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Transcriptome Analysis of Immune Response Process in Yellow Catfish (*Pelteobagrus fulvidraco*) During *Edwardsiella tarda* Exposure

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Abstract

Edwardsiella tarda, the causative agent of ascites disease, is a major fish pathogen and has caused significant economic losses in aquaculture. To decipher the immune response process challenged by *E. tarda* in yellow catfish (*Pelteobagrus fulvidraco*), the transcriptomic profiles of the spleens infected with bacteria at 6 h, 24 h, and 72 h were obtained using the Illumina sequencing platform. After de novo assembly, a total of 158,124 unigenes were detected. To further investigate the immune-related DEGs, gene ontology (GO) enrichment and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis were performed. Immune pathways about antigen processing and presentation pathway, complement and coagulation cascades pathway, and apoptosis pathway were combined to discussed in this study. Additionally, 10 immune-related DEGs in these three immune pathways were randomly selected for expression verification by quantitative Real-time PCR (qRT-PCR). The data generated in this study provides a valuable resource for further immune response research and offers efficient strategies against *E. tarda* infection in yellow catfish.

Introduction

Edwardsiella tarda, a typical gram negative bacterium, has been widely reported in important economic fishes, including *Paralichthys olivaceus* (Bang et al., 1992), *Ictalurus punctatus* (Meyer & Bullock, 1973), *Scophthalmus maximus* (Nougayrede et al., 1994), *Pagrus major* (Mohanty & Sahoo, 2007)and *Seriola quinqueradiata (*Yasunaga et al., 1982*)*. The infection of *E. tarda* can lead to crack-head disease with high morbidity and mortality, threatening the sustainability and productivity of the yellow catfish aquaculture industry. Thus, it is imperative to research the yellow catfish immune response and its related immune genes in response to bacterial infection.

Next generation sequencing (NGS) technology has emerged as a fast and cost-effective way for transcriptome analysis of immune responses against bacterial infections in various aquaculture species. There are several reports of immunological reaction to bacteria using transcriptome analysis in teleost fishes, such as common carp (*Cyprinus carpio*) (Jiang et al., 2016), Antarctic notothenioid fish (*Notothenia coriiceps*) (Ahn et al., 2016) and barramundi (*Lates calcarifer*) (E. Zoccola et al., 2017; Emmanuelle Zoccola et al., 2017). Moreover, we have analyzed the immune response of grass carp (*Ctenopharyngodon idella*) with transcriptome analysis method (Yang et al., 2016), which suggests it is a reliable technology. There are an

increasing number of transcriptome analyses of *E. tarda* infection in teleost fish. For example, using transcriptomic techniques, Zan Li et al., (2018) and Bin Sun et al.（2020）analyzed the gene changes in blood and spleen red blood cells of Japanese flounder (Paralichthys olivaceus) infected with *E. tarda*. Min Cao et al. systematically analyzed circular RNAs (circRNAs), microRNAs (miRNAs) and messenger RNAs (mRNAs) the intestine of black rockfish（*Sebastes schlegelii*）after infection with *E. tarda* at different time points (2, 6, 12, and 24 h) (Li et al., 2018; Sun et al., 2020). However, only a few transcriptome studies have focused on yellow catfish infected with *E. tarda*. Zhu et al. (2017) studied the gene expression profile of yellow catfish resistant to *E. tarda* infection within 72 h (Zhu *et al*., 2017), and Hua Liu et al. (2017) studied mirNa-MRnain gene expression of yellow catfish infected with *E. tarda* at 6 h (Liu et al., 2021). However, they only studied a certain time point after infection with *E. tarda*, and the gene expression of the whole immune response period of yellow catfish needs further study.

Therefore, we tested the differentiation of transcriptome of yellow catfish spleen during bacterial pathogen *E. tarda* infection in four time points, to provide new insights into the immune system and defense mechanisms of yellow catfish in response to *E. tarda*. In the present study, the Illumina HiSeq 4000™ platform was employed for transcriptome profiling analysis of the yellow catfish with *E. tarda* infection at 0 h, 6 h, 24 h and 72 h. Subsequently, the differentially expressed genes (DEGs) were found and the immune-related DEGs were identified via GO and KEGG enrichment analysis. Finally, several differential expressions of selected immune-related genes were verified by qRT-PCR.

Materials and Methods

Fish and Bacteria

Healthy juvenile yellow catfish with body weights of 25 g ± 2 g were kindly provided by Hold-one aquatic breeding center in Foshan (Guangdong, China), and reared at 28 ℃ for two weeks in 200 L aquaria with Eheim biofilters (Liu et al., 2021) . The bacterial strain *E. tarda* used in the experiment was isolated from diseased yellow catfish and stored in our laboratory. The bacteria were incubated to mid-logarithmic stage in Luria-Bertani (LB) medium at 28 ℃, then harvested by centrifugation at 4000 rpm for 5 min, washed twice with phosphate-buffered saline (PBS) and centrifuged again. Bacterial pellets were then resuspended in PBS with concentration of 5×10^7 colony forming units (CFU) ml^{-1}. The fish were randomly divided into two groups (30 fish per group). One group was infected (IG) by an intraperitoneal injection of 0.1 mL of the *E. tarda* suspension (described above), and the other group was a control (CG), injected with an equal volume of PBS.

Challenge experiments and RNA Preparation

The spleen samples were collected at 0 h, 6 h, 24 h, and 72 h after infection and immediately stored in liquid nitrogen at -80 ℃ until used for RNA extraction. The total RNA of each sample was extracted using TRIzol Reagent (Invitrogen, USA) following the manufacturer's instructions. The quality and concentration of the total RNA was accurately examined using the Agilent 2100 Bioanalyzer (Agilent Technologies) and the integrity was assessed by electrophoresis on 1 % agarose gel. Three high-quality RNA samples (RNA integrity number (RIN) > 8.0 and Total RNA concentration > 250ng/μL) were selected from each group at each time point for the construction of RNA-Seq library construction.

Library Construction and Illumina Sequencing

After DNase I treatment, mRNA was purified using oligo (dT)25 magnetic beads (Dynabeads® oligo (dT)25, Invitrogen) and subsequently interrupted to short fragments of 150-200 nucleotides using RNA fragmentation reagent (Ambion, USA). Then sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations. The cDNAs were checked by Agilent 2100 Bioanalyzer (Agilent, USA) and ABI StepOnePlus Real-Time PCR System (ABI, USA). The mixed DNA libraries were sequenced with Illumina Hiseq 4000 ™ platform (Chen et al., 2019).

