INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–0403/2020/24–4–783–793
DOI: 10.17957/IJAB/15.1501
http://www.fspublishers.org

Full Length Article

Transcriptome Signatures Focusing on Immune-Related Genes in the Livers, Spleens and Kidneys of Male and Female *Takifugu rubripes*

Jun Cui[1,3], Lei Men[2], Zhicheng Wang[1], Xuemei Qiu[1] and Xiuli Wang[1*]
[1]*College of Fisheries and Life Science, Dalian Ocean University, Dalian, 116023, China*
[2]*College of Life Science, Dalian Minzu University, Dalian, 116600 China*
[3]*School of Bioengineering, Dalian University of Technology, Dalian 116024, China*
[*]For correspondence: xiuliwang417@sina.com
Received 14 March 2020; Accepted 07 May 2020; Published 16 August 2020

Abstract

To obtain information regarding immune-related genes and provide a genetic basis for the immune response in fish, we performed transcriptomic comparison analysis of the livers, spleens and kidneys of male and female *Takifugu rubripes*. The GO terms associated with cell surface receptor signaling were the most significantly enriched, and among the KEGG pathways, cytokine-cytokine receptor interaction, an immune-related pathway, was present in all the male groups. In addition, expression level of *complement C1q tumor necrosis factor-related protein 4-like* (CTRP4-like) gene was upregulated in all the male samples, while the expression levels of *voltage-dependent calcium channel subunit alpha-2/delta-2-like* (CACNA2D2-like) and *protein L-Myc-1b-like* genes were upregulated in all the female samples. These results demonstrate the regulatory mechanisms of immune-related genes and provide candidates for the enhancement of pathogen resistance in *T. rubripes.*

Keywords: *Takifugu rubripes*; Immune-related genes; Liver; Spleen; Kidney; RNA-Seq

Introduction

Pufferfish, *Takifugu rubripes* is one of marine commercial fishes in Asia (Hamasaki *et al*. 2017; Kong *et al*. 2019). This species has high nutritional and economic value. In China, its production is approximately 70% of the total global production per year, and most are exported to Japan and Korea (Guo *et al*. 2017). However, various diseases caused by *Edwardsiella tarda*, *Flexibacter maritimus*, *Myxococcus* sp. and red sea bream iridovirus, have hindered its production and caused economic losses (Cui *et al*. 2014a; Ma *et al*. 2014). Therefore, it is urgent and significant to understand molecular immune-related mechanisms of *T. rubripes*.

RNA-Seq sequencing has developed rapidly over the past decade and has been widely used for transcriptome expression profiling (Zhou *et al*. 2016a, 2019), regulatory network construction (Zhao *et al*. 2016; Wang *et al*. 2018), noncoding RNA identification (Cui *et al*. 2017, 2020; Jiang *et al*. 2018), genetic diversity determination (Cui *et al*. 2014a; Zhou *et al*. 2016b) and other applications. RNA-Seq allows the identification of many immune-related genes and elucidation of mechanisms of immune evasion in fishes. For example, after infection with *Aeromonas hydrophila*, 2,900 differentially expressed genes (DEGs) are identified from common carp spleen, and these genes are mainly involved in immune response (Jiang *et al*. 2016). During channel catfish-*Flavobacterium columnare* interaction, the RNA-Seq results have shown that fish gill is involved in the immune response to the pathogen (Peatman *et al*. 2013; Sudhagar *et al*. 2018). Meanwhile, some defense-related genes and pathways have been identified form *Lateolabrax japonicus* and *Cynoglossus semilaevis* challenged with *Vibrio anguillarum* (Zhang *et al*. 2015; Zhao *et al*. 2016), *Larimichthys crocea*, *Megalobrama amblycephala* and *Ctenopharyngodon idella* challenged with *A. hydrophila* (Mu *et al*. 2010; Tran *et al*. 2015; Yang *et al*. 2016; Song *et al*. 2017), tilapia challenged with *Streptococcus agalactiae* and *S. iniae* (Zhu *et al*. 2017; Wang *et al*. 2016) and zebrafish challenged with *Salmonella typhimurium*, *E. tarda* and *Mycobacterium marinum* (Stockhammer *et al*. 2010; Ordas *et al*. 2011; Yang *et al*. 2012; Chen *et al*. 2017) by RNA-Seq.

In fishes, immune organs include liver, spleen, kidneys and gills. In our previous study, many immune-related genes and pathways have been found in the gills of *T. rubripes* (Cui *et al*. 2014b), and it is also found that these immune-related genes contain a number of single-nucleotide polymorphisms (SNPs) (Cui *et al*. 2014c). In addition, fish livers, spleens and kidneys, as important immune organs, play important roles in the response of fish against various pathogens. Chemokines are key immune regulators, acting as

bridges between innate and adaptive immunity. Some catfish β and α chemokines are responsive to *E. ictaluri* infection in the lever (Fu *et al*. 2017a, b). Meanwhile, fish toll-like receptors, which are important immune-related proteins, can recognize pathogen-associated molecular patterns. During *A. hydrophila* challenge, the expression levels of genes encoded toll-like receptors are significantly changed in the kidneys and spleen of common carp, suggesting that the toll-like receptor genes affect in the host immune response to *A. hydrophila* infection (Gong *et al*. 2017).

Previous studies have shown that immune responses are different between males and females, which likely contributes to differences in the response of the two sexes to pathogens (Pennell *et al.* 2012; Klein *et al.* 2015). Females exhibit lower burdens of pathogen infection (Pennell *et al.* 2012). In previous studies, it has been found that a number of immune-related genes are differentially expressed between males and females. For example, the expression levels of some cytokines genes, including TNF-α, IL-1β, IL-6 and CXCL10, are more up-regulated in male mice than in females (Kahlke *et al*. 2000; Asai *et al*. 2001; Moxley *et al*. 2002; Marriott *et al*. 2006; Klein *et al.* 2015). However, the studies on the differences between the immune responses of male and female fishes are limited.

In this study, RNA-Seq is performed to investigate the differences in genome-wide gene expression in important organs, namely, livers, spleens and kidneys, between male and female *T. rubripes*. Sets of DEGs were identified. The important pathways and key immune-related genes are screened by Gene Ontology (GO), KEGG pathway enrichment and protein-protein interaction (PPI) network analysis. These results will not only provide insight into the molecular immune-related mechanisms of *T. rubripes* but also help future molecular breeding.

Materials and Methods

Ethics statement

All the fish experiments were conducted in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology. This study was approved by the Animal Care and Use Committee of the College of Fisheries and Life Science, Dalian Ocean University. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Sample collection

A total of 20 *T. rubripes* (10 males and 10 females) were sampled from Dalian Tianzheng Industrial Co., Ltd. (Dalian, China). Each individual was 15–18 cm long. *T. rubripes* were transferred to experimental tanks. These individuals were kept in sea water at 20℃ for 7 days to acclimate them to the experimental conditions before the study. The livers, spleens and kidneys of these fish were collected. The same tissue types from male or female fish were pooled. The tissues were placed in RNAlater (Ambion), stored at room temperature for 24 h, and then moved to -80℃ for storage until RNA isolation.

RNA extraction, library construction and sequencing

Total RNA of the livers, spleens and kidneys was extracted using RNAiso Plus (TaKaRa) according to the manufacturer's instructions. Total RNA was sent for next generation-sequencing to Biomarker Biotechnology Corporation (Beijing, China). The library was sequenced on an Illumina HiSeq 2000 with 101-bp paired-end reads.

Read mapping

Based on the method of Kong *et al*. (2018), the low-quality reads were removed. The clean reads mapped to the *T. rubripes* genome (https://www.ncbi.nlm.nih.gov/genome/63?genome_assembly_id=22739) by TopHat.

