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GHRH-SST-GH-IGF axis regulates crosstalk between growth and immunity in rainbow trout (*Oncorhynchus mykiss*) infected with *Vibrio anguillarum*

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ABSTRACT

An energy trade-off is existed between immunological competence and growth. The axis of growth hormone releasing hormone, somatostatin, growth hormone, insulin-like growth factor (GHRH-SST-GH-IGF axis) regulates growth performances and immune competences in rainbow trout (*Oncorhynchus mykiss*). The salmonid-specific whole genome duplication event is known to result in duplicated copies of several key genes in GHRH-SST-GH-IGF axis. In this study, we evaluated the physiological functions of GHRH-SST-GH-IGF axis in regulating crosstalk between growth and immunity. Based on principal components analysis (PCA), we observed the overall expression profiles of GHRH-SST-GH-IGF axis were significantly altered by *Vibrio anguillarum* infection. Trout challenged with *Vibrio anguillarum* showed down-regulated *igf1s* subtypes and up-regulated *igfbp1a1*. The brain *sst* genes (*sst1a*, *sst1b*, *sst3b* and *sst5*) and *igfpbs* genes (*igfbp4s* and *igfbp5b2*) were significantly affected by *V. anguillarum* infection, while the *igfbp4s, igfbp5s, igfbp6s* and *igf2bps* genes showed significant changes in peripheral immune tissues in response to *V. anguillarum* infection. Gene enrichment analyses showed functional and signaling pathways associated with apoptosis (such as p53, HIF-1 or FoxO signaling) were activated. We further proposed a possible model that describes the IGF and IGFBPs-regulated interaction between cell growth and programmed death. Our study provided new insights into the physiological functions and potentially regulatory mechanisms of the GHRH-SST-GH-IGF axis, indicating the pleiotropic effects of GHRH-SST-GH-IGF axis in regulating crosstalk between growth and immunity in trout.

1. Introduction

Growth is orchestrated by the multi-hormone crosstalk of growth hormone releasing hormone (GHRH), somatostatin (SST), growth hormone (GH), insulin-like growth factor (IGF) and other neuroendocrine and endocrine regulators [1]. GHRH, which is secreted from the hypothalamus, triggers the release of GH from the pituitary, while SST inhibits GH release. GH activates its receptor (GHR) located in the target tissues and stimulates the release of IGF, an important governor of vertebrate growth and metabolism. The physiological functions of IGF are co-modulated by the receptor (IGFR) and the binding proteins (IGFBPs).

In addition to the canonical functions of regulating growth, there is a growing body of evidences showing that GHRH-SST-GH-IGF axis plays an important role in regulating immune responses. In both mammals and teleosts, genes of GH and IGF systems are reported to be involved in the regulation of the immune cell proliferation and cytokine-regulated inflammation [2–10]. For example, GH overexpression (GH transgenesis) results in enhanced growth but disruptions in muscle immune functions in coho salmon (*Oncorhynchus kisutch*) [11]. The following transcriptomic study with wild type, domesticated, and GH transgenic coho salmon further showed the pleiotropic effects of GH on

Abbreviations: 4 rounds of genome duplication, (4R); teleost-specific whole genome duplication, (tsWGD); salmonid-specific whole genome duplication, (ssWGD); Brain-sympathetic-chromaffin, (BSC) axis; Growth hormone, (GH); Growth hormone receptor, (GHR); Growth hormone releasing hormone, (GHRH); Hypothalamus-pituitary-interrenal, (HPI) axis; Hypothalamus-pituitary-adrenal, (HPA) axis; Insulin-like growth factor, (IGF); Insulin-like growth facto binding proteins, (IGFBPs); Somatostatin, (SST); Terms of RNA-Seq, Differentially expressed genes (DEGs); Gene Ontology, (GO); Kyoto Encyclopedia of Genes and Genomes, (KEGG).