Filtering of Sequencing Reads and De Novo Transcriptome Assembly

Clean data was obtained by removing low-quality reads and reads contain adapter or ploy-N from raw data. Preprocessed clean reads were de novo assembled into transcripts using Trinity software package with default values (Manfred G, 2011). Transcripts shorter than 150 bp paried-end read were removed and the remaining sequences were clustered using TGICL software (version v2.1) to reduce redundancy (Pertea et al., 2003). The longest sequence in each gene cluster was preserved and designated as a unigene. Q20, Q30, GC-content and sequence duplication level of the clean reads were calculated.

Unigenes Functional Annotation and Classification

The assembled unigenes were scanned using the following databases: NCBI nucleotide sequences (NT), NCBI non-redundant rotein (NR), Gene Ontology (GO), Clusters of Orthologous Groups (COG), KEGG and Swiss-prot. Unigenes with NR annotation were submitted to Blast2GO software (Conesa et al., 2005) to obtain their GO annotations, and were further classified according to

GO functions using the Web Gene Ontology (WEGO) program (Jia, 2006; Ye et al., 2006). Additionally, these sequences were queried against KOG database for functional classification and aligned to KEGG database for pathway assignment (Moriya et al., 2007; Tatusov et al., 2003). All the genes were searched against the Swiss-Prot, NR, NT, GO, KEGG, and COG databases using the BLASTx algorithm (E-value <1E-5).

Differentially Expressed Genes and Enrichment Analysis

Expression datas from each library were generated by Bowtie2 software (Langmead & Salzberg, 2012) , which were mapped to the transcriptome assembly. To identify DEGs among the libraries, the fragments in kilobase of transcripts per million fragments mapped (FPKM) values were calculated using RSEM (version v1.2.21) (Li & Dewey, 2011). The false discovery rate (FDR) was used to calculate the threshold *p*-value in multiple tests. Genes with an absolute value log2 Ratio ≥ 2 and Q-value ≤ 0.01 were defined as DEGs. All identified DEGs were subjected to GO functional enrichment and KEGG pathway analysis.

qRT-PCR of Selected Immune-related Genes Expression

To examine the reliability of the RNA-Seq results, 10 immune-related DEGs in previously discussed immune pathways were randomly selected for validation using quantitative real-time RT-PCR (qRT-PCR). The housekeeping gene β-actin was used as the reference gene (Liu et al., 2021). Suitable primers were designed using Primer premier 5.0 program (Table 1) and synthesized by Sangon Biotech Co., Ltd (Shanghai, China). QRT-PCR with SYBR Green dye (TaKaRa, Dalian, China) was performed on an ABI PRISM 7500 Fast Real-Time PCR System following the manufacturer's protocol. The samples collected at different time points were pooled, and used for qPCR analysis. Each group was created by combining equal amounts of RNA from three replicate pools (three individual fish per pool). The RNA samples selected for qRT-PCR assay were the same as those used for the transcriptomic sequencing. All reactions were performed in triplicates. The qRT-PCR reaction system (25 µl) consisted of 12.5 µL of 2 × SYBR qPCR Mix, 1 µL of forward and reverse primers, 1 µL of cDNA, and 10.5 µL of RNase-free H_2O. The qRT-PCR procedure was as follows: 95 ℃ for 5 min, followed by 40 cycles of 95 ℃ for 10 s, and 60 ℃ for 1 min. The melting curves of each reaction from 60 ℃ to 95 ℃ were analyzed after PCR amplification. The amplification efficiency of each pair of primers ranged from 90(%) to 100(%). The relative expression ratio of the target genes versus β-actin gene was calculated using $2^{-\triangle\triangle CT}$ method and all data were given in terms of relative mRNA expression (Livak & Schmittgen, 2001). The threshold for significance was set at P <0.05, and high significance was set at P <0.01.

Results

Sequencing and De Novo Assembly

The yellow catfish infected with *E. tarda* showed clinical signs including 'hole in the head' lesion, hemorrhages in the lower jaw and at the base of fins, abdominal distension, and yellowish fluid in the intestine. After filtering to remove low-quality sequences, a total of 569,584,794 clean reads were generated with a higher Q20(%) and Q30(%) (Table 2) with equal GC content. A total of 158,124 unigenes were obtained by de novo assembly. The length of assembled unigenes ranged from 200 bp to 27,705 bp, with an average length of 1,166.50 bp and an N50 of 2,408 bp (Table 3). Raw sequencing reads data has been submitted to Sequence Read Archive in NCBI, SRA accession nos. SRP155088.

Annotation of Assembled Unigenes

In order to obtain comprehensive genes function information, 158,124 unigenes were annotated using six databases including NT, NR, GO, COG, KOG and Swiss-Port.

Results showed that 80,820 unigenes, that accounted for almost 51.11% of the total number of unigenes, were annotated in at least one database. Of these, 62,786 (39.71%), 66,702 (42.18%), 36,344 (22.98%), 24,213(15.31%), 55,594 (35.16%) and 58,022 (36.70%) unigenes were annotated by NT, NR, GO, COG, KEGG and Swiss-Prot databases, respectively (Figure 1).

Differential Expression Analysis

The differences in gene expression after the *E. tarda* infection were assessed to explore the transcriptomic response of yellow catfish. The numbers of total DEGs in spleen at 6 h, 24 h, and 72 h were 6,866 (4,005 up-regulated and 2,861 down-regulated genes), 3,028 (2,245 up-regulated and 783 down-regulated genes), and 737 (549 up-regulated and 188 down-regulated genes), respectively (Figure. 2).

GO and KEGG Enrichment Analysis of Differently Expressed Genes

GO is a standardized system for categorizing genes and gene products across species. The results of the GO enrichment analysis of differentially expressed genes were classified into three categories: biological process, cellular component and molecular function. The significant GO terms in these three categories and DEGs of them at each time were showed in Figure 3. In order to better understand the pathway, DEGs were compared to the KEGG database for pathway enrichment. At 6 h, 4,396 of 6,866 DEGs were annotated to 301 signaling pathways in KEGG. At 24 h, 2,014 of 3,028 DEGs were annotated to 287 signaling pathways

Table 1. List of primers used for qRT-PCR validation of the RNA-Seq data.

