were obtained. After filtering, 20.41%–37.77% reads were matched to the gene of *P. f. martensii*, and 47.40%–61.57% clean reads were successfully matched to the genome of *P. f. martensii* (Supple Table 1). Compared with the control group, 2556–5878 DEG (fold change > 2, FDR < 0.05) were obtained at various time points from the allograft and xenograft groups (Fig. 2). The DEG number in the xenograft group was greater than in the allograft group at each time point, except for 6 h (Fig. 2). Therefore, the distant mantle piece in the xenograft group may have triggered stronger responses than in the allograft group. Among the down-regulated genes, the DGE number was highest after 30 d, and such result may be caused by the drop in temperature at 30 d.

3.3. DEG annotation and functional enrichment

All the DEGs at each time point were assigned to various GO terms. At 6 h in the xenograft group and 12 h in the allograft group, GO terms related to the "sensory perception of mechanical stimulus" was significantly enriched (P valve < 0.05) (Supple Table 2). Among the related genes, two transient receptor potential cation channel (TRP) genes was induced at 6 and 12 h, and gradually returned to the normal level at 6–12 d after the transplantation in the two groups (Fig. 3a). To date, 27 TRP channel members have been identified in human [12]. In the genome of pearl oyster, 49 TRP channel genes were found (Supple Table 3) and 22 (RPKM > 1) were expressed in the hemocytes. The two up-regulated TRP channel genes after transplantation belong to TRPA (ankyrin-like with transmembrane domains 1) and TRPP (polycystin) families. These genes are tandem repeats in the genome and may have the same regulatory factor and similar expression trends after transplantation (Fig. 3a/b). Most identified TPR channels in mammals are Ca^{2+} permeable, and TRPA and TRPP are established as Ca^{2+}-permeable cation channels [13,14]. The control group of intracellular Ca^{2+} is crucial for various physiological activities. Excessive calcium or loss of control in calcium signaling can lead to cell death. Calmodulin (CaM) is a Ca^{2+} binding protein that acts as a Ca^{2+} sensor. Corresponding to the expansion and up-regulation of TRP channels in the genome of pearl oyster, 59 CaM genes were observed in the genome of pearl oyster (Supple Table 3), compared with 15 in the human genome. A total of 43 CaM genes were detected (RPKM > 1) in the hemocytes of pearl oyster. After transplantation, 11 CaM genes were up-regulated in both groups, 16 CaM genes were only induced in the xenograft group, and two were specifically up-regulated in the allograft group (Fig. 3c). These results indicated the Ca^{2+} signal is important in regulating the cellular homeostasis after transplantation.

GO terms related to the "small molecule metabolic process" ($p < 0.05$) were also significantly enriched at 6 h and 1 d in both groups (Supple Table 2). Among the related genes, some aminoacyl-tRNA synthetase genes (ARSs), such as tyrosyl-tRNA synthetase (tyrS), tryptophanyl-tRNA synthetase, and asparaginyl-tRNA synthetase, were over-expressed at 1 d after the surgical transplantation xenograft and allograft groups (Fig. 4a). In addition, GO term-related "DNA replication" was significantly enriched at 6 h in both groups (Supple Table 2). The expression trend of the related genes revealed that the "DNA replication" process was down-regulated after 6–12 h, increased between 1 and 6 d, and returned to the normal level at 12 d after transplantation in the two groups (Fig. 4b). The inhibited DNA replication at 6–12 h may be induced by the cellular damage caused by surgical transplantation, and the pearl oyster must inhibit cell metabolism to help itself for recovery. DNA replication is correlated with cell proliferation. The up-regulation of DNA replication-related genes indicated that the cell proliferation process was induced between 1 and 6 d. This result corresponded to the agminated hemocytes in the pearl sac after 3 d.

Similar with GO enrichment analysis, the KO pathway related to "cell cycle" and "DNA replication" were also significantly enriched at 6 h–6 d compared with the control in the two groups (Supple Table 2). The "DNA mismatch repair" pathway-related genes were also up-regulated at 3 d (Fig. 4b). This up-regulation was favorable to the increase in DNA replication and helpful for the recovery of damaged DNA caused by transplantation. In addition, the "apoptosis" pathway was enriched at 3 d in the xenograft group. The pathway was also indicated in Wei's report [8,15]. The apoptosis-inducible-related genes in the xenograft group were over-expressed compared with those in the allograft group (Fig. 4b). The induced apoptosis after transplantation may help clear virus-infected, activated, or stressed cells.