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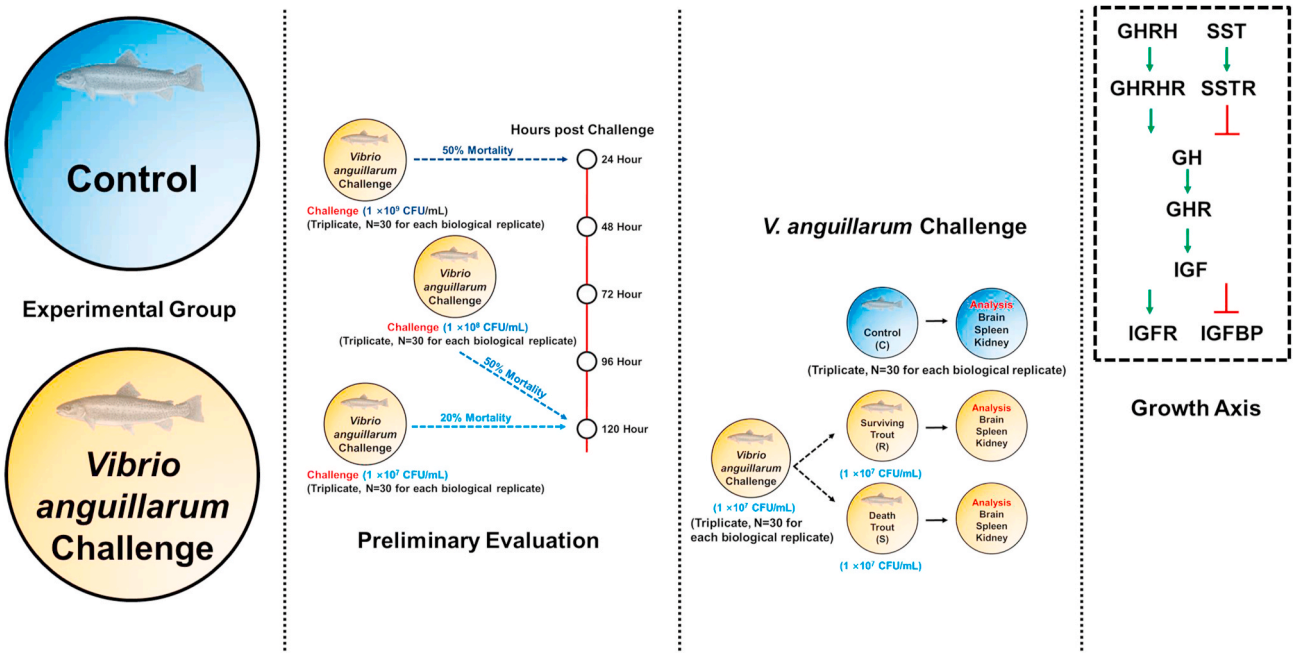

Fig. 1. Experimental setup. **Preliminary Evaluation:** 30 individuals (triplicates) were challenged with 1 mL of 10^7, 10^8 and 10^9 CFU/mL of *V. anguillarum* strain and mortality was monitored for 120 h. Mortality of trout challenged with 10^9 CFU/mL of bacteria reached to ~70% within 24 h after challenge. After 120 h of challenge, *V. anguillarum* of 10^8 and 10^7 CFU/mL resulted in ~50% and ~20% mortality, respectively. ***V. anguillarum* Challenge:** Trout were treated with or without *V. anguillarum* challenge. Each treatment had three replicates and each replicate contains 30 individuals. The challenged trout was intraperitoneally injected to 1 mL *V. anguillarum* at 10^7 CFU/mL, while the same volume of saline was used in control group. For the trout challenged by *V. anguillarum*, the first three dead trout (triplicate) were sampled as the sensitive group (S) and the three individuals of surviving trout (triplicate) were sampled as the resistant group (R). Each biological replicate contained three pooled samples to reduce the individual variation for further analysis.
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immunomodulation is probably resulted from the energy trade-off between growth and immunity [9]. Regarding IGF systems, rainbow trout (*Oncorhynchus mykiss*) fry challenged with *Aeromonas salmonicida* (*A. salmonicida*) or viral hemorrhagic septicemia virus (VHSv) exerts slightly up-regulated *igf1ra1* and *igf1ra2* expressions, while the adult trout challenged with *Yersinia ruckeri* shows down-regulated head kidney and spleen *igf1ra1* and *igf1ra2* expressions, with down-regulated *igf1* (head kidney and spleen) and *igf2* (only in spleen) [12]. In addition, the *igfpbs* are also involved in immunomodulation in teleost [13,14]. For example, the trout *igfbps*, especially the *igfbp1a1* and *igfbp6a2*, are also involved in host defense responses [4,15].

The neuroendocrine and endocrine systems have been reported to interact with immune systems [16]. Most studies in aquatic animals focused on the hypothalamus-pituitary-interrenal (HPI) axis and/or brain-sympathetic-chromaffin (BSC) axis. For example, in invertebrates, catecholamine could increase the pathogen virulence in prawn (*Macrobrachium rosenbergii*) [17], and significantly suppress the immune responses against *V. anguillarum* in scallop (*Chlamys farreri*) [18]. In teleosts, glucocorticoid could suppress the immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*) [19], and induce anti-inflammatory responses in sea bass (*Dicentrarchus labrax*) [20]. A growing body of evidence revealed that immune responses disturb the endocrine homeostasis and energy balance between growth and immunity, leading to poor growth performance in teleost [21–23]. However, studies on GHRH-SST-GH-IGF axis-regulated crosstalk between immunity and growth are still limited, especially in the Salmonidae species with duplicated genes in GHRH-SST-GH-IGF axis due to the genome duplication [12,24–27].