Gene	Forward primer (5'-3')	TM(℃)	Reverse primer (5'-3')	TM(℃)	Amplicon length (bp)	Accession Number
β-actin	ATTGCCGCACTGGTTGTT	58.15	CCTGTTGGCTTTAGGGTT	54.32	336	M25013.1
MHC Ⅰ	ACTTGGTCAGGTGGATGGAT	58.33	CTCACAGCCGTACATCACCT	59.47	235	M25013.1
MHC Ⅱ	CTCCTCCTGTCCGTATCCAC	58.97	ACACCACAAAACACAGACGG	58.91	244	M25013.1
HSP90	GCAAAGGTCCGCATTGAGAA	59.12	ACCCACAGACCCGAATCAAT	59.01	230	M25013.1
C6	GCTGTGCAAAACCTCTTCCA	58.97	ATCTCTCCAGGTTTGCAGCT	59.01	217	M25013.1
C7	CTATGCCCTGTATGCCAAAT	55.54	CCTCCAGTGCCCTCTTCA	58.19	143	M25013.1
F3	GGATGTTTTCGGGGATGAGC	58.97	GTTTGCAGCACGTGAAGGTA	59.06	169	M25013.1
F7	GAGCTGAAGACGGGGAATCT	59.17	AATCGATTGCTCACAGTGGC	58.91	240	M25013.1
CASP3	TGACTTGCTGTGGTCCTCTT	58.87	AACGGAACTGATGTTGATGC	56.72	141	M25013.1
CASP6	AACCACAAGAGACGGGGAAT	58.94	AAAAGACGCAGACAAGGCAG	59.05	238	M25013.1
TNFα	GCAACTGGGCTCAAGCTTAC	59.48	GGTCCTGGTTCACTCTCCAA	58.94	2617	M25013.1

Table 2. Summary of sequencing reads after filtering.

Type	Total Clean Reads	Total Clean Bases	GC(%)	Q20(%)	Q30(%)	Total Clean Reads Ratio(%)
PF_Dm1(0 h)	45984780	6897717000	46.26	92.97	84.45	99.55
PF_Dm2(0 h)	52545358	7881803700	46.8	94.99	88.66	99.31
PF_Dm3(0 h)	45687904	6853185600	46.35	94.95	88.54	99.15
PF_6m1(6 h)	48385674	7257851100	46.02	94.84	88.61	99.36
PF_6m2(6 h)	43708058	6556208700	45.84	94.22	87.37	99.31
PF_6m3(6 h)	44524184	6678627600	46.16	94.27	87.32	99.26
PF_24m1(24 h)	46556698	6983504700	46.19	95.04	88.91	99.14
PF_24m2(24 h)	44453980	6668097000	46.05	94.76	88.17	99.15
PF_24m3(24 h)	44716168	6707425200	45.91	94.25	87.27	99.15
PF_72m1(72 h)	53210664	7981599600	45.91	94.02	86.8	99.39
PF_72m2(72 h)	56036240	8405436000	46.78	91.97	82.76	99.75
PF_72m3(72 h)	43775086	6566262900	46.18	94.8	87.91	99.7

Table 3. Statistics of assembled unigenes.

Sample	Total Number (bp)	Min_Length (bp)	Max_length (bp)	Mean_Length (bp)	N50 length (bp)	GC(%)
All	158124	200	27705	1166.50	2408	43.8

in KEGG. At 72 h, 542 of 737 DEGs were annotated to 247 signaling pathways in KEGG. The top30 most enriched pathway terms and DEGs of them at 6 h, 24 h, and 72 h were showed in Figure 4 and Figure 3, respectively. Among these pathways, 16 pathways associated with immune system were identified.

qRT-PCR Validation for RNA-Seq Data

We tested the expression of 10 immune-related DEGs at three time points using qRT-PCR to validate the DEGs identified by RNA-Seq. The qRT-PCR analysis demonstrated a single product for all validated DEGs. Fold changes at each time point of qRT-PCR were compared with the RNA-Seq expression profiles. The selected genes are involved in the immune signaling pathway, including antigen processing and presentation pathway (major histocompatibility complex, class I (MHC Ⅰ), major histocompatibility complex, class II (MHC Ⅱ), heat shock protein 90(HSP90)), complement and coagulation cascades pathway (complement component 6 (C6), complement component 7 (C7), coagulation factor III (F3), coagulation factor VII (F7)), and apoptosis pathway (caspase 3 (CASP3), caspase 6 (CASP6), tumor necrosis factor alpha (TNFα)). As showed in Figure 5, all trends of qRT-PCR results were consistent with the RNA-Seq results, and they displayed trend patterns identical with those of DEGs in both methods.

Discussion

In this research, transcriptome profiling of yellow catfish was used to gain insights into the molecular mechanisms underlying host responses to *E. tarda*. We focused on screening immune-related genes and pathways. The sequencing results showed that DEGs had a linear downward trend over time. Most DEGs appeared at 6 h, indicating the immune response was activated at the early stage. Taking the advantages of GO annotation and KEGG pathway classification, the regulations of selected immune related genes in antigen processing and presentation pathway, complement and coagulation cascades pathway, and apoptosis pathway were combined to analyzed in detail (Table 4).

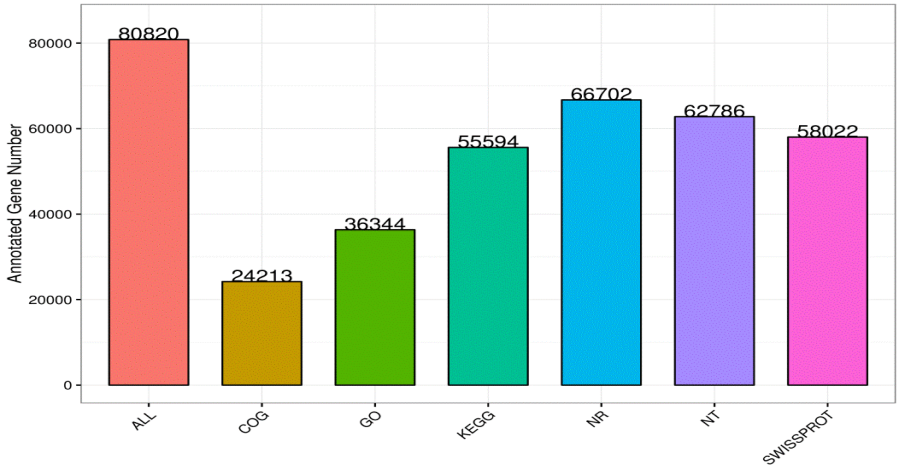

Figure 1. The annotated gene number in different databases. The x-axis represents the union of all data and the y- axis represents the number of unigenes on the corresponding database annotation.