The "lysosome" pathway was enriched at 6 and 12 h in the two

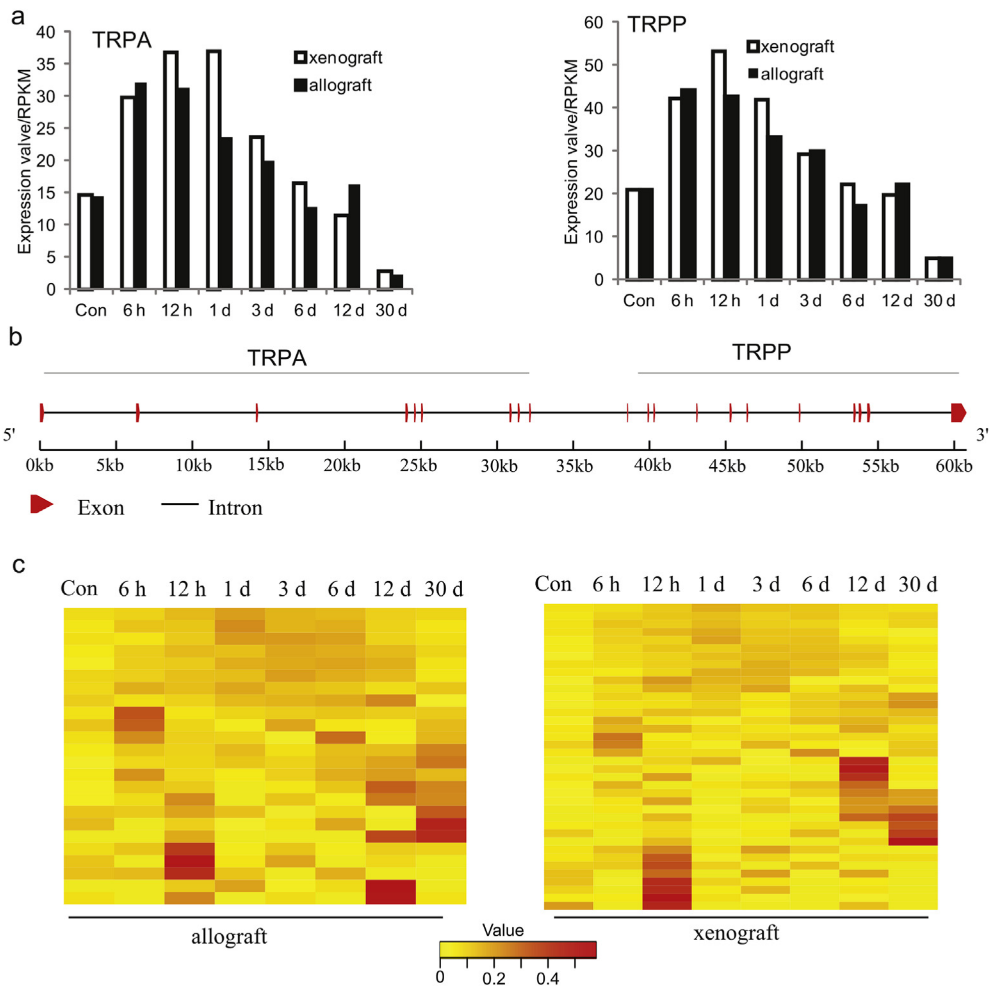

Fig. 3. TRP channel and CaM expression after transplantation, a. TRPA and TRPP expressions after the transplantation in the allograft and xenograft groups, b. The position of TRPA and TRPP in the scaffold of *P. f. martensii* genome, c. Expression of CaM after transplantation in both groups.

groups (P < 0.01) (Supple Table 2). By contrast, most protease-related genes in lysosomes, such as cathepsins, were down-regulated. Therefore, the function of protein degradation in lysosomes is down-regulated after the transplantation in xenograft and allograft groups. Some sugar and lipid metabolism-related genes, such as arylsulfatase I/J (ARSI_J), *N*-acetylglucosamine-1-phosphodiester alpha-*N*-acetylglucosaminidase, and lysosomal acid lipase, were up-regulated in the two groups (Fig. 5a). Some Niemann-Pick C protein 1 were up-regulated in the xenograft group.

In contrast to the down-regulation of proteases in lysosomes, the proteasome subunit-related genes were up-regulated after transplantation in both groups (Fig. 5b). Proteasomes are large multimeric self-compartmenting proteases that play a crucial role in the clearance of misfolded proteins, breakdown of regulatory proteins, processing of proteins by specific partial proteolysis, cell cycle control, and preparation of peptides for immune presentation. Proteasome-related genes were immediately induced at 6 h after the transplantation in xenograft and allograft groups. These genes gradually recovered to normal level after 12 d in the allograft group but not in the xenograft group. Therefore, the cellular damage after transplantation in the xenograft group was more serious than in the allograft group. At 30 d, proteasome-related genes were up-regulated again, and such activity may be caused by the drop in temperature (Fig. 5b).

3.4. Analysis of responsive genes in allograft and xenograft groups

To further understand the underlying mechanism after transplantation, the responsive genes were screened using the method as reference [16]. A total of 7027 responsive genes were obtained, and 2767 genes responding to allograft and xenograft transplantations were overlapped (Fig. 6a). GO terms related to "lipid metabolic process" and "lipid homeostasis" are highly enriched among the overlapped 2767 genes (p < 0.05) (Supple Table 4). The related gene elongation of long chain fatty acids protein 4 (ELOVL4) were up-regulated (Fig. 6b). KO terms related to "Glutathione (GSH) metabolism" pathway were significantly enriched among the overlapped 2767 genes (Supple Table 4), and most related genes in this pathway were up-regulated (Fig. 6c). GSH plays important roles in antioxidant defense, nutrient metabolism, and the regulation of cellular events. Glutathione-S-transferase (GST) is the detoxication of electrophiles by GSH conjugation. In the genome of pearl oyster, 39 GST genes exist (Supple Table 3), compared with the 22 GST genes in human [17]. Of the 39 GST genes, 31 were detected in the transcriptomes of hemocytes (RPKM > 1), 17 were induced in the allograft and xenograft groups, five were up-regulated only in the xenograft group, and two were over-expressed after allograft transplantation (Fig. 6c). Two glutathione synthases (GSas) were found in the transcriptomes of hemocytes (RPKM > 1). Both were up-regulated in the xenograft group, whereas only one was over-expressed in the allograft group (Fig. 6d). These results suggest GSH metabolism is important in regulating the homeostasis of pearl oysters after transplantation.