Identification of new genes

To improve the annotation of the *T. rubripes* genome, we used the RNA-Seq data to identify new genes. First, Cufflinks was used to assemble all the mapped reads and map them to the genome. Then, sequences encoding short peptide chains (less than 50 amino acid residues) and those containing single exons were removed. Lastly, the new genes identified above were used as query sequences to search the Pfam, NR, eggnog, KOG, Swiss-Prot, GO, KEGG databases, and COGusing BLASTX.

Analysis of DEGs

The expression levels of the genes were measured as read counts normalized to the respective lengths of the genes using Cufflinks. DEGs of nine groups, namely, ♂liver *vs.* ♂spleen, ♂liver *vs*. ♂kidney, ♂spleen *vs*. ♂kidney, ♀liver *vs*. ♀spleen, ♀liver *vs.* ♀kidney, ♀spleen *vs*. ♀kidney, ♂liver *vs*. ♀liver, ♂spleen *vs.* ♀spleen and ♂kidney *vs.* ♀kidney, were identified using DEGseq with a false discovery rate (FDR) < 0.01 and fold change > 2 or < -2.

Gene ontology (GO) and KEGG enrichment analyses

We used the GOseq R packages to conduct GO enrichment analysis of the DEGs (Young *et al.* 2010), and KOBAS software was used to perform the KEGG pathway enrichment analysis (Mao *et al.* 2005; Kanehisa *et al*. 2008).

Protein-Protein interaction (PPI) analysis

The sequences of the DEGs were compared by BLASTX against the genome of a related species (information about

the PPIs of which can be found in the STRING database: http://string-db.org/) to obtain the predicted PPIs associated with these DEGs. Then, the PPIs associated with these DEGs were visualized using Cytoscape (Shannon *et al*. 2003).

Results

RNA-Seq data from the livers, spleens and kidneys of *T. rubripes*

RNA-Seq was performed on six RNA samples (♂liver, ♂spleen, ♂kidney, ♀liver, ♀spleen and ♀kidney) from *T. rubripes*. A total of 274,074,082 clean reads were generated (Table 1). We obtained clean reads for approximately 41 Gb in total. The number of clean sequences from each library ranged from 41,722,196 to 52,936,660. The Q30 value of each library was more than 92%. The accession number of all clean data in the NCBI were SRR6925615, SRR6925526, SRR6925454, SRR6925406, SRR6925404 and SRR6925377.

Mapping to *T. rubripes* genome and identification of new genes

TopHat was used to align all the clean reads to *T. rubripes* genome (Trapnell *et al*. 2009). The mapping ratio ranged from 81 to 84% (Table 1). The number of unique mapped reads from ♂liver, ♂spleen, ♂kidney, ♀liver, ♀spleen and ♀kidney samples were 42,013,959, 34.293,686, 35,711,201, 33,536,438, 36,814,376 and 35,030,165, respectively. Sets of unique mapped reads were mapped to exons (88.09%/84.76%/86.43%/88.68%/85.39%/85.39%, ♂liver/♂spleen/♂kidney/♀liver/♀spleen/♀kidney), introns (6.17%/8.48%/7.03%/5.9%/7.97%/7.8%, ♂liver/♂spleen/♂kidney/♀liver/♀spleen/♀kidney) and intergenic regions (5.74%/6.76%/6.54%/5.42%/6.64%/6.81%, ♂liver/♂spleen/♂kidney/♀liver/♀spleen/♀kidney) (Fig. 1).

In addition, after analysis of the assembly and mapping data, we identified 1,443 new genes which were annotated by a BLASTX search against eight databases, namely, NR, Swiss-Prot, GO, COG, KOG, Pfam, eggNOG and KEGG. A total of 1,041 new genes had significant hits in at least one database.

DEGs analysis

The FPKM value of each gene was used to represent the normalized expression value. Scaling normalization of the count data indicated equal variation in the data for the transcriptomes of the six samples, as indicated in Fig. 2. A total of 3,673, 3,919, 2,912, 3,912, 3,840, 2,971, 369, 323 and 263 genes showed significantly differential expression in nine groups, namely, ♂liver *vs.* ♂spleen, ♂liver *vs.* ♂kidney, ♂spleen *vs.* ♂kidney, ♀liver *vs.* ♀spleen, ♀liver *vs.* ♀kidney, ♀spleen *vs.* ♀kidney, ♂liver *vs.* ♀liver, ♂spleen *vs.* ♀spleen and ♂kidney *vs.* ♀kidney (Table 2),

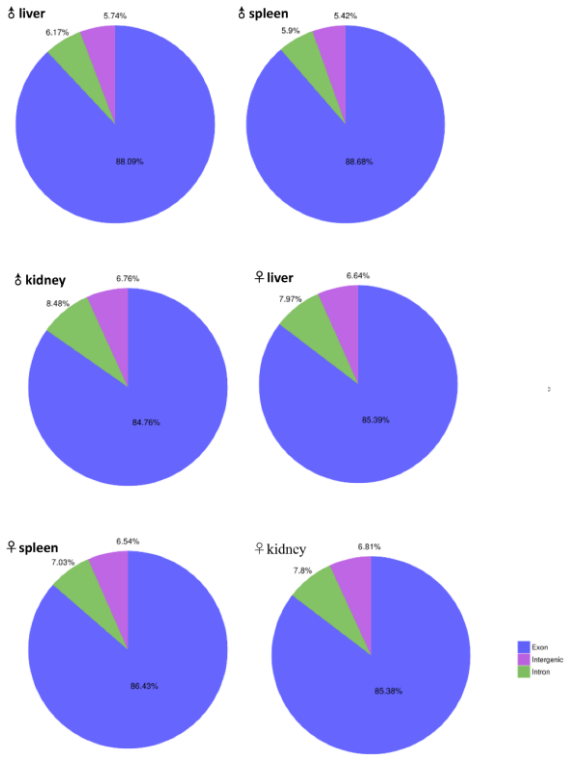

Fig. 1: Unique reads mapped to various genomic regions

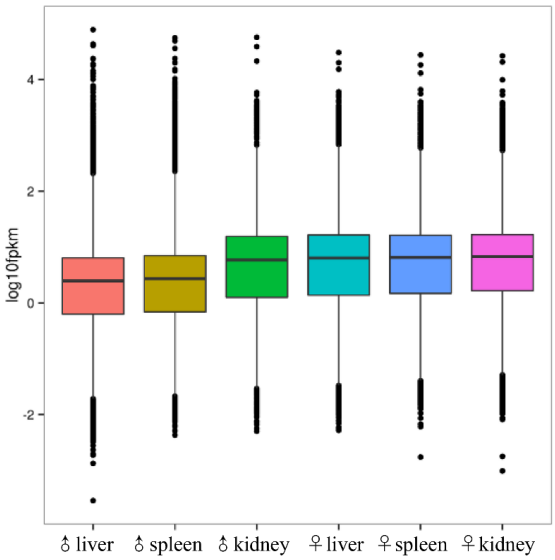

Fig. 2: Scaling normalization box plot of the gene mapping counts in the six samples. The similarity in transcript expression and variability of the six corresponding transcriptome libraries indicates that these libraries were comparable for the identification of DEGs at the transcriptome level. The *y*-axis represents the logarithm of the FPKM value. The *x*-axis represents the samples

in which 3,555, 3,840, 2,844, 3,815, 3,754, 2,911, 352, 307 and 252 DEGs, respectively, were annotated by a BLASTX search against eight databases. The Fig. 3 showed that MA