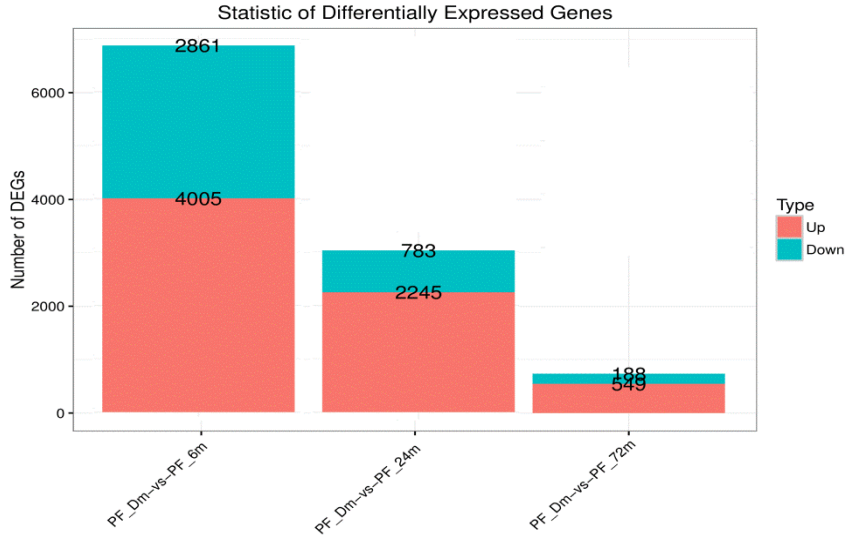

Figure .2. The number of up-regulated and down-regulated DEGs compared between different time points under *E. tarda* challenge at 0 h (PF_Dm), 6 h (PF_6m), 24 h (PF_24m), and 72 h (PF_72m).

Apoptosis

Apoptosis, also called programmed cell death, plays a considerable role in immune response. In the present study, the caspase pathway is an important pathway involved in *E. tarda*-triggered apoptosis. Caspase activity has been thought to be a useful marker for assaying stress-induced apoptosis in the early immune stages of fishes, during which caspase-3 is confirmed as a key executor to be activated down-stream in apoptosis pathways (Liu et al., 2007). Generally speaking, there are two pathways of caspase activation involved in apoptosis. The first one is initiated by the stress-mediated release of cytochrome c from the mitochondria, which promotes downstream effector caspase-3 activation through formation of the cytochrome c/Apaf-1/caspase-9-containing apoptosome complex then leading to the characteristic phenotype of apoptosis (S. Fulda, 2009). In the second pathway, stimulation of Fas, tumour necrosis factor receptor (TNFR) or TNF-related apoptosis-inducing ligand receptors (TRAILR) results in activation of the initiator caspase-8 (Simone Fulda, 2009). In our study, except for caspase-9, Fas, tumor necrosis factor superfamily (TNFα), TRAILR, caspase 3, caspase 6, caspase 7, and caspase 8 were DEGs, indicating the second caspase-dependent apoptotic pathway may contribute greatly to the *E. tarda*-induced apoptosis in the early immune response of yellow catfish.

Current studies have shown that, by inhibiting apoptosis, microbial pathogens can replicate in cells and avoid inflammatory and antimicrobial effects outside the host cell (Faherty & Maurelli, 2008; Raymond et al., 2013). Ze-Jun Zhou *et al*. also suggested that *Edwardsiella tarda* takes the inhibition of apoptosis as its cellular survival strategy, but the specific mechanism of action needs to be further studied (Zhou & Sun, 2016). Some lipopolysaccharides (LPS) in the cell wall of

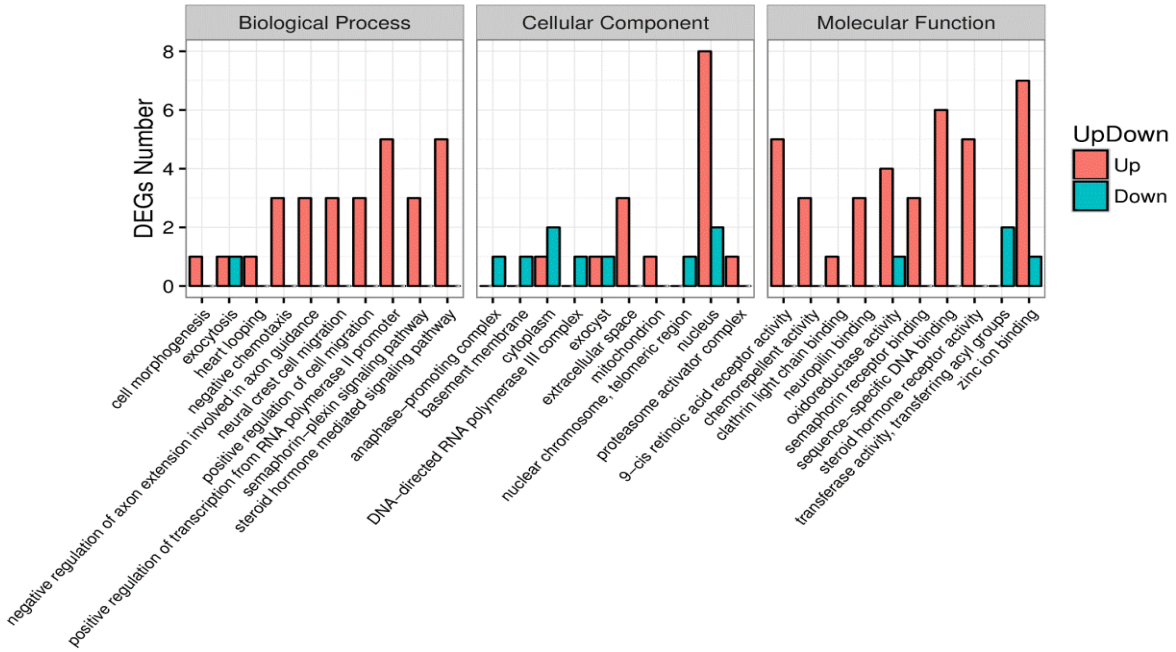

Figure 3. GO enrichment analysis of differentially expressed genes at 6 h among three categories: biological process, cellular component and molecular function. The x-axis is gene functional classification of GO, y-axis is the corresponding number of up-regulated and down-regulated genes.