Of the 7027 responsive genes, 2818 genes were only responsive to xenograft transplantation. "Ribosome" and "Cytosolic DNA-sensing pathway" were observed in the enriched KO terms of the 2818 genes (Supple Table 4). In the "Ribosome" pathway, many of the early-stage

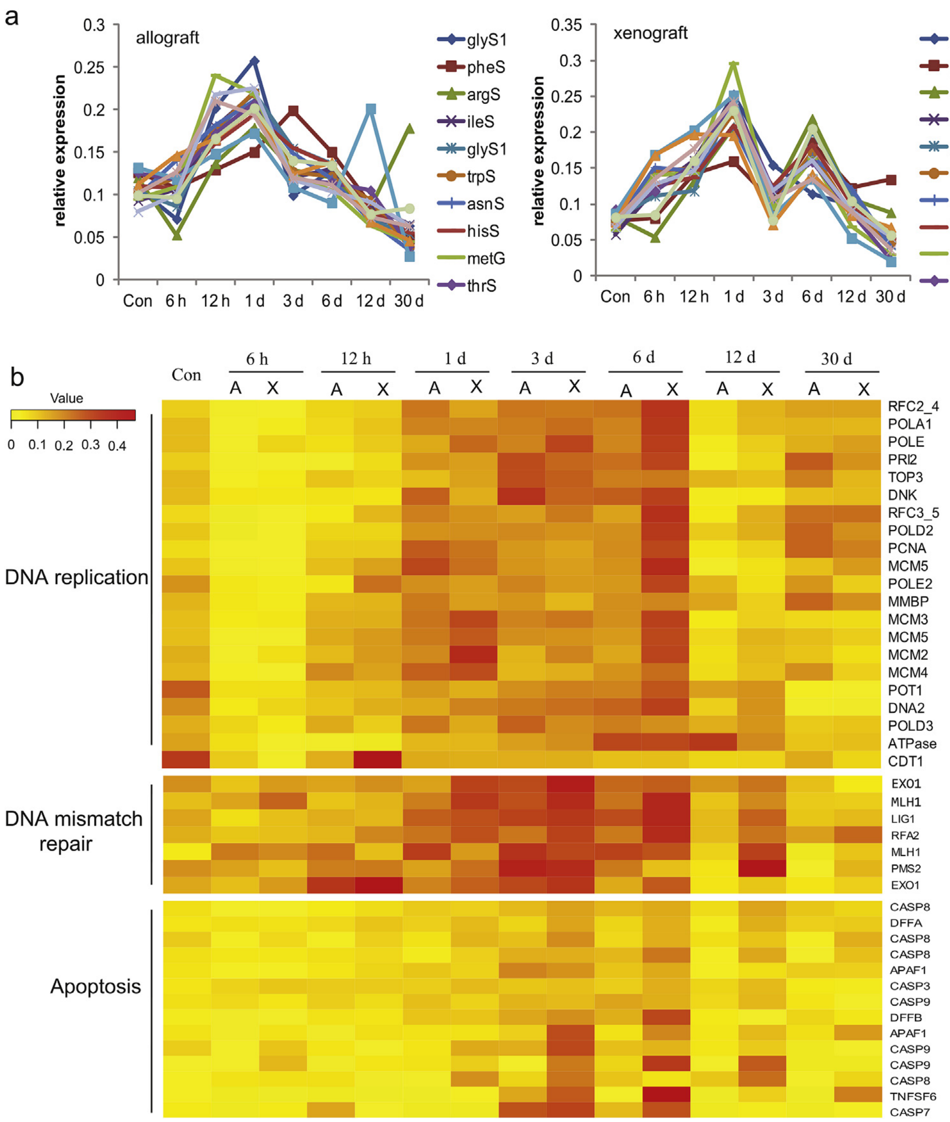

Fig. 4. Expression of ARSs and cell cycle-related genes after transplantation; a. Expression of ARSs after transplantation in allograft and xenograft groups; b. Expression of DNA replication, DNA repair, and cell apoptosis-related genes after transplantation in allograft (A) and xenograft (X) groups.

induced ribosome-related genes were mitochondrial-like ribosome. Among the cytosolic ribosomes, some were up-regulated, whereas some were down-regulated (Fig. 7a). In the “Cytosolic DNA-sensing pathway,” two cyclic GMP-AMP synthase (cGAS) genes were up-regulated at 3 d in the xenograft and allograft groups. In the allograft group, the expression of cGAS quickly returned to normal. In the xenograft group, cGAS genes remained at a high expression level at 30 d (Fig. 7b). cGAS is a widely expressed, essential molecule for cytoplasmic DNA recognition. DNA is usually confined within the nucleus and mitochondria of eukaryotic cells. The presence of cytosolic DNA, either through infections or cellular damage, triggers robust immune responses [18,19]. The high expression of cGAS in the xenograft suggested that the effect of transplantation sustained up to 30 d.

Furthermore, we obtained 1442 genes that were only responsive to the allograft transplantation, among which “Notch signaling pathway” is specifically enriched (Supple Table 4), and Notch gene is significantly induced at 6 h after the transplantation in allograft but not in xenograft (Supple Fig. 3).

4. Discussion

In this study, combined with transcriptome, genomic, and histological analyses after allograft and xenograft transplantations, we conducted a detailed analysis of the immune response after transplantation. First, surgery transplantation is an external stimulus for pearl oysters. We observed 50 TRP channels in the genome of pearl oyster, compared with 27 in humans [12]. Most mollusks have simple nervous systems comprising few neurons, thereby allowing mollusks to receive, process, and respond to stimuli in limited ways [20]. TRP channels are cellular sensors for various internal and external stimuli. These channels are also linked to sensory stimuli, including phototransduction, thermosensation, and mechanosensation. In addition, TRP channels exist in various receptor cells, especially in those involved in mechanosensation [21–23]. The expansion of TRP channels in the pearl oyster may complement the simple nervous systems of mollusks and may help them cope with various stimuli from internal or external environment. Moreover, the induced TRP channels after transplantation belong to Ca^{2+} permeable TRP family. Thus, hemocytes can sense the mechanical stimuli by the surgery transplantation and induce the influx of Ca^{2+}. Pearl oysters involved an expansion of CaM, which may be helpful in sensing the concentration of Ca^{2+} and avoiding the cellular damage of high concentration of Ca^{2+}. Ca^{2+} is an essential second messenger involved in the regulation of many eukaryotic cellular functions, such as chemotaxis, adhesion, the secretion of pro- and anti-inflammatory