Table 1: Statistical data of RNA-Seq reads for six samples

Sample	Clean reads	Clean bases	Q30 (%)	Mapped Reads	Unique Mapped Reads
♂liver	52,936,660	7,920,730,888	92.55	44,708,721 (84.46%)	42,013,762 (79.37%)
♂spleen	43,129,860	6,439,115,608	92.58	35,581,354 (82.50%)	34,293,686 (79.51%)
♂kidney	45,024,504	6,723,621,318	92.53	36,914,930 (81.99%)	35,711,201 (79.32%)
♀liver	41,722,196	6,248,786,778	92.65	35,299,378 (84.61%)	33,536,438 (80.38%)
♀spleen	46,960,728	7,033,459,874	92.27	38,094,434 (81.12%)	36,814,376 (78.39%)
♀kidney	44,300,134	6,626,705,206	92.44	36,099,012 (81.49%)	35,030,165 (79.07%)

Table 2: Number of differentially expressed genes in immune organs

DEG set	DEG number	Up-regulated	Down-regulated
♂liver *vs.* ♂spleen	3,673	1,067	2,606
♂liver *vs.* ♂kidney	3,919	1,691	2,228
♂spleen *vs.* ♂kidney	2,912	1,855	1,057
♀liver *vs.* ♀spleen	3,912	1,417	2,495
♀liver *vs.* ♀kidney	3,840	1,830	2,010
♀spleen *vs.* ♀kidney	2,971	2,037	934
♂liver *vs.* ♀liver	369	172	197
♂spleen *vs.* ♀spleen	323	137	186
♂kidney *vs.* ♀kidney	263	175	88

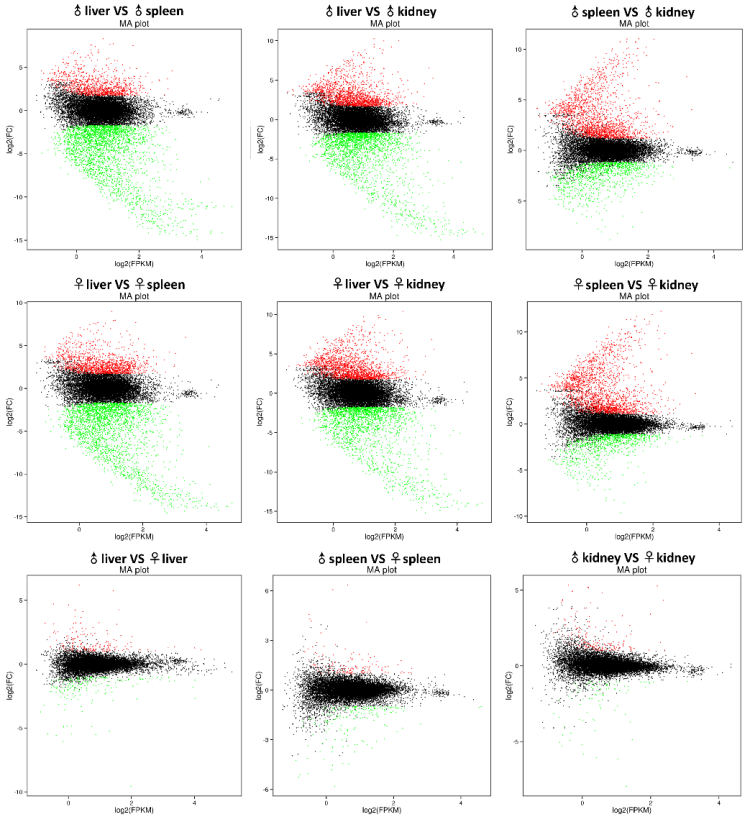

Fig. 3:MA (log ratios–mean average) plots showing gene expression. The *y*-axis represents the log fold change, and the *x*-axis represents the log transcript counts. M is the log ratio of the two dyes used in the hybridization experiment, and A is the average of the log intensities. Red, green and black points represent upregulated, down-regulated and normally expressed genes, respectively

plots were drafted using "eps" format files. Compared to ♂spleen and ♂kidney, 1,067 and 1,691 DEGs, respectively, were upregulated and 2,606 and 2,228 DEGS, respectively, were downregulated in ♂liver. Between ♂spleen and ♂kidney, 1,855 DEGs were upregulated in ♂spleen and 1,057 DEGs were upregulated in ♂kidney. Meanwhile, compared with ♀spleen and ♀kidney, 1,417 and 1,830 DEGs were upregulated, respectively, and 2,495 and 2,010 DEGs were downregulated, respectively, in ♀liver. In ♀spleen *vs.* ♀kidney, 2,037 DEGs were upregulated in ♀spleen and 934 were upregulated in ♀kidney.

In addition, compared to female *T. rubripes*, 172, 137 and 175 DEGs were upregulated in the livers, spleens and kidneys of male *T. rubripes*. A Venn diagram of the DEGs showed that a majority of these genes were not shared by these nine groups. Only 553 DEGs were present in ♂liver *vs*. ♂spleen, ♂liver *vs*. ♂kidney and ♂spleen *vs*. ♂kidney, and 621 DEGs were present in ♀liver *vs*. ♀spleen, ♀liver *vs*. ♀kidney and ♀spleen *vs*. ♀kidney (Fig. 4). In addition, the DEGs were not shared between males and females; 323, 271 and 210 DEGs were present in only ♂liver *vs*. ♀liver, ♂spleen *vs*. ♀spleen and ♂kidney *vs*. ♀kidney, respectively (Fig. 4); 547 were found in only ♂liver *vs*. ♂spleen; and 786 DEGs were found in only ♀liver *vs*. ♀spleen (Fig. 4). Meanwhile, 808 and 710 DEGs were found in only ♂liver *vs*. ♂kidney and ♂spleen *vs*. ♂kidney, respectively, and 729 and 739 DEGs were found in only ♀liver *vs*. ♀kidney and ♀spleen *vs*. ♀kidney, respectively (Fig. 4).

GO enrichment analysis

GO enrichment analysis was performed, and the most significant enriched GO terms under Biological Process (BP) and Cellular Component (CC) were "G-protein coupled receptor signaling pathway (GO:0007186)" and "integral component of membrane (GO:0016021)" in ♂liver *vs*. ♂spleen, ♂liver *vs*. ♂kidney and ♂spleen *vs*. ♂kidney. On the other hand, the most significant enriched GO term under Molecular Function (MF) was "G-protein coupled receptor activity (GO:0004930)" in ♂liver *vs*. ♂spleen and ♂spleen *vs*. ♂kidney and "olfactory receptor activity (GO:0004984)" in ♂liver *vs*. ♂kidney. The enriched BP GO terms "one-carbon metabolic process (GO:0006730)" and "alpha-amino acid catabolic process (GO:1901606)" were present in only ♂liver *vs*. ♂spleen, and "regulation of cyclin-dependent protein serine/threonine kinase activity (GO:0000079)" and "killing of cells of other organism (GO:0031640)" were present in only ♂spleen *vs*. ♂kidney. The CC GO terms "postsynaptic membrane (GO:0045211)" and "connexin complex (GO:0005922)" were significantly enriched in ♂liver *vs*. ♂spleen and ♂spleen *vs*. ♂kidney, respectively. The enriched MF GO term "electron carrier activity (GO:0009055)" was present in only ♂liver *vs*. ♂spleen, "cation-transporting ATPase activity (GO:0019829)" in only ♂liver *vs*. ♂kidney, and "structural constituent of cytoskeleton (GO:0005200)" and "voltage-gated potassium channel activity GO:0005249" in only ♂spleen *vs*. ♂kidney.