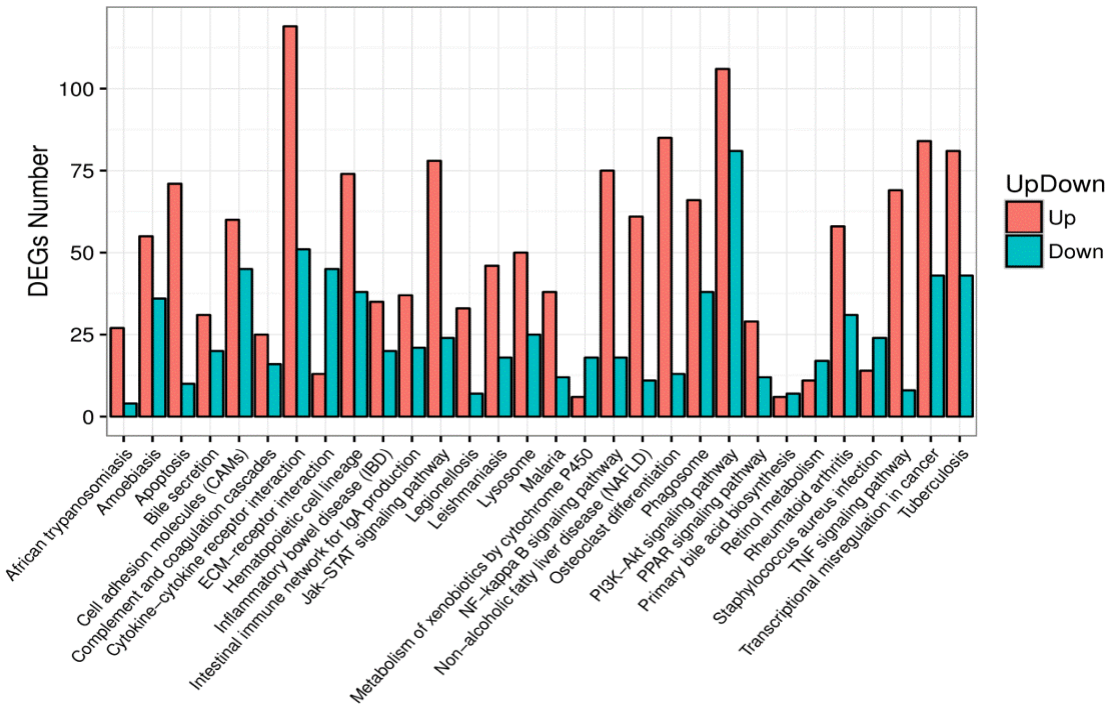

Figure 4. The top30 most enriched KEGG pathways at 6 h. The x-axis is KEGG pathway classification and y-axis is the corresponding number of up-regulated and down-regulated genes.

Gram-negative bacteria can inhibit apoptosis by activating the nuclear factor-κB (NF-κB) during infection (Pålsson-McDermott & O'Neill, 2004). NF-κB is mainly activated through two pathways: the canonical and non-canonical NF-κB signaling pathways (Sun, 2017). In the canonical pathway, the NF-κB pathway responds to stimulation from various immune receptors, such as interleukin 1 receptor type I (IL-1R) and tumor necrosis factor receptor superfamily member 1A (TNF-R1), which activate inhibitor of nuclear factor kappa-B kinase subunit alpha (IKK), phosphorylate NF-kappa-B inhibitor alpha (IκBα) and promote its ubiquitination, which ultimately activates NF-κB to enter the nucleus for transcriptional regulation (Vallabhapurapu & Karin,

2009). The non-canonical pathways rely on IKK homodimer and NF-κB induced kinase (NIK) (Dejardin, 2006; Senftleben et al., 2001).

In this study, interleukin 1 alpha (IL-1) and TNFα, as the signature pro-inflammatory cytokines, were significantly upregulated at 6h, indicating that the inflammatory response was triggered at the early stage of infection. Proinflammatory cytokines IL-1R and TNF-R1 were significantly up-regulated at 6h, while IKK, IκBα and NF-κB were significantly up-regulated, and NIK did not show any significant differences. At 6h, proinflammatory cytokines IL-1R and TNF-R1 were significantly up-regulated, IKK, IκBα and NF-κB were significantly up-regulated, while NIK did not change significantly. The present results suggest that the *Edwardsiella* may activate NF-κB through the canonical pathway. Then the activated NF-κB enters the nucleus and binds to the associated genetic elements, regulating the expression of a wide range of anti-apoptotic molecules such as Bcl-2-like 1 (Bcl-XL), apoptosis regulator BAX (Bax) (Chen & Chen, 2013; DiDonato et al., 1997) . In mammals, NF-κB can regulate the anti-apoptotic molecules Bcl-XL to inhibit cell apoptosis. And Guoqin Qi et al. have preliminarily confirmed that Bcl-XL