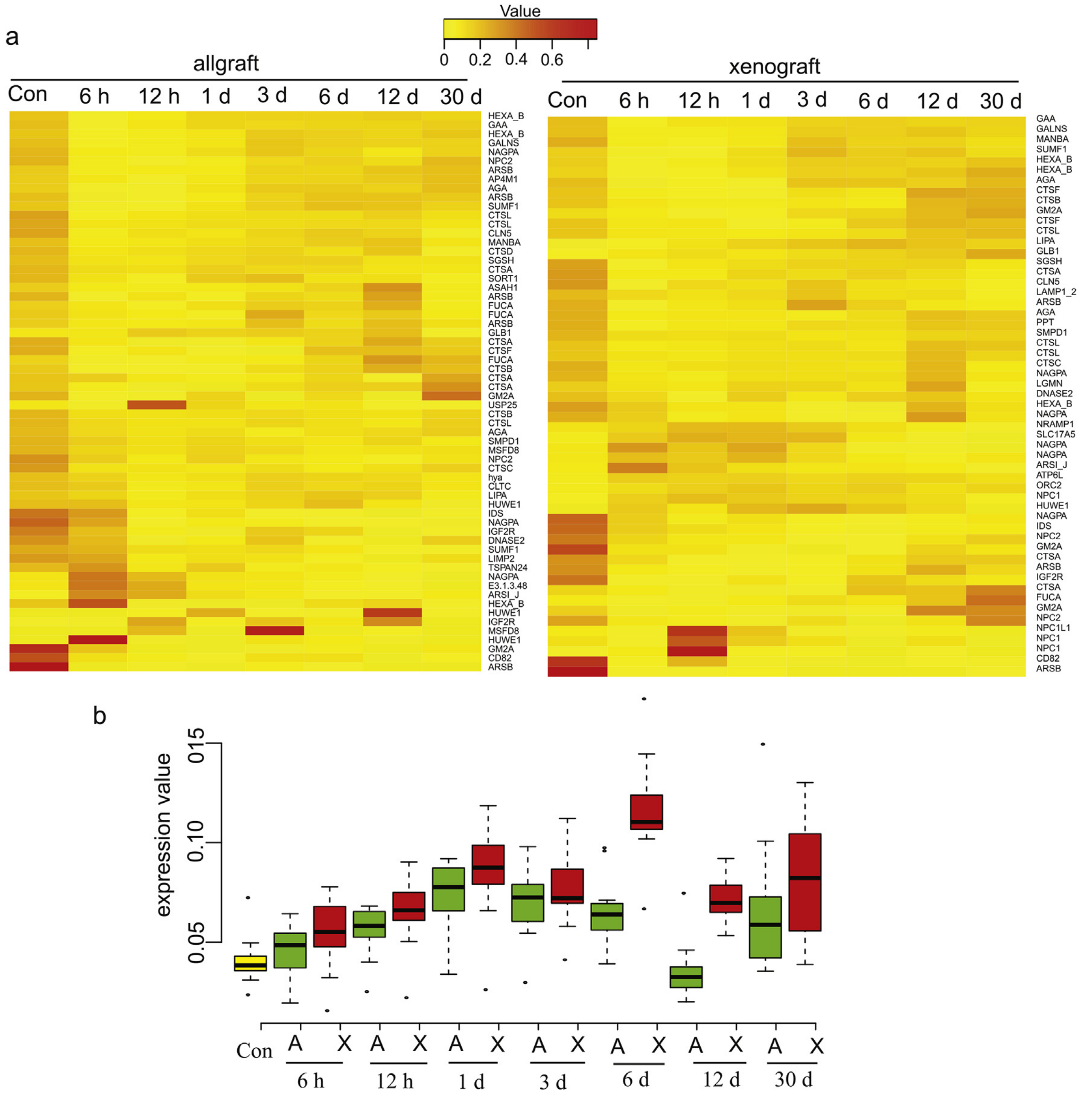

Fig. 5. Expression of protein lysis-related genes after transplantation, a. Expression of the enriched lysosome-related genes after transplantation in allograft (left) and xenograft (right) groups, b. Expression of the enriched proteasome-related genes after transplantation in allograft (A) and xenograft (X) groups.

cytokines, and cell cycle progression [24,25]. Cell cycle-related genes were initially down-regulated at 6–12 h and up-regulated between 1 and 6 d, indicating this process is probably connected with the concentration of Ca^{2+} despite further validation is needed. The increased cell number of hemocytes helps the pearl oyster cope with the transplanted foreign mantle piece and nucleus. Therefore, many hemocytes gathered around the transplanted mantle piece and nucleus by histological analysis after 3 d in both groups. This response may be caused by some molecules in the "small molecule metabolic process." In this process, ARSs are highly induced at 1 d in the allograft and xenograft groups. ARSs are essential enzymes that add amino acids to tRNAs. ARSs participate in various functions, including transcription, translation, splicing, inflammation, angiogenesis, and apoptosis [26]. In humans, tyrS can be secreted and cleaved into *N*- and C-domains that have pro-angiogenic and immune activation functions, respectively [27,28]. The up-regulated tyrS after transplantation may have similar functions but must be further elucidated.

Second, proteolysis system lysosome- and proteasome-related genes are present in different expression changes after transplantation. Lysosomal proteolysis-related genes, such as cathepsins, were down-regulated, whereas proteasome-related genes were up-regulated. Proteasomes and lysosomes are essential to protein quality and quantity control and can degrade soluble damaged, oxidized, or misfolded proteins and regulatory proteins [29]. In contrast to proteasomes, lysosomes can degrade a wide spectrum of substrates, which may be long-lived and bulky [30]. In vertebrates, immune cells and cells activated by the inflammatory cytokine express variant proteasomes called immunoproteasomes, which present antigen, regulate cytokine production, prevent the accumulation of harmful protein aggregates, and protect cells against stress and inflammation [31–33]. The up-regulated proteasomes in pearl oysters may help clean up the damaged proteins and protect hemocytes against inflammatory conditions caused by transplantation.