Meanwhile, in female samples, the most significant enrichment GO term of BP and CC was also "G-protein coupled receptor signaling pathway (GO:0007186)" and "integral component of membrane (GO:0016021)". While the most significant enrichment GO term of MF "G-protein coupled receptor activity (GO:0004930)" was in ♀liver *vs*. ♀kidney and ♀spleen *vs*. ♀kidney, and olfactory receptor activity (GO:0004984) was in ♀liver *vs*. ♀spleen. The enrichment GO terms of BP "alpha-amino acid catabolic process (GO:1901606)" and "transmembrane transport (GO:0055085)" were enriched in only ♀liver *vs*. ♀spleen and ♀spleen *vs*. ♀kidney, respectively; the GO terms of CC "proton-transporting V-type ATPase complex (GO:0033176)" and "intermediate filament (GO:0005882)" were only present in ♀liver *vs*. ♀kidney and ♀spleen *vs*. ♀kidney, respectively; The enrichment GO terms of MF "electron carrier activity (GO:0009055)" and "endopeptidase inhibitor activity (GO:0004866)" were only present in ♀liver *vs*. ♀spleen. "NADP binding (GO:0050661)" in only ♀liver *vs*. ♀kidney and "structural constituent of cytoskeleton (GO:0005200)" and "voltage-gated potassium channel activity GO:0005249" in only ♀spleen *vs*. ♀kidney.

The enriched GO terms were also compared between male and female *T. rubripes*. The enriched BP GO terms were not differentially expressed in ♂liver *vs*. ♀liver, ♂spleen vs. ♀spleen and ♂kidney *vs*. ♀kidney. The enriched CC GO term "myosin complex (GO:0016459)"and MF GO term "voltage-gated potassium channel activity (GO:0005249)" were present in only ♂spleen *vs*. ♀spleen.

KEGG pathway enrichment analysis

The top 20 pathways identified from each group by KEGG enrichment analysis are shown in Fig. 5. In ♂liver *vs*. ♂spleen, ♂liver *vs*. ♂kidney and ♂spleen *vs*. ♂kidney, the *q*-values of 20, 18 and 7 pathways, respectively, were lower than 0.05. The pathway categories "cytokine-cytokine receptor interaction" and "glycine, serine and threonine metabolism" were identified as being significantly enriched in ♂liver *vs*. ♂spleen, ♂liver *vs*. ♂kidney and ♂spleen *vs*. ♂kidney. "Fatty acid degradation", "fatty acid metabolism", "glycerolipid metabolism" and "tryptophan metabolism" were identified as significantly enriched categories in only ♂liver *vs*. ♂spleen; "one-carbon pool by folate" and "renin-angiotensin system" were significantly enriched in only ♂liver *vs*. ♂kidney; "carbon metabolism", "ECM-receptor interaction", "pentose phosphate pathway", "neuroactive ligand-receptor interaction", "pentose phosphate pathway" and "taurine and hypotaurine metabolism" were significantly enriched in only ♂spleen *vs*. ♂kidney.

In ♀liver *vs*. ♀spleen, ♀liver *vs*. ♀kidney and ♀spleen *vs*. ♀kidney, the *q*-values of 20, 16 and 6 pathways, respectively, were lower than 0.05. Only one category, namely, "glycine, serine and threonine metabolism", was significantly enriched in ♀liver *vs*. ♀spleen, ♀liver *vs*. ♀kidney and ♀spleen *vs*. ♀kidney. "Biosynthesis of unsaturated fatty acids", "carbon metabolism", "cytokine-cytokine receptor interaction", "fatty acid degradation", "glycerolipid metabolism" and "peroxisome" were significantly enriched in only ♀liver *vs*. ♀spleen; "porphyrin and chlorophyll metabolism" and "renin-angiotensin system" were significantly enriched in only ♀liver *vs*. ♀kidney; "cytokine-cytokine receptor interaction", "ECM-receptor interaction", "focal adhesion", "glutathione metabolism" and "primary bile acid biosynthesis" were significantly enriched in only ♀spleen *vs*. ♀kidney.

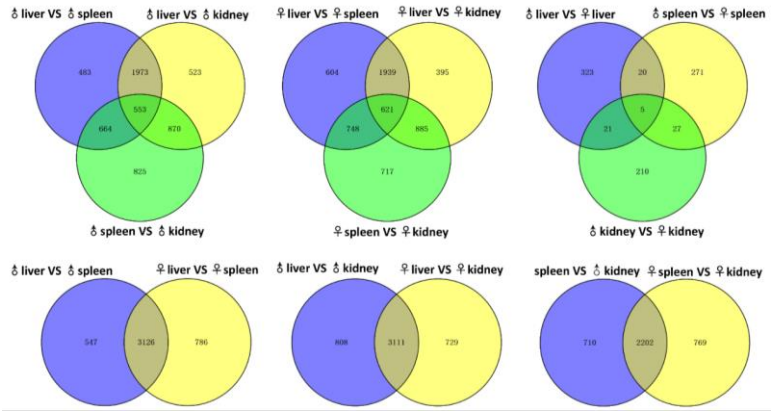

Fig. 4: Comparative Venn diagram of DEGs among different immune organs

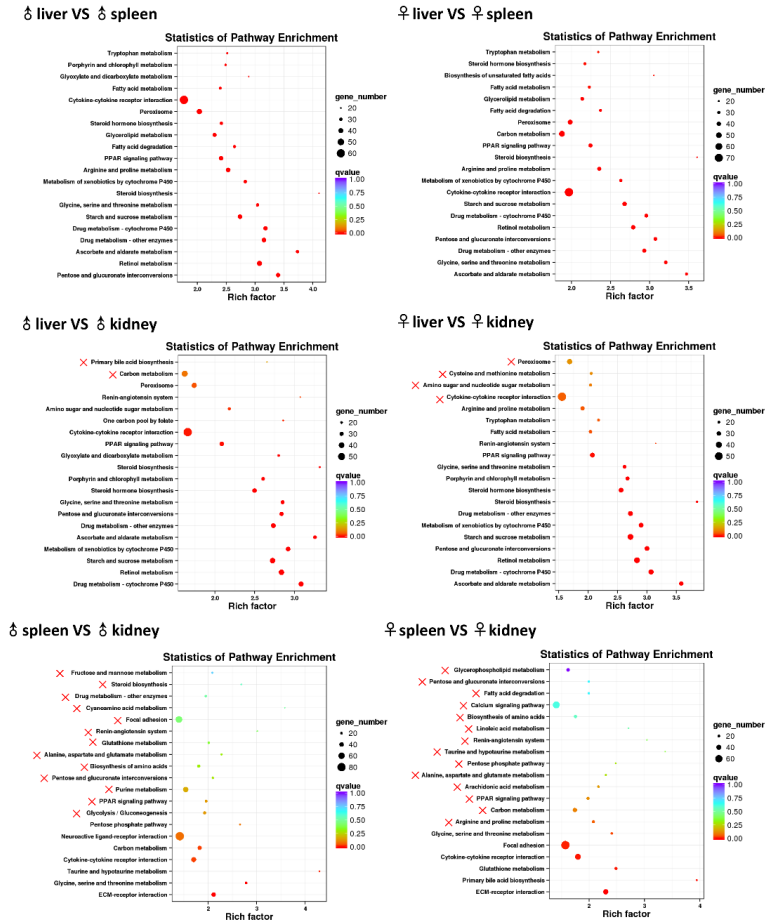

Fig. 5: Scatter diagram of the top 20 enriched KEGG pathways of the DEGs from six groups. A red cross indicates that the *q*-value of the pathway is lower than 0.05

The enriched KEGG categories were also compared between male and female *T. rubripes*. In ♂liver vs. ♀liver, only two categories, namely, "PPAR signaling pathway" and "base excision repair", were significantly enriched. In ♂spleen *vs.* ♀spleen, only one category, namely, "neuroactive ligand-receptor interaction", was significantly enriched. In ♂kidney *vs.* ♀kidney, there was no KEGG category with a *q*-value < 0.05.