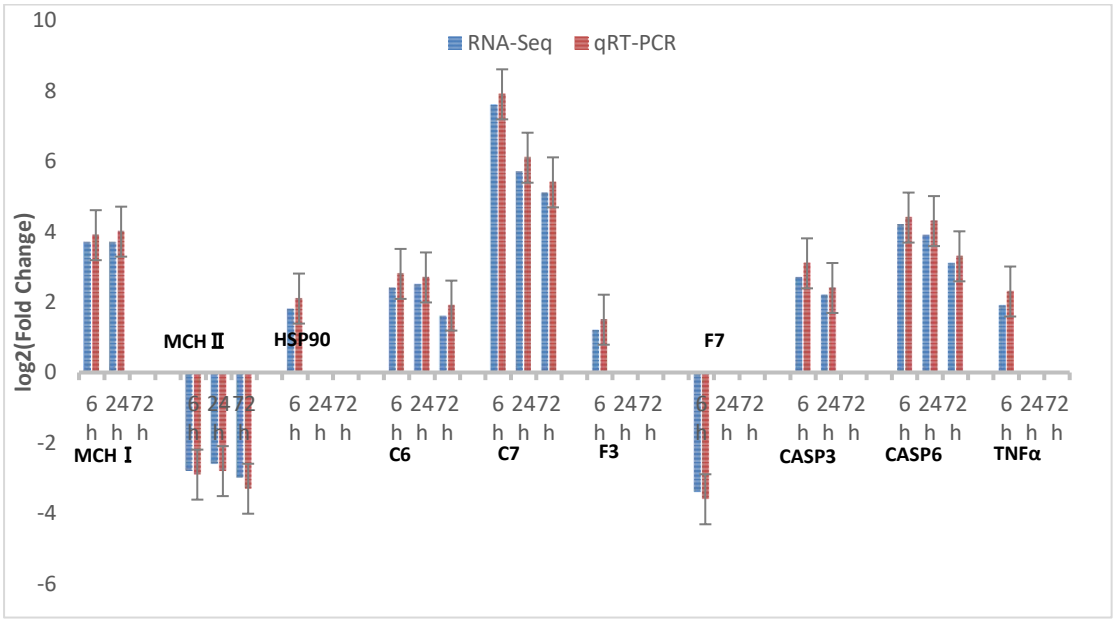

Figure 5. Comparison of the expressions of RNA-Seq and qRT-PCR results at each time point. The transcript expression levels of the selected genes were each normalized to that of the β-actin gene.

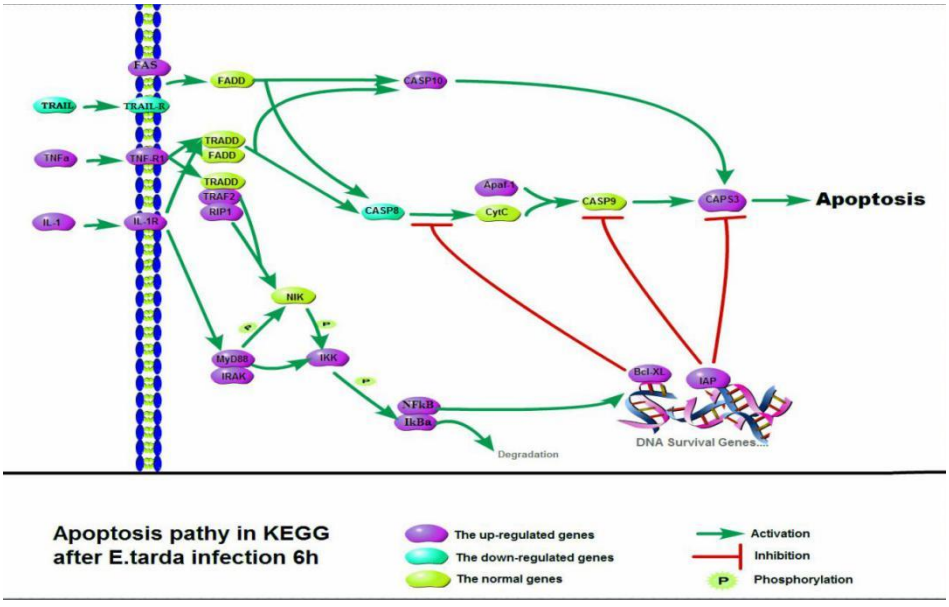

Figure 6. Pathway diagram of KEGG cell apoptosis. Apoptosis pathway 6 h after *E.tarda* infection.

of grass carp is involved in the apoptotic signaling pathway of NF-κB (Qi et al., 2020). In our study, both NF-κB and Bcl-XL were up-regulated at 6h. Therefore, we propose that *Edwardsiella tardiness* intervenes cell apoptosis of yellow catfish through NF-κB pathway in the initial immune stage.

Moreover, the activities of the caspase were rapidly decreased at 72 h upon *E. tarda* treament. Combined with our previous experiment (Yang et al., 2019), which showed the spleen of yellow catfish was severely damaged at 72 h after being injected with *E. tarda*. Thus, we may speculate that the spleen is an important potential target organ for *E. tarda* in yellow catfish and *E. tarda*-induced apoptosis may result in depressing immune cell, eventually disturbing the immune response of yellow catfish (Table 4 and Figure 6).

Complement and Coagulation Cascades

The complement system is the bridge between innate and adaptive immune responses against pathogen invasion. The three complement pathways (classical, lectin, and alternative pathways) converge to the lytic pathway, opsonization or direct killing of pathogens. In our results, complement component (3b/4b) receptor 1 (C1R), C6 and C7 showed up-regulation at 6 h and 24 h, indicating the activation of classical pathway. At 24 h and 72 h, C3 was up-regulated, indicating the alternative pathway was activated. The differential genes expression analyses showed that upon exposure to *E. tarda* complement factor responses were higher at 24 h compared to 6 h and 72 h. This result suggested that the complement system tagged and killed invading microbes and promoted inflammatory responses mainly at 24 h. In summary, our results suggest that the complement system recognizes the pathogenic bacterium and activates subunits of the membrane attack complex (MAC), which is a prerequisite for formation of a MAC complex at the surface of the microbe and thereafter cell lysis (Markiewski et al., 2007; Nakao et al., 2011). Moreover, complement receptors involved in phagocytosis, degranulation and chemotaxis such as C6, C7, C5a anaphylatoxin chemotactic (C5R1) and CR1 were upregulated, which are related to the need to recruit host phagocytic cells for clearance of the bacterium. On the other hand, the coagulation pathway is a complex process to stop blood loss from injury through a series of sequentially activated serine kinases (Palta et al., 2014). In our study, several factors, such as tissue factor pathway inhibitor (TFPI), fibrinogen alpha chain (FG), and urokinase plasminogen activator (PLAU), were significantly up-regulated at 6 h and 24 h, revealing the inhibition of blood coagulation, (Lin et al., 2011; Winslow et al., 2007). This may be related to the haemorrhagic septicaemia observed in yellow catfish caused by *E. tarda* infection. In summary, all these molecules were observed after *E. tarda* challenge, implying their potential roles in immune responses of yellow catfish (Table 4).