Although proteolysis-related genes in lysosomes were down-regulated, the sugar and lipid metabolism-related genes in lysosomes were up-regulated. For a long time, lysosomes were considered merely cellular incinerators involved in the degradation and recycling of cellular waste. However, compelling evidence indicates that lysosomes have a broad function. They degrade the carbohydrate portion of glycoproteins and glycosaminoglycans to produce monosaccharides, which may be

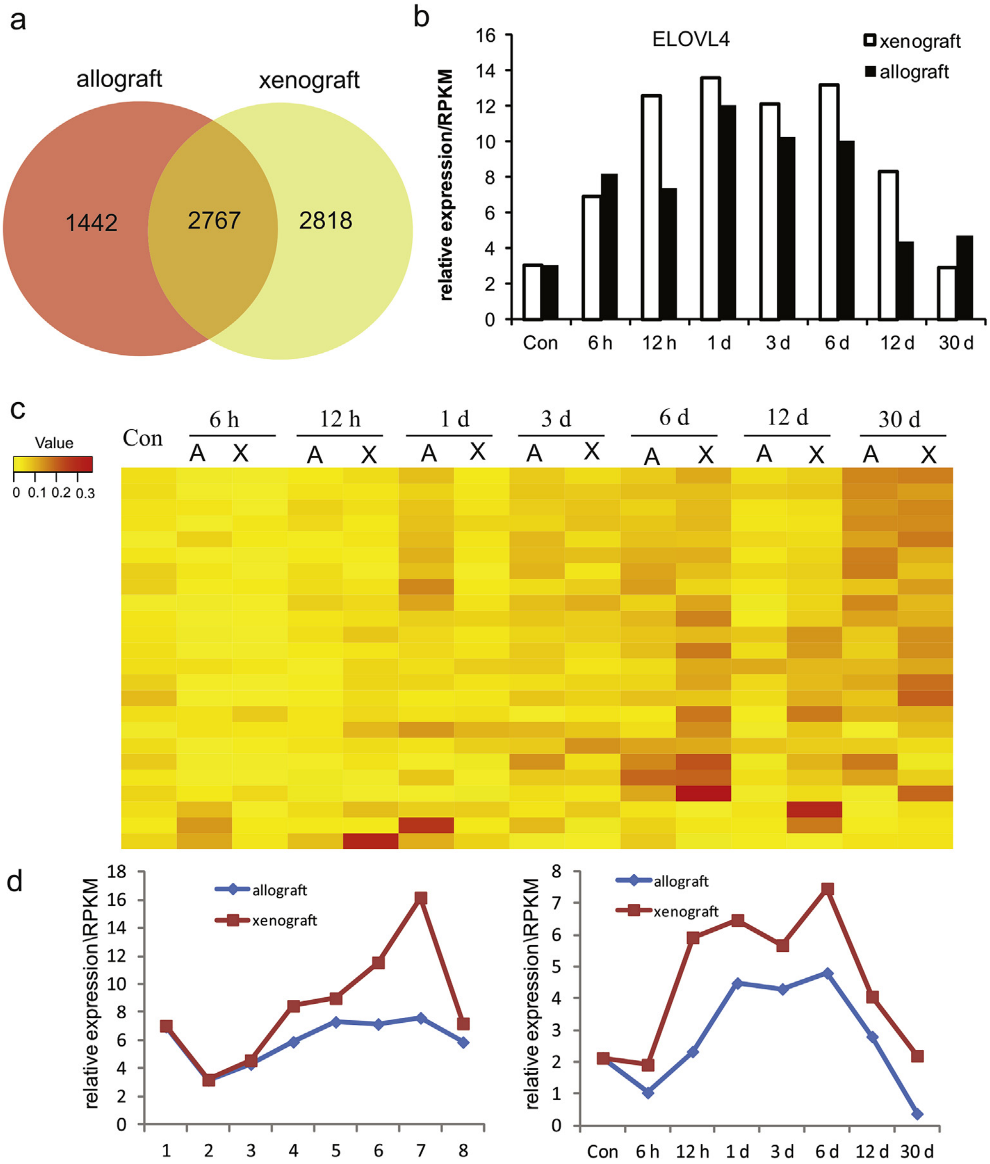

Fig. 6. Analysis of responsive genes in allograft (A) and xenograft (X) groups, a. Gene number of the responsive genes in allograft (A) and xenograft (X) groups, b. ELOVL4 expression after transplantation in allograft and xenograft groups, c. GSH expression after transplantation in allograft (A) and xenograft (X). d. GSa expression after transplantation in allograft and xenograft.

reused for energy production [34]. Lysosomes can also process and sort exogenous and endogenous lipids [35,36]. The up-regulated NPC genes after xenograft transplantation serve an important function in lipid and cholesterol trafficking and homeostasis [37–39]. ELOVL4 is a fatty acyl elongase that participates in the biosynthesis of long-chain fatty acids and has a potential role in DHA synthesis [40]. ELOVL4 was up-regulated in the xenograft and allograft groups. Fatty acids can influence the immune system, and DHA-derived lipid mediators belong to resolving family, which refers to anti-inflammatory and inflammation resolving [41]. The increased fatty acids and DHA may help pearl oysters cope with the immune response caused by the surgical transplantation. Given these results, we proposed that lipid metabolism is important for the recovery of pearl oysters after transplantation.

Furthermore, the transcriptomes and histological analyses revealed that the physiological response in the xenograft group was greater than that in the allograft group. In addition, no formed pearl sac was found 30 days after the transplantation in the xenograft. Generally, twelve days after transplantation, the immune response in the host pearl oyster gradually disappears, and pearl sac is formed, similar to what we observed in the allograft group in this study. In the xenograft group 30 d after transplantation, many hemocytes gathered around the nucleus, and many immune-related genes, such as proteasome and cGAS, were still up-regulated at 12 d. Therefore, the pearl oyster has not recovered at 12 d in the xenograft group. The pathway related to ribosome was affected in the xenograft group, not in the allograft group. An increased expression of ribosome-related genes was also observed among the genes after transplantation in other reports [2,8]. The up-regulated cytosolic ribosomes may help the pearl oyster to produce specific and necessary protein. The mitochondrial ribosome is responsible for the biosynthesis of protein components that is crucial to the generation of ATP in the eukaryotic cell [42]. Thus, we proposed that the mitochondrial-like ribosome over-expressed in the xenograft group may help the pearl oyster to produce additional energy.