PPI network analysis

The PPI networks of DEGs were determined by BLAST

analysis against the STRING database. In these PPI networks, there were 40, 50, 35, 44, 46, 37, 12, 15 and 4 modules, and 145,05, 20,167, 11,393, 17,492, 18,432, 11,955, 128, 113 and 66 nodes in liver *vs*. ♂spleen, ♂liver *vs*. ♂kidney, ♂spleen *vs*. ♂kidney, ♀liver *vs*. ♀spleen, ♀liver *vs*. ♀kidney, ♀spleen *vs*. ♀kidney, ♂liver *vs*. ♀liver, ♂spleen *vs*. ♀spleen and ♂kidney *vs*. ♀kidney, respectively.

Discussion

In this study, we performed a comparative RNA-Seq analysis of the important immune organs (livers, spleens and kidneys) of *T. rubripes* to find the key immune-related regulated networks, immune-related genes and DEGs of both the male and female fish using GO enrichment, KEGG pathway enrichment and PPI network analyses.

The most significantly enriched GO terms were "G-protein coupled receptor signaling pathway (GO:0007186)", "integral component of membrane (GO:0016021)", "G-protein coupled receptor activity (GO:0004930)" and "olfactory receptor activity (GO:0004984)" in the categories BP, CC and MF. Interestingly, all these GO terms are involved in cell surface receptor signaling. Cell surface receptors could bind cytokines, cell adhesion molecules and hormones and allow communication between cells and the external environment. In this study, a set of DEGs exhibited significant enrichment of these four GO terms. Previously, a transcriptomic study of common carp spleen infected with *A. hydrophila* also revealed that a number of genes associated with cell surface receptor signaling exhibit significantly different expression patterns after bacterial infection (Jiang *et al*. 2016). These results indicate that cell surface receptors are involved in immune-related signal transduction on cell surfaces and bind to extracellular ligands to activate, perpetuate, or inhibit an immune response.

In addition to GO enriched terms, we also found that the pathway "glycine, serine and threonine metabolism" was identified in all male and female groups (Fig. 5). Another important enriched pathway, namely, "cytokine-cytokine receptor interaction", was identified in all the male groups, while in the female groups, this pathway was present in ♀liver *vs*. ♀spleen and ♀spleen *vs*. ♀kidney but not ♀liver *vs*. ♀kidney (Fig. 5). Cytokine-cytokine receptor interactions are involved in the innate immune response, a fundamental defense mechanism in fish. These interactions can stimulate the recruitment, activation, and adhesion of cells to sites of infection or injury (Laing and Secombes 2004). In this study, 107 DEGs, including chemokines and chemokine receptors (20 DEGs), ILs and IL receptors (15 DEGs), TNFs and TNF receptors (20 DEGs), were identified in this pathway (Table 3). Chemokines are a family of small cytokines and include the CC, CXC, CX3C and XC subfamilies. Cytokines exert their biological effects by interacting with chemokine receptors (Vicari *et al*. 1997). Members of the CXC chemokine, CC chemokine, CC receptor, and CXC receptor superfamilies have been identified from the channel catfish genome and are involved in defense against disease and hypoxia response (Fu *et al*. 2017a, b, c). Our previous study has also identified a set of chemokines and chemokine receptors in the gills of *T. rubripes* (Cui *et al*. 2014b). In tongue sole, 9 CC chemokines, including CCL20, CCL21, and CCL27, are cloned and characterized, and they act in the response to *E. tarda* and *Vibrio harveyi* (Hao and Li 2015). Four fish-specific CC chemokine receptors, namely, CCR4La, CCR4Lc1, CCR4Lc2 and CCR11, are identified in rainbow trout (*Oncorhynchus mykiss*), and the expression of these receptors change after challenges with *Yersinia ruckeri* (Qi *et al*. 2017). Meanwhile, CXC chemokines and CXC chemokine receptors are induced by the pathogens. CXC_F from *Salmo trutta*, osgCXCL12 and osgCXCR4 from *Epinephelus coioides*, and CXCR4 from *Oplegnathus fasciatus* are responsive to viral challenge with hemorrhagic septicemia virus and *Y. ruckeri*; nervous necrosis virus and *E. tarda*; *S. iniae*; and rock bream iridovirus, respectively (Thulasitha *et al*. 2015; Wu *et al*. 2015; Gorgoglione *et al*. 2016). ILs may trigger the protective defenses of the immune system and help eradicate pathogens (Jiang *et al*. 2016). In this study, 5 ILs and 10 IL receptor genes were identified (Table 3). A number of ILs and IL receptor genes have been identified in fish species. For example, IL-1β, IL-1RI and IL-1RII expression is induced by LPS and poly (I:C) in miiuy croaker, and overexpression of IL-1β increase IL-1RI expression (Yang *et al*. 2017). Seven IL17 ligands and four IL17 receptor homologs are identified and characterized from the transcriptomic and genomic databases for channel catfish, and three IL17A/Fs and their putative receptors, IL17RA and IL17RC, are induced following experimental challenge with *E. ictaluri* and *F. columnare* (Wang *et al*. 2014). Meanwhile, rock bream RbIL-15 and RbIL-15Rα are highly induced in the kidneys and spleen after infection with *E. tarda*, *S. iniae* and red sea bream iridovirus (Bae *et al*. 2013). TNF is another large class of inflammatory cytokines involved in cytokine-cytokine receptor interactions. In this study, 12 TNFs and 8 TNF receptor genes were identified (Table 3). In *T. rubripes*, ten novel TNFSF genes are identified and highly expressed in livers. In addition, the expression levels of three TNFSF10 genes are seen to be increased in poly (I:C)-treated head kidney cells (Biswas *et al*. 2015). The expressions of TNF-α and TNF-N in *T. rubripes* are significantly elevated by both the probiotic bacterial stimulants tested compared with the expression observed in the unstimulated control (Biswas *et al*. 2013). In addition, a TNF13B gene is cloned from *Takifugu obscurus*, and this gene may be a factor for the enhancement of immunological efficacy in fish (Ai *et al*. 2011).

DEG analysis between male and female *T. rubripes* showed that there were 5 DEGs in ♂liver *vs*. ♀liver, ♂spleen *vs*. ♀spleen and ♂kidney *vs*. ♀kidney (Fig. 4).