Antigen Processing and Presentation

Antigen processing refers to the antigen exposed to the surface of the presenting cells in the form of immune peptides by antigen presenting cell uptake, after which it is identified by the immunocompetent cells. This process, as the initial stage of the immune response, involves a variety of cellular mechanisms, including complement and coagulation cascades, apoptosis, and finally triggers the immune response. The two major classes of MHC molecules execute this function differently. For MHC class I molecules, the goal is to report on intracellular events (such as viral infection, the presence of intracellular bacteria or cellular transformation) to CD8+ T cells (Germain & Margulies, 1993; Vyas et al., 2008). In MHC-I antigen processing and presentation pathway, MHC I, TAP binding protein (TAPBP), and beta-2-microglobulin (β2M) was up-regulated at 6 h. In addition, several important genes related to cytotoxic T lymphocytes (CTL), including TNFα, proteasome activator subunit 1 (PA28), HSP90, also showed varying degrees of up-regulation before 24h, further confirming the induction of CTL effector function. For MHC class II molecules, the goal is to sample the extracellular milieu and present antigens to CD4+ T cells4. In this study, MHC-II and CD4 showed a continuously down-regulated trend during the whole immunization phase. Therefore, we speculated that *E. tarda* might inhibit the function of MHC-II. Activated cytotoxic T lymphocytes released toxic particles such as perforin to kill host cells infected with *E.tarda*. This suggests that the antigen processing and presentation signaling pathway may serve as a bridge to communicate the complement and coagulation cascades and apoptosis signaling pathway and plays an essential role in fish early immune system (Table 4).

Conclusion

This study provided the first glimpse regarding the transcriptome of yellow catfish immune response upon *E. tarda* infection at three time points. A total of 158,124 unigenes were identified, including differentially-expressed genes related to immune responses, especially those in the antigen processing and presentation pathway, complement and coagulation cascades pathway, and apoptosis pathway. These pathways contribute to anti-bacterial responses after *E. tarda* challenge in yellow catfish. Overall, the data offers a platform to understand the molecular defense mechanisms underlying yellow catfish - *E. tarda* interactions, as well as to develop strategies for efficient immune protection against *E. tarda* infection to improve the yellow catfish aquaculture industry.

Table 4. List of the differently expressed genes (DEGs) involved in immune responses.

Gene name	Description	Log2 Fold	Diff	Log2 Fold	Diff	Log2 Fold	Diff
	Antigen processing and presentation	6h		24h		72h	
TNFα	tumor necrosis factor alpha	1.9	up	-	-	-	-
PA28	proteasome activator subunit 1 (PA28 alpha)	1.4	up	1.2	up	-	-
HSP90	heat shock protein 90	1.8	up	-	-	-	-
CANX	calnexin	1.5	up	-	-	-	-
MHC Ⅰ	major histocompatibility complex, class I	3.7	up	3.7	up	-	—
CALR	calreticulin	1.3	up	-	-	-	-
β2M	beta-2-microglobulin	1.9	up	-	-	-	-
TAPBP	TAP binding protein (tapasin)	1.6	up	-	-	-	-
TAP1/2	ATP-binding cassette, subfamily B (MDR/TAP), member 2	2.2	up	1.3	up	-	-
TCR	T cell receptor alpha chain V region	-2.4	down	-4.3	down	4.3	up
AEP	legumain	4.9	up	-	-	-	-
CTSB	cathepsin B	-1.8	down	-	-	-	-
MHC Ⅱ	major histocompatibility complex, class II	-2.8	down	-2.6	down	-3.0	down
CTSB/L/S	cathepsin B	6.7	up	3.9	up		
CD4	T-cell surface glycoprotein CD4	-2.3	down	-1.7	down	-	-
CIITA	class II, major histocompatibility complex, transactivator	-4.5	down	-	-	-	-
RFX	regulatory factor X 5	-2.0	down	-	-	-	-
Ii	CD74 antigen	-	-	-1.3	down	-	-
SLIP	CD74 antigen	-	-	-1.3	down	-	-
CLIP	CD74 antigen	-	-	-1.3	down	-	-
NFY	nuclear transcription factor Y, alpha	-	-	-4.8	down	-	-
	Complement and coagulation cascades	6h		24h		72h	
TFPI	tissue factor pathway inhibitor	1.2	up	1.7	up	-	-
F3	coagulation factor III (tissue factor)	1.2	up	-	-	-	-
F7	coagulation factor VII	-3.4	down	-	-	-	-
F10	coagulation factor X	2.4	up	2.4	up	2.6	up
F8	coagulation factor VIII	8.4	up	5.0	up	3.5	up
FG	fibrinogen alpha chain	5.8	up	3.6	up	-	-
A2M	alpha-2-macroglobulin	-6.1	down	-2.5	down	-4.6	down
THBD	thrombomodulin	1.3	up	-	-	-	-
SERPINE1	plasminogen activator inhibitor 1	5.4	up	2.9	up	1.6	up
PLAU	urokinase plasminogen activator	5.4	up	2.9	up	1.2	up
PLAUR	plasminogen activator, urokinase receptor	-3.1	down	-2.6	down	-	-
PLG	plasminogen	-3.8	down	-	-	-	-
BDKR	bradykinin receptor B1	5.1	up	4.0	up	2.5	up
HF1	complement factor H	1.6	up	2.9	up	2.8	up
MCP	membrane cofactor protein	4.0	up	4.1	up	-	-
CD59	CD59 antigen	6.3	up	4.7	up	4.1	up
C6	complement component 6	2.4	up	2.5	up	1.6	up
C7	complement component 7	7.6	up	5.7	up	5.1	up
C1Q	complement component 1q	-1.7	down	1.2	up	-	-
C1R	complement C1r subcomponent	2.0	up	2.4	up	-	-
C1S	complement component 1s	2.6	up	-	-	-	-
C4BP	complement component 4 binding protein, alpha	3.6	up	3.5	up	-	-
C5R1	C5a anaphylatoxin chemotactic receptor 1	2.3	up	2.2	up	-	-
CR1	complement component (3b/4b) receptor 1	3.8	up	3.1	up	-	-
C3	complement component 3	-	-	2.2	up	2.0	up
C4	complement component 4	-	-	1.2	up	-	-
DF	component factor D	-	-	-	-	1.9	up
	Apoptosis	6h		24h		72h	
TRAIL	tumor necrosis factor ligand superfamily member 10	-1.6	down	-	-	-	-
TNFα	tumor necrosis factor superfamily, member 2	1.9	up	-	-	-	-
IL-1	interleukin 1 alpha	9.0	up	5.7	up	3.6	up
Fas	tumor necrosis factor receptor superfamily member 6	2.2	up	1.2	up	-	-

Table 4. List of the differently expressed genes (DEGs) involved in immune responses (continued).