The pearl oyster's quick recovery in the allograft may benefit from the specifically induced Notch signal at 6 h after transplantation. Notch signaling is a conserved pathway determining cell fate, including cell growth, differentiation, and survival [43]. Notch signaling also suppresses the TLR-triggered production of proinflammatory cytokines (IL-

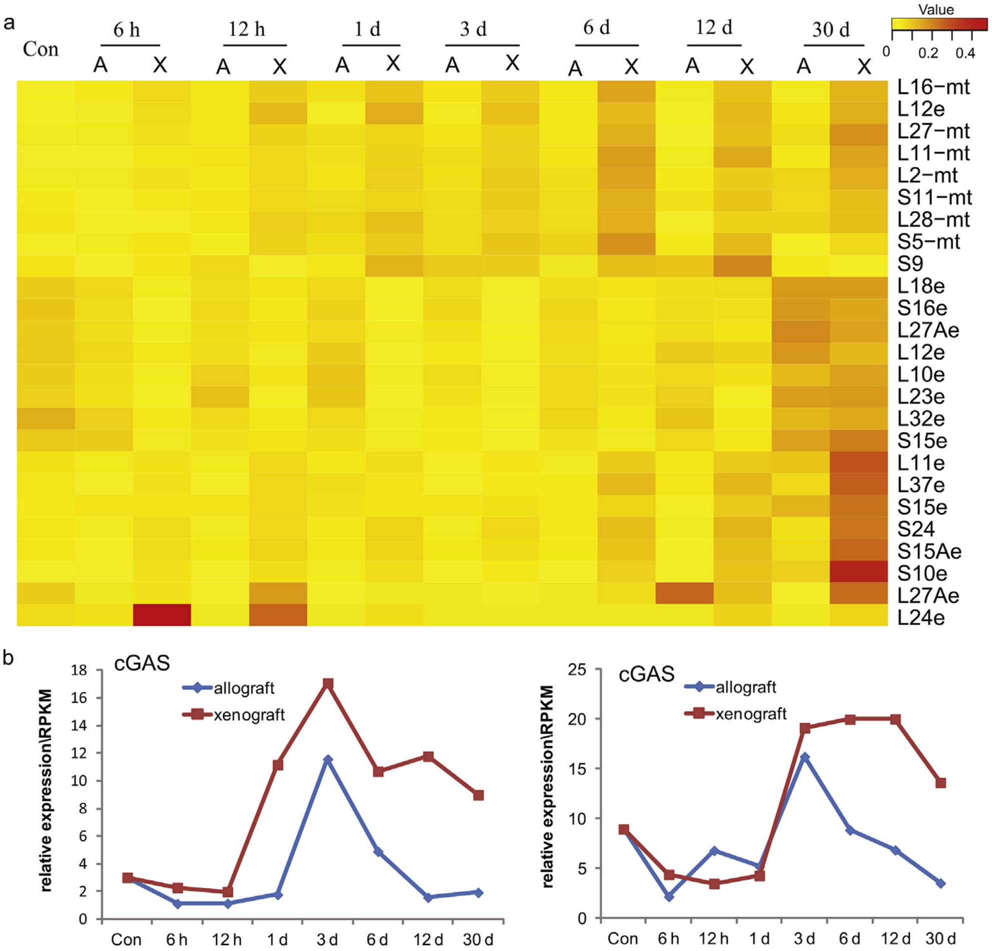

Fig. 7. Expression of ribosome-related genes and cGAS after transplantation, a. Expression of ribosome-related genes after transplantation in allograft (A) and xenograft (X). b. Expression of cGAS after transplantation in allograft and xenograft. mt: mitochondria.

6 and TNF-a) by inhibiting ERK1/2-mediated NF-kB activity in macrophages [44]. We concluded that the activated "Notch signaling pathway" can help the pearl oyster suppress the induced inflammation response in the allograft, thus preventing the damage caused by excess inflammation. The mechanism underlying the activation of "Notch signaling pathway" need to be further elucidated in the future, which may be helpful for preventing the excessive inflammation response after transplantation.

In this study, we obtained some different results with previous research [8]. For example, oxidation-reduction reaction and MAPK signaling pathway were found to be involved in response to transplantation in the research of Wei and et al. [8], while we found cell cycle, Ca^{2+} signal and lipid metabolic process were activated after transplantation. These difference may be caused by the different method used in the DGEs analysis, and the different process to obtain the experimental pearl oysters. In the research of Wei and et al. [8], the pearl oysters were cultured in tanks with re-circulating filtered seawater and were fed daily with unicellular algae during the experiment. The process we prepared for the experimental pearl oysters was the same as that used in normal pearl production, and the pearl oysters were cultured in the sea after transplantation. Even thought, both of us found ribosome, lysosome and apoptosis involved in the response to transplantation, indicating their important function after transplantation.

5. Conclusions

In conclusion, the transcriptomic, histological, and genomic analyses of the hemocytes obtained from pearl oysters after allograft and xenograft transplantations reveal many crucial genes, such as TRP, CaM, ARSs, GSH, cGAS, DNA replication- proteasome- and sugar and lipid metabolism-related genes. These genes are involved in the physiological response to allograft and xenograft transplantations. The only difference between xenograft and allograft groups is the transplanted mantle piece. Reorganization systems in pearl oysters can recognize the allografted or xenografted mantle piece, that is, our next project.

Acknowledgments

The studies were financially supported by grants of the National Natural Science Foundation of China (grant numbers 31672626), the Guangdong Marine and Fishery Bureau (grant numbers Z2015004), Graduate education innovation program of Guangdong Ocean University (grant numbers 201839).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.04.061.