Table 3: Identification of DEGs in Cytokine-cytokine receptor interaction

Gene	♂liver *vs.* ♂spleen	♂liver *vs.* ♂kidney	♂spleen *vs.* ♂kidney	♀liver *vs.* ♀kidney	♀liver *vs.* ♀spleen	♀spleen *vs.* ♀kidney
Chemokine and chemokine receptor						
LOC101061975 (CC25)			+			
LOC101077134 (CC13)				+		+
ccl19l1	+					
LOC105417015 (CC21)						+
cxcl11						+
cxcl14				+		
cxcl12						+
ccl19			+			
cxcl13		+				
ccr10	+	+	+	+		+
ccr9	+	+		+	+	
LOC101061903 (CCR4)	+	+		+	+	
LOC101066745 (CCR9)	+	+		+		
LOC101071120 (CCR4)		+		+		
LOC101077667 (XCR1)		+		+	+	
LOC101077892 (XCR1)	+	+		+	+	
cxcr4		+	+	+	+	+
cxcr5	+	+	+	+		+
LOC101071867 (CXCR2)		+	+	+	+	
LOC101079665 (CXCR)				+	+	
TNF and TNF receptor						
tnfa	+		+			
tgfb2	+					+
tgfb3				+		
LOC101066955 (TNFL6)	+	+		+	+	
LOC101061420 (TNFL10)	+	+	+	+	+	
LOC101075168 (TNFL13B)	+			+		
tnfsf12			+			
tnfsf11	+	+		+		
tnfsf12						+
tnfsf13b	+			+		
tnfsf10	+	+			+	
LOC101061564 (TNF11B)	+	+		+	+	
tnfrsf19	+	+		+	+	+
tnfrsf21	+	+		+	+	
ngfr	+		+	+		+
edar	+			+		+
tgfbr2				+		
tnfrsf11a		+	+		+	+
tnfrsf13b	+	+	+	+	+	+
VEGF and VEGF receptor						
LOC101070662 (VEGFA)		+	+		+	+
vegfa	+		+	+		+
vegfb	+	+	+	+		+
vegfc	+	+	+	+	+	+
LOC101071220 (VEGFR)	+		+	+		+
flt1						+
flt3	+	+		+	+	+
flt4						+
kdr						+
BMP and BMP receptor						
LOC101076733 (BMP7)	+	+		+	+	
LOC101076832 (BMP7)	+		+	+		+
bmp7	+	+		+	+	
bmpr1b			+	+		+
bmpr2		+				
LOC101061264 (BMPR1B)	+	+		+	+	
IL and IL receptor						
il1b				+		
il-18	+		+	+		+
il8	+	+		+	+	
newGene_1959 (IL18)	+		+	+		+
il6st		+		+	+	
il18rap	+	+		+	+	
il22ra2		+	+		+	+

Table Continue

Table 3: Continued

il2rg	+	+		+	+	
il7r	+	+		+	+	
LOC101078915 (IL21)	+	+		+	+	
LOC105417588 (IL31Rα)		+	+	+	+	+
LOC105419406 (IL2Rβ)				+		
LOC778023 (IL8R1)		+	+	+	+	
LOC101065602 (IL17RA)	+	+		+	+	
LOC101075482 (IL12Rβ2)	+					+
Acv receptor						
acvr1	+		+			
acvr2b				+		
LOC101064200 (acvr1b)	+		+			+
class I helical cytokine receptor						
newGene_449 (class I CR)	+	+	+	+	+	+
newGene_1895 (class I CR)	+	+	+	+	+	+
LOC101075512 (class I CR)	+	+		+	+	
PDGF						
LOC101063539 (PDGFA)				+	+	
pdgfa			+			
pdgfra			+			
LOC101074724 (PDGFRβ)			+	+		+
Others						
LOC101061818 (LIFR)	+	+		+	+	
LOC101065739 (AMCF2)	+	+		+	+	
LOC101071352 (EPOR)			+			+
LOC101073748 (MRTPK)	+	+		+	+	+
LOC101079667 (PR-like)	+		+	+	+	+
LOC101069994 (M-CSF1R1)	+	+	+	+	+	
LOC101080143 (M-CSF1R2)					+	
LOC101075211 (G-CSF)			+			+
csf3r		+	+	+	+	
LOC101077955 (TGFβ3)	+			+		
LOC101078862 (HGF)	+	+				
hgf	+	+	+	+	+	+
newGene_2384 (TPO)	+	+		+	+	
mpl	+		+	+		+
egf	+	+				
egfr		+	+		+	+
ghr1	+	+		+	+	
kit			+	+		+
lepr			+			+
met	+	+	+	+	+	+
prlr		+	+		+	+
scf	+					
LOC101067230				+	+	
LOC101067572	+	+	+	+	+	+
LOC105417838	+	+		+	+	
LOC105418164	+	+		+	+	
LOC101075732		+	+		+	+
LOC101073224		+		+	+	

These 5 DEGs encoded complement C1q TNF-related protein 4-like, hepcidin-like, voltage-dependent calcium channel subunit alpha-2/delta-2-like, protein L-Myc-1b-like and apolipoprotein A-IV1 precursor. Expression analysis showed that the expression of *complement C1q TNF-related protein 4-like* (CTRP4-like) was upregulated in all the male samples and the expression of *voltage-dependent calcium channel subunit alpha-2/delta-2-like* (CACNA2D2-like) and *L-Myc-1b-lik*e was upregulated in all the female samples. Complement C1q TNF-related protein 4 (CTRP4) is a secreted cytokine that is homologous to adiponectin, which plays an important role in immunity and metabolism (Huang *et al*. 2016). CTRP4 stimulates IL-6 synthesis and STAT3 and NFkB activation (Schäffler and Buechler 2012). Voltage-dependent calcium channels (VDCCs), also known as voltage-gated calcium channels (VGCCs), are a group of voltage-gated ion channels found in the membranes of excitable cells. In humans, it is found that VDCCs can mediate resistance to chemotherapy in small cell lung cancer (Yu *et al.* 2018). Human L-Myc is a proto-oncogenic transcription factor and acts by targeting the CCL6 chemokine gene. *Mycl1*is selectively expressed in the dendritic cells of the immune system and is controlled by IRF8 (Wumesh*et al*. 2014). An MYCL1 SNP, rs3134613, is associated with susceptibility to diffuse-

type gastric cancer and with differentiation of gastric cancer in a southeast Chinese population (Chen *et al.* 2010).

Conclusion

In this study, RNA-Seq analysis of the livers, spleens and kidneys of *T. rubripes* was performed, and a number of DEGs were identified. The GO terms associated with cell surface receptor signaling were the most significantly enriched, and an immune-related pathway (cytokine-cytokine receptor interaction) was present in all the male groups. In addition, the DEGs between male and female *T. rubripes* were also analyzed, and *CTRP4-like* was found to be upregulated in all the male samples, while the expression of *CACNA2D2-like* and *L-Myc-1b-like* was upregulated in all the female samples. These results not only provide information regarding the molecular immune mechanisms in *T. rubripes* livers, spleens and kidneys but also identify candidates for breeding to enhance pathogen resistance in *T. rubripes.*

Acknowledgements

This study was supported by the National Key R&D Program of China (2018YFD0900301), the Science and Technology Innovation Fund Project of Dalian city (2018J12SN070), the High Level Talents Innovation Support Program of Dalian (2017RQ015) and the China Agriculture Research System (CARS-47).

References

Ai H, Y Shen, C Min, S Pang, J Zhang, S Zhang, Z Zhao (2011). Molecular structure, expression and bioactivity characterization of TNF13B (BAFF) gene in mefugu, *Takifugu obscurus*. *Fish Shellf Immunol* 30:1265–1274

Asai K, N Hiki, Y Mimura, T Ogawa, K Unou, M Kaminishi (2001). Gender differences in cytokine secretion by human peripheral blood mononuclear cells: Role of estrogen in modulating LPS-induced cytokine secretion in an *ex vivo* septic model. *Shock* 16:340–343

Bae JS, SH Shim, SD Hwang, JW Kim, DW Park, CI Park (2013). Molecular cloning and expression analysis of interleukin (IL)-15 and IL-15 receptor α from rock bream, *Oplegnathus fasciatus*. *Fish Shellf Immunol* 35:1209–1215

Biswas G, S Kinoshita, T Kono, J Hikima, M Sakai (2015). Evolutionary evidence of tumor necrosis factor super family members in the Japanese pufferfish (*Takifugu rubripes*): Comprehensive genomic identification and expression analysis. *Mar Genomics* 22:25–36

Biswas G, H Korenaga, R Nagamine, H Takayama, S Kawahara, S Takeda, Y Kikuchi, B Dashnyam, T Kono, M Sakai (2013). Cytokine responses in the Japanese pufferfish (*Takifugu rubripes*) head kidney cells induced with heat-killed probiotics isolated from the Mongolian dairy products. *Fish Shellf Immunol* 34:1170–1177