Gene name	Description	Log2	Gene name	Description	Log2	Gene name	Description
IL-1R	interleukin 1 receptor type I	4.1	up	2.9	up	1.8	up
RIP1	receptor-interacting serine/threonine-protein kinase 1	1.1	up	-	-	-	-
TRAF2	TNF receptor-associated factor 2	5.1	up	3.3	up	1.3	up
MyD88	myeloid differentiation primary response protein MyD88	1.8	up	-	-	-	-
IRAK	interleukin-1 receptor-associated kinase 1	2.8	up	2.2	up	-	-
FLIP	CASP8 and FADD-like apoptosis regulator	1.3	up	-	-	-	-
Bcl-2/XL	apoptosis regulator Bcl-2	3.1	up	-	-	-	-
CASP10	caspase 10	2.5	up	-	-	-	-
CASP8	caspase 8	-3.3	down	-	-	-	-
CASP3	caspase 3	2.7	up	2.2	up	-	-
CASP7	caspase 7	2.1	up	1.4	up	-	-
CASP6	caspase 6	4.2	up	3.9	up	3.1	up
IAP	baculoviral IAP repeat-containing protein 2/3	1.4	up	-	-	-	-
DFF40	DNA fragmentation factor, 40 kD, beta subunit	-1.7	down	-	-	-	-
AIF	apoptosis-inducing factor 1	1.7	up	-	-	2.2	up
Apaf-1	apoptotic protease-activating factor	1.2	up	-	-	-	-
IKK	inhibitor of nuclear factor kappa-B kinase subunit alpha	1.1	up	-5.0	down	1.2	up
IκBα	NF-kappa-B inhibitor alpha	2.0	up	-	-	-	-
NF-κB	nuclear factor NF-kappa-B p105 subunit	1.1	up	-	-	-	-
Bcl-XL	Bcl-2-like 1	3.1	up	-	-	-	-
NGF	nerve growth factor, beta	1.9	up	2.3	up	-	-
IL-3R	interleukin 3 receptor alpha	3.5	up	-	-	-	-
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit	-3.5	down	2.0	up	-	-
PKA	protein kinase A	-4.2	down	-3.8	down	-	-
Bax	apoptosis regulator BAX	2.1	up	1.6	up	1.1	up
p53	tumor protein p53	3.3	up	2.5	up	-	-
Calpain	calpain-1	3.2	up	-	-	-	
TRAIL-R	tumor necrosis factor receptor superfamily member 10A/B	-4.2	down	-	-	-	-
TNF-R1	tumor necrosis factor receptor superfamily member 1A	2.6	up	2.3	up	-	-
IL-1R	interleukin 1 receptor type I	4.1	up	2.9	up	1.8	up
RIP1	receptor-interacting serine/threonine-protein kinase 1	1.1	up	-	-	-	-
TRAF2	TNF receptor-associated factor 2	5.1	up	3.3	up	1.3	up
MyD88	myeloid differentiation primary response protein MyD88	1.8	up	-	-	-	-
IRAK	interleukin-1 receptor-associated kinase 1	2.8	up	2.2	up	-	-
FLIP	CASP8 and FADD-like apoptosis regulator	1.3	up	-	-	-	-
Bcl-2/XL	apoptosis regulator Bcl-2	3.1	up	-	-	-	-
CASP10	caspase 10	2.5	up	-	-	-	-
CASP8	caspase 8	-3.3	down	-	-	-	-
CASP3	caspase 3	2.7	up	2.2	up	-	-
CASP7	caspase 7	2.1	up	1.4	up	-	-
CASP6	caspase 6	4.2	up	3.9	up	3.1	up
IAP	baculoviral IAP repeat-containing protein 2/3	1.4	up	-	-	-	-
DFF40	DNA fragmentation factor, 40 kD, beta subunit	-1.7	down	-	-	-	-
AIF	apoptosis-inducing factor 1	1.7	up	-	-	2.2	up
Apaf-1	apoptotic protease-activating factor	1.2	up	-	-	-	-

Table 4. List of the differently expressed genes (DEGs) involved in immune responses (continued).

Gene name	Description	Log2 Fold	Diff	Log2 Fold	Diff	Log2 Fold	Diff
IKK	inhibitor of nuclear factor kappa-B kinase subunit alpha	1.1	up	-5.0	down	1.2	up
IκBα	NF-kappa-B inhibitor alpha	2.0	up	-	-	-	-
NF-κB	nuclear factor NF-kappa-B p105 subunit	1.1	up	-	-	-	-
Bcl-XL	Bcl-2-like 1	3.1	up	-	-	-	-
NGF	nerve growth factor, beta	1.9	up	2.3	up	-	-
IL-3R	interleukin 3 receptor alpha	3.5	up	-	-	-	-
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit	-3.5	down	2.0	up	-	-
PKA	protein kinase A	-4.2	down	-3.8	down	-	-
Bax	apoptosis regulator BAX	2.1	up	1.6	up	1.1	up
p53	tumor protein p53	3.3	up	2.5	up	-	-
Calpain	calpain-1	3.2	up	-	-	-	

"-" represents the gene was not significantly different.

Ethical Statement

Animal experiments were carried out according to animal welfare standards and approved by the Ethical Committee for Animal Experiments of Foshan University. All animal experiments complied with the guidelines of the Animal Welfare Council of China.

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Author Contribution

Ying Yang and Jun Zhou finished the experiments and wrote the article. Hui Yu gave the idea and experimental support. Pengfei Mao and Jiafang Xie provided assistance and guidance throughout the research. All authors assisted in writing the article and manuscript editing.

Conflict of Interest

The authors declare no conflicts of interest.

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