References

[1] X. Zhao, Q. Wang, Y. Jiao, R. Huang, Y. Deng, H. Wang, et al., Identification of genes potentially related to biomineralization and immunity by transcriptome analysis of pearl Sac in pearl oyster Pinctada Martensii, Mar. Biotechnol. (New York, NY) 14 (2012) 730–739, https://doi.org/10.1007/s10126-012-9438-3.

[2] W. Wang, Y. Wu, Q. Lei, H. Liang, Y. Deng, Deep transcriptome profiling sheds light on key players in nucleus implantation induced immune response in the pearl oyster

Pinctada martensii, Fish Shellfish Immunol. 69 (2017) 67–77, https://doi.org/10.1016/j.fsi.2017.08.011.

[3] P. Kishore, P.C. Southgate, Haemocyte persistence after grafting for pearl production in Pinctada margaritifera (Linnaeus, 1758), Fish Shellfish Immunol. 42 (2015) 530–532 https://doi.org/10.1016/j.fsi.2014.11.035.

[4] Y. Jiao, S. Shi, X. Du, Y. Lu, Q. Wang, Histology and histochemistry on the pearl sac formation of pearl oyster Pinctada martensii, J. Guangdong Ocean Univ. 30 (2010) 4, https://doi.org/10.3969/j.issn.1673-9159.2010.04.002.

[5] E.L. McGinty, B.S. Evans, J.U.U. Taylor, D.R. Jerry, Xenografts and pearl production in two pearl oyster species, P. maxima and P. margaritifera: effect on pearl quality and a key to understanding genetic contribution, Aquaculture 302 (2010) 175–181 https://doi.org/10.1016/j.aquaculture.2010.02.023.

[6] E. Fukushima, T. Iwai, C. Miura, F.T. Celino, S. Urasaki, T. Miura, A xenograft mantle transplantation technique for producing a novel pearl in an akoya oyster host, Mar. Biotechnol. (New York, NY) 16 (2014) 10–16, https://doi.org/10.1007/s10126-013-9525-0.

[7] S. Panha, P. Kosavititkul, Mantle transplantations in freshwater pearl mussels in Thailand, Aquacult. Int. 5 (1997) 267–276, https://doi.org/10.1023/a:1018347703780.

[8] J. Wei, S. Fan, B. Liu, B. Zhang, J. Su, D. Yu, Transcriptome analysis of the immune reaction of the pearl oyster Pinctada fucata to xenograft from Pinctada maxima, Fish Shellfish Immunol. 67 (2017) 331–345, https://doi.org/10.1016/j.fsi.2017.06.030.

[9] X. Du, Y. Deng, Q. Wang, S. Xie, D. Liu, Haixuan NO 1" strain of pearl oyster Pinctada fucata martensii, China Fish. 10 (2015) 4.

[10] F. Liang, S. Xie, S. Fu, Y. Deng, Growth characteristics of pearl oyster Pinctada maxiam seeds with broodstocks introduced from Indonesia cultured in the Xuwen sea of Zhanjiang, J. Guangdong Ocean Univ. 31 (2011) 5, https://doi.org/10.3969/j.issn.1673-9159.2011.06.007.

[11] X. Du, G. Fan, Y. Jiao, H. Zhang, X. Guo, R. Huang, et al., The pearl oyster Pinctada fucata martensii genome and multi-omic analyses provide insights into biomineralization, GigaScience 6 (2017) 1–12, https://doi.org/10.1093/gigascience/gix059.

[12] Z. Pan, H. Yang, P.S. Reinach, Transient receptor potential (TRP) gene superfamily encoding cation channels, Hum. Genom. 5 (2011) 108, https://doi.org/10.1186/1479-7364-5-2-108.

[13] A. Giamarchi, F. Padilla, B. Coste, M. Raoux, M. Crest, E. Honoré, et al., The versatile nature of the calcium-permeable cation channel TRPP2, EMBO Rep. 7 (2006) 787–793, https://doi.org/10.1038/sj.embor.7400745.

[14] L.J. Moilanen, M. Hämäläinen, L. Lehtimäki, R.M. Nieminen, K. Muraki, E. Moilanen, Pinosylvin inhibits TRPA1-induced calcium influx in vitro and TRPA1-mediated acute paw inflammation in vivo, Basic Clin. Pharmacol. Toxicol. 118 (2016) 238–242, https://doi.org/10.1111/bcpt.12485.

[15] J. Wei, B. Liu, S. Fan, H. Li, M. Chen, B. Zhang, et al., Differentially expressed immune-related genes in hemocytes of the pearl oyster Pinctada fucata against allograft identified by transcriptome analysis, Fish Shellfish Immunol. 62 (2017) 247–256, https://doi.org/10.1016/j.fsi.2017.01.025.

[16] G. Zhang, X. Fang, X. Guo, L. Li, R. Luo, F. Xu, et al., The oyster genome reveals stress adaptation and complexity of shell formation, Nature 490 (2012) 49–54, https://doi.org/10.1038/nature11413.

[17] R.C. Strange, M.A. Spiteri, S. Ramachandran, A.A. Fryer, Glutathione-S-transferase family of enzymes, Mutat. Res. 482 (2001) 21–26 11535245 http://www.ncbi.nlm.nih.gov/pubmed/11535245.

[18] T. Li, Z.J. Chen, The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer, J. Exp. Med. 215 (2018) 1287–1299, https://doi.org/10.1084/jem.20180139.

[19] X. Cai, Y.H. Chiu, Z.J. Chen, The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling, Mol. Cell 54 (2014) 289–296, https://doi.org/10.1016/j.molcel.2014.03.040.

[20] R.P. Croll, A.J.G. Dickinson, Form and function of the larval nervous system in molluscs, Invertebr. Reprod. Dev. 46 (2004) 173–187, https://doi.org/10.1080/07924259.2004.9652620.