Chen L, Z Liu, Y Su, D Wang, B Yin, B Shu, J Zhang, X Zhu, C Jia (2017). Characterization of *Mycobacterium marinum* infections in zebrafish wounds and sinus tracts. *Wound Repair Regen* 25:536–540

Chen SQ, XD Lin, JW Zhu, Y Tang, JY Lin (2010). Association of a *MYCL1* single nucleotide polymorphism, rs3134613, with susceptibility to diffuse-type gastric cancer and with differentiation of gastric cancer in a southeast Chinese population. *DNA Cell Biol* 29:739–743

Cui J, N Jiang, X Hou, S Wu, Q Zhang, J Meng, Y Luan (2020). Genome-wide identification of lncRNAs and analysis of ceRNA networks during tomato resistance to *Phytophthora infestans*. *Phytopathology* 110:456–464

Cui J, Y Luan, N Jiang, H Bao, J Meng (2017). Comparative transcriptome analysis between resistant and susceptible tomato allows the identification of lncRNA16397 conferring resistance to *Phytophthora infestans* by co-expressing glutaredoxin. *Plant J* 89:577–589

Cui J, H Wang, S Liu, L Zhu, X Qiu, Z Jiang, X Wang, Z Liu (2014a). SNP discovery from transcriptome of the swim bladder of *Takifugu rubripes*. *PLoS One* 9; Article e92502

Cui J, S Liu, B Zhang, H Wang, H Sun, S Song, X Qiu, Y Liu, X Wang, Z Jiang, Z Liu (2014b). Transciptome analysis of the gill and swim bladder of *Takifugu rubripes* by RNA-Seq. *PLoS One* 9; Article e85505

Cui J, H Wang, S Liu, X Qiu, Z Jiang, X Wang (2014c). Transcriptome analysis of the gill of *Takifugu rubripes* using Illumina sequencing for discovery of SNPs. *Compar Biochem Physiol D Genomics Proteomics* 10:44–51

Fu Q, Q Zeng, Y Li, Y Yang, C Li, S Liu, T Zhou, N Li, J Yao, C Jiang, D Li, Z Liu (2017a). The chemokinome superfamily in channel catfish: I. CXC subfamily and their involvement in disease defense and hypoxia responses. *Fish Shellf Immunol* 60:380–390

Fu Q, Y Yang, C Li, Q Zeng, T Zhou, N Li, Y Liu, Y Li, X Wang, S Liu, D Li, Z Liu (2017b). The chemokinome superfamily: II. The 64 CC chemokines in channel catfish and their involvement in disease and hypoxia responses. *Dev Compar Immunol* 73:97–108

Fu Q, Y Yang, C Li, Q Zeng, T Zhou, N Li, Y Liu, S Liu, Z Liu (2017c). The CC and CXC chemokine receptors in channel catfish (*Ictalurus punctatus*) and their involvement in disease and hypoxia responses. *Dev Compar Immunol* 77:241–251

Gong Y, S Feng, S Li, Y Zhang, Z Zhao, M Hu, P Xu, Y Jiang (2017). Genome-wide characterization of Toll-like receptor gene family in common carp (*Cyprinus carpio*) and their involvement in host immune response to *Aeromonas hydrophila* infection. *Compar Biochem Physiol D Genomics Proteomics* 24:89–98

Gorgoglione B, E Zahran, NG Taylor, SW Feist, J Zou, CJ Secombes (2016) Comparative study of CXC chemokines modulation in brown trout (*Salmo trutta*) following infection with a bacterial or viral pathogen. *Mol Immunol* 71:64–77

Guo R, X Wang, H Su, X Zhang, H Liu (2017). Analysis and comparison of nutritional compositions in muscle, liver and skin of *Takifugu rubripes*. *J Agric Univ Hebei* 40:77–82 (in Chinese)

Hamasaki M, Y Takeuchi, R Yazawa, S Yoshikawa, K Kadomura, T Yamada, K Miyaki, K Kikuchi, G Yoshizaki (2017). Production of tiger puffer *Takifugu rubripes* offspring from triploid grass puffer *Takifugu niphobles* parents. *Mar Biotechnol* 19:579–591

Hao LX, MF Li (2015). Molecular characterization and expression analysis of nine CC chemokines in half-smooth tongue sole, *Cynoglossus semilaevis*. *Fish Shellf Immunol* 47:717–724

Huang H, X Wu, L Cao, L Wang (2016). Preparation and Identification of Monoclonal Antibody Against C1q/TNF-Related Protein 4. *Monoclon Antib Immunodiagn Immunother* 35:280–284

Jiang N, J Meng, J Cui, G Sun, Y Luan (2018). Function identification of miR482b, a negative regulator during tomato resistance to *Phytophthora infestans*. *Hortic Res* 5; Article 9

Jiang Y, S Feng, S Zhang, H Liu, J Feng, X Mu, X Sun, P Xu (2016). Transcriptome signatures in common carp spleen in response to *Aeromonas hydrophila* infection. *Fish Shellf Immunol* 57:41–48

Kahlke V, C Dohm, K Brötzmann, S Schreiber, J Schröder (2000). Gender-related therapy: Early IL-10 administration after hemorrhage restores immune function in males but not in females. *Shock* 14:354–359

Kanehisa M, M Araki, S Goto, M Hattori, M Hirakawa, M Itoh, T Katayama, S Kawashima, S Okuda, T Tokimatsu, Y Yamanishi (2008). KEGG for linking genomes to life and the environment. *Nucl Acids Res* 36:480–484

Klein SL, I Marriott, EN Fish (2015). Sex-based differences in immune function and responses to vaccination. *Trans Roy Soc Trop Med Hyg* 109:9–15

Kong D, Z Wang, L Zang, J Cui, H Li, H Sun, X Qiu, H Liu, X Wang (2019). Expression, purification and characterization of recombinant interferon-γ of *Takifugu rubripes* in Pichia pastoris. *Intl J Agric Biol* 21:19–24

Kong D, J Cui, Z Wang, L Zang, H Sun, Z Hu, X Li, X Qiu, C Jiang, H Liu, T Zhang, S Liu, Z Jiang, X Meng, X Wang (2018). The regulatory networks conferred by IFN-γ in the kidney of *Takifugu rubripes*. *Intl J Agric Biol* 20:2189–2195

Laing KJ, CJ Secombes (2004). Trout CC chemokines: Comparison of their sequences and expression patterns. *Mol Immunol* 41:793–808

Ma A, W Li, X Wang, L Yue, Z Zhuang, X Meng, S Liu, L Tang, S Hou (2014). Research progress and outlook of *Takifugu rubripes* culture techniques. *Mar Sci* 38:116–121

Mao X, T Cai, JG Olyarchuk, L Wei (2005). Automated genome annotation and pathway identification using the KEGG orthology (KO) as a controlled vocabulary. *Bioinformatics* 21:3787–3793

Marriott I, KL Bost, YM Huet-Hudson (2006). Sexual dimorphism in expression of receptors for bacterial lipopolysaccharides in murine macrophages: A possible mechanism for gender-based differences in endotoxic shock susceptibility. *J Reprod Immunol* 71:12–27

Moxley G, D Posthuma, P Carlson, E Estrada, J Han, LL Benson, MC Neale (2002). Sexual dimorphism in innate immunity. *Arthritis Rheum* 46:250–258

Mu Y, F Ding, P Cui, J Ao, S Hu, X Chen (2010). Transcriptome and expression profiling analysis revealed changes of multiple signaling pathways involved in immunity in the large yellow croaker during *Aeromonas hydrophila* infection. *BMC Genomics* 11; Article 506

Ordas A, Z Hegedus, CV Henkel, OW Stockhammer, D Butler, HJ Jansen, P Racz, MMink, HP Spaink, AH Meijer (2011). Deep sequencing of the innate immune transcriptomic response of zebrafish embryos to Salmonella infection. *Fish Shellf Immunol* 31:716–724

Peatman E, C Li, BC Peterson, DL Straus, BD Farmer, BH Beck (2013). Basal polarization of the mucosal compartment in *Flavobacterium columnare* susceptible and resistant channel catfish (*Ictalurus punctatus*). *Mol Immunol* 56:317–327

Pennell LM, CL Galligan, EN Fish (2012). Sex affects immunity. *J Autoimmun* 38:282–291

Qi Z, JW Holland, Y Jiang, CJ Secombes, P Nie, T Wang (2017). Molecular characterization and expression analysis of four fish-specific CC chemokine receptors CCR4La, CCR4Lc1, CCR4Lc2 and CCR11 in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellf Immunol* 68:411–427

Schäffler A, C Buechler (2012). CTRP family: Linking immunity to metabolism. *Trends Endocrinol Metab* 23:194–204

Shannon P, A Markiel, O Ozier, NS Baliga, JT Wang, D Ramage, N Amin, B Schwikowski, T Ideker (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res* 13:2498–2504

Song X, X Hu, B Sun, Y Bo, K Wu, L Xiao, C Gong (2017). A transcriptome analysis focusing on inflammation-related genes of grass carp intestines following infection with *Aeromonas hydrophila*. *Sci Rep* 7; Article 40777

Stockhammer OW, H Rauwerda, FR Wittink, TM Breit, AH Meijer, HP Spaink (2010). Transcriptome analysis of Traf6 function in the innate immune response of zebrafish embryos. *Mol Immunol* 48:179–190

Sudhagar A, G Kumar, M El-Matbouli (2018). Transcriptome analysis based on RNA-Seq in understanding pathogenic mechanisms of diseases and the immune system of fish: A comprehensive review. *Intl J Mol Sci* 19:245–271

Thulasitha WS, N Umasuthan, KS Revathy, I Whang, J Lee (2015). Molecular characterization, genomic structure and expressional profiles of a CXC chemokine receptor 4 (CXCR4) from rock bream *Oplegnathus fasciatus*. *Fish Shellf Immunol* 44:471–477

Tran NT, ZX Gao, HHZhao, SK Yi, BX Chen, YH Zhao, L Lin, XQ Liu, WM Wang (2015). Transcriptome analysis and microsatellite discovery in the blunt snout bream (*Megalobrama amblycephala*) after challenge with *Aeromonas hydrophila*. *Fish Shellf Immunol* 45:72–82

Trapnell C, L Pachter, SL Salzberg (2009). TopHat: Discovering splice junctions with RNA-Seq. *Bioinformatics* 25:1105–1111

Vicari AP, DJ Figueroa, JA Hedrick, JS Foster, KP Singh, S Menon, NG Copeland, DJ Gilbert, NA Jenkins, KB Bacon, A Zlotnik (1997). TECK: A novel CC chemokine specifically expressed by thymic dendritic cells and potentially involved in T cell development. *Immunity* 7:291–301

Wang B, Z Gan, S Cai, Z Wang, D Yu, Z Lin, Y Lu, Z Wu, J Jian (2016). Comprehensive identification and profiling of Nile tilapia (*Oreochromis niloticus*) microRNAs response to *Streptococcus agalactiae* infection through high-throughput sequencing. *Fish Shellf Immunol* 54:93–106

Wang X, C Li, W Thongda, Y Luo, B Beck, E Peatman (2014). Characterization and mucosal responses of interleukin 17 family ligand and receptor genes in channel catfish *Ictalurus punctatus*. *Fish Shellf Immunol* 38:47–55

Wang Z, J Cui, J Song, H Wang, K Gao, X Qiu, M Gou, X Li, Z Hu, X Wang, Y Chang (2018). Comparative Transcriptome analysis reveals growth-related genes in juvenile Chinese sea cucumber, Russian sea cucumber, and their hybrids. *Mar Biotechnol* 20:193–205

Wu CS, TY Wang, CF Liu, HP Lin, YM Chen, TY Chen (2015). Molecular cloning and characterization of orange-spotted grouper (*Epinephelus coioides*) CXC. chemokine ligand 12. *Fish Shellf Immunol* 47:996–1005

Wumesh KC, AT Satpathy, AS Rapaport, CG Briseño, X Wu, JC Albring, EV Russler-Germain, NM Kretzer, V Durai, SP Persaud, BT Edelson, J Loschko, M Cella, PM Allen, MC Nussenzweig, M Colonna, BP Sleckman, TL Murphy, KM Murphy (2014). L-Myc expression by dendritic cells is required for optimal T-cell priming. *Nature* 507:243–247

Yang D, Q Liu, M Yang, H Wu, Q Wang, J Xiao, Y Zhang (2012). RNA-seq liver transcriptome analysis reveals an activated MHC-I pathway and an inhibited MHC-II pathway at the early stage of vaccine immunization in zebrafish. *BMC Genomics* 13; Article 319

Yang Q, Q Chu, X Zhao, T Xu (2017). Characterization of IL-1β and two types of IL-1 receptors in miiuy croaker and evolution analysis of IL-1 family. *Fish Shellf Immunol* 63:165–172

Yang Y, H Yu, H Li, A Wang (2016). Transcriptome profiling of grass carp (*Ctenopharyngodon idellus*) infected with *Aeromonas hydrophila*. *Fish Shellf Immunol* 51:329–336

Young MD, MJ Wakefield, GK Smyth, A Oshlack (2010). Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biol* 11:14–25

Yu J, S Wang, W Zhao, J Duan, Z Wang, H Chen, Y Tian, D Wang, J Zhao, T An, H Bai, M Wu, J Wang (2018). Mechanistic exploration of cancer stem cell marker voltage-dependent calcium channel α2δ1 subunit-mediated chemotherapy resistance in small cell lung cancer. *Clin Cancer Res* 24:2148–2158

Zhang X, S Wang, S Chen, Y Chen, Y Liu, C Shao, Q Wang, Y Lu, G Gong, S Ding, Z Sha (2015). Transcriptome analysis revealed changes of multiple genes involved in immunity in *Cynoglossus semilaevis* during *Vibrio anguillarum* infection. *Fish Shellf Immunol* 43:209–218

Zhao C, M Fu, C Wang, Z Jiao, L Qiu (2016). RNA-Seq analysis of immune-relevant genes in *Lateolabrax japonicus* during *Vibrio anguillarum* infection. *Fish Shellf Immunol* 52:57–64

Zhou X, J Cui, X Qiu, Y Chang, X Wang (2019). Whole genomic SNPs and SSRs development based on high-throughout transcript sequencing in sea cucumber (*Apostichopus japonicus*). *Intl J Agric Biol* 22:877–881

Zhou X, J Cui, S Liu, D Kong, H Sun, C Gu, H Wang, X Qiu, Y Chang, Z Liu, X Wang (2016a). Comparative transcriptome analysis of papilla and skin in the sea cucumber, *Apostichopus japonicus*. *Peer J* 4; Article e1779

Zhou X, H Wang, J Cui, X Qiu, Y Chang, X Wang (2016b). Transcriptome analysis of tube foot and large scale marker discovery in sea cucumber, *Apostichopus japonicus*. *Compar Biochem Physiol D Genomics Proteomics* 20:41–49

Zhu J, Q Fu, Q Ao, Y Tan, Y Luo, H Jiang, C Li, X Gan (2017). Transcriptomic profiling analysis of tilapia (*Oreochromis niloticus*) following *Streptococcus agalactiae* challenge. *Fish Shellf Immunol* 62:202–212