[21] S.-Y. Lin, D.P. Corey, TRP channels in mechanosensation, Curr. Opin. Neurobiol. 15 (2005) 350–357 https://doi.org/10.1016/j.conb.2005.05.012.

[22] M.M. Moran, H. Xu, D.E. Clapham, TRP ion channels in the nervous system, Curr. Opin. Neurobiol. 14 (2004) 362–369 https://doi.org/10.1016/j.conb.2004.05.003.

[23] D.E. Clapham, TRP channels as cellular sensors, Nature 426 (2003) 517, https://doi.org/10.1038/nature02196.

[24] J.T. Taylor, X.B. Zeng, J.E. Pottle, K. Lee, A.R. Wang, S.G. Yi, et al., Calcium signaling and T-type calcium channels in cancer cell cycling, World J. Gastroenterol. 14 (2008) 4984–91. 18763278.

[25] P. Nunes, N. Demaurex, The role of calcium signaling in phagocytosis, J. Leukoc. Biol. 88 (2010) 57–68, https://doi.org/10.1189/jlb.0110028.

[26] S.G. Park, K.L. Ewalt, S. Kim, Functional expansion of aminoacyl-tRNA synthetases and their interacting factors: new perspectives on housekeepers, Trends Biochem. Sci. 30 (2005) 569–574 https://doi.org/10.1016/j.tibs.2005.08.004.

[27] K. Wakasugi, B.M. Slike, J. Hood, K.L. Ewalt, D.A. Cheresh, P. Schimmel, Induction of angiogenesis by a fragment of human tyrosyl-tRNA synthetase, J. Biol. Chem. 277 (2002) 20124–20126, https://doi.org/10.1074/jbc.C200126200.

[28] K. Wakasugi, P. Schimmel, Two distinct cytokines released from a human aminoacyl-tRNA synthetase, Science (New York, NY) 284 (1999) 147–151 10102815.

[29] X. Wang, J. Robbins, Proteasomal and lysosomal protein degradation and heart disease, J. Mol. Cell. Cardiol. 71 (2014) 16–24 https://doi.org/10.1016/j.yjmcc.2013.11.006.

[30] R.A. Dunlop, U.T. Brunk, K.J. Rodgers, Oxidized proteins: mechanisms of removal and consequences of accumulation, IUBMB Life 61 (2009) 522–527, https://doi.org/10.1002/iub.189.

[31] S. van Deventer, J. Neefjes, The immunoproteasome cleans up after inflammation, Cell 142 (2010) 517–518, https://doi.org/10.1016/j.cell.2010.08.002.

[32] A. Angeles, G. Fung, H. Luo, Immune and non-immune functions of the immunoproteasome, Front. Biosci. (Landmark edition) 17 (2012) 1904-16. 22201844.

[33] H. Kimura, P. Caturegli, M. Takahashi, K. Suzuki, New insights into the function of the immunoproteasome in immune and nonimmune cells, J. Immunol. Res. 2015 (2015) 541984, https://doi.org/10.1155/2015/541984.

[34] C. Settembre, A. Ballabio, Lysosomal adaptation: how the lysosome responds to external cues, Cold Spring Harbor Perspect. Biol. (2014 6), https://doi.org/10.1101/cshperspect.a016907.

[35] A.M. Thelen, R. Zoncu, Emerging roles for the lysosome in lipid metabolism, Trends Cell Biol. 27 (2017) 833–850 https://doi.org/10.1016/j.tcb.2017.07.006.

[36] C. Settembre, A. Ballabio, Lysosome: regulator of lipid degradation pathways, Trends Cell Biol. 24 (2014) 743–750, https://doi.org/10.1016/j.tcb.2014.06.006.

[37] D.S. Ory, The niemann-pick disease genes; regulators of cellular cholesterol homeostasis, Trends Cardiovasc. Med. 14 (2004) 66–72, https://doi.org/10.1016/j.tcm.2003.12.003.

[38] S. Mukherjee, F.R. Maxfield, Lipid and cholesterol trafficking in NPC, Biochim. Biophys. Acta 1685 (2004) 28–37, https://doi.org/10.1016/j.bbalip.2004.08.009.

[39] E.D. Carstea, J.A. Morris, K.G. Coleman, S.K. Loftus, D. Zhang, C. Cummings, et al., Niemann-pick C1 disease gene: homology to mediators of cholesterol homeostasis, Science (New York, NY) 277 (1997) 228–231 9211849.

[40] Ó. Monroig, J. Rotllant, J.M. Cerdá-Reverter, J.R. Dick, A. Figueras, D.R. Tocher, Expression and role of Elovl4 elongases in biosynthesis of very long-chain fatty acids during zebrafish Danio rerio early embryonic development, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1801 (2010) 1145–1154 https://doi.org/10.1016/j.bbalip.2010.06.005.

[41] K. Fritsche, Fatty acids as modulators of the immune response, Annu. Rev. Nutr. 26 (2006) 45–73, https://doi.org/10.1146/annurev.nutr.25.050304.092610.

[42] M.R. Sharma, E.C. Koc, P.P. Datta, T.M. Booth, L.L. Spremulli, R.K. Agrawal, Structure of the mammalian mitochondrial ribosome reveals an expanded functional role for its component proteins, Cell 115 (2003) 97–108 https://doi.org/10.1016/S0092-8674(03)00762-1.

[43] G. Dontu, K.W. Jackson, E. McNicholas, M.J. Kawamura, W.M. Abdallah, M.S. Wicha, Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells, Breast Canc. Res. 6 (2004) R605, https://doi.org/10.1186/bcr920.

[44] Q. Zhang, C. Wang, Z. Liu, X. Liu, C. Han, X. Cao, et al., Notch signal suppresses Toll-like receptor-triggered inflammatory responses in macrophages by inhibiting extracellular signal-regulated kinase 1/2-mediated nuclear factor kappaB activation, J. Biol. Chem. 287 (2012) 6208–6217, https://doi.org/10.1074/jbc.M111.310375.