Two rounds (2R) of whole genome duplication (WGD) occurred in early vertebrate ancestor evolution, thus resulting in increased complexity and genome size [28,29]. Based on the model of "one-two-four" rule, the ancestral genome was duplicated to two copies after the first genome duplication (1R), and further duplicated to four copies after the second (2R) duplication [30–32]. Around 350 million years ago, the common teleost fish ancestor experienced three rounds of genome duplication (3R, also termed as teleost-specific WGD, tsWGD), extending the "one-two-four" to a "one-two-four-eight" rule [15,30,33,34]. Compared to the common teleost fish ancestor, the Salmonids ancestor experienced four rounds of genome duplication (4R, also termed as salmonid-specific WGD, ssWGD), with ~50% of paralogs functionally retained [24,27,35–37]. The fourth WGD event occurred when all extant salmonids were derived (around 25–100 million years ago), causing significant genetic expansions of GH-IGF system, including genes encode GH, GHR, IGF, IGFR and IGFBPs [12,24–27]. For example, previous studies revealed gene duplications occurred in *gh and ghr* genes in rainbow trout, Atlantic salmon and other salmonid species [38–45]. Regarding IGF systems, 9 *igfbp* genes are identified in zebrafish with the absence of *igfbp4* due to the 3R, and at least 19 *igfbp* genes have been identified in Atlantic salmon due to the 4R [4,15,35,46]. The Salmoninae members also retained two *igf1a* paralogs and one single *igf2* gene (*igf2b1*); and two *igf-ira* paralogs and a single *igf-irb* [12,15,25].

Rainbow trout is one of the most important Salmonidae species with high economic values and high global production (~800,000 tons in 2016) (FAO, 2019). The *Vibrio anguillarum* (*V. anguillarum*) is reported to cause vibriosis over 50 teleost species [8], causing severely economic losses in aquaculture [47–49]. Moreover, the Salmonidae species, such as rainbow trout, are served as typically evolutionary models due to the genetic expansions resulting from ssWGD [4,24,27,35–37]. In this study, we reported ssWGD has expanded the gene copies of trout *sstr* and *igf2bps*. We further reported the transcriptional profiles of GHRH-SST-GH-IGF axis in response to *V. anguillarum* infection, revealing that *sst*, *igfpbs* and *igf2bps* genes were involved in crosstalk between immunity and growth. We also proposed a potential model, indicating that the mechanisms of cell growth and programmed death were regulated by crosstalk between SST and IGF systems.

2. Materials and methods

2.1. Ethics statement

All experiments in this study were conducted in conformity to Guidelines of Animal Research and Ethics Committees of Ocean University of China (Permit Number: 20141201), National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). No endangered or protected species were involved in our studies. In this study, trout juveniles were immature, and the influence of sex was not considered.

2.2. V. anguillarum and culture conditions

The pathogenic *V. anguillarum* strain was provided by Laboratory of Pathology and Immunology of Aquatic Animals, KLMME, Ocean University of China. Previous studies confirmed the *V. anguillarum* strain could result in vibriosis in teleosts including sole (*Cynoglossus semilaevis*) [50] and flounder (*Paralichthys olivaceus*) [51,52]. The bacteria were cultured at 28 °C for 24 h with 2216E medium. Subsequently, bacterial suspension was centrifuged and resuspended with 0.01 M phosphate-buffered saline (PBS, pH = 7.2). Based on the McFarland standard, the densities of the bacterial suspension were measured spectrophotometrically. The density of the bacteria suspension was adjusted to 10^9, 10^8 or 10^7 colony forming units (CFU)/mL for challenge test.

2.3. Animals

Rainbow trout (~108 g, ~20 cm) were provided by Linqu Salmon and Trout Aquatic Breeding LLC (Weifang, Shandong, China) and reared at the Experimental Fish Facility in Key Laboratory of Mariculture, KLMME, Ocean University of China. These trout juveniles were from the same full-sibling family batch and spawned at the same day with same day-age and synchronized development. Trout were acclimated with natural photoperiod for fourteen days and fed with commercial feed (~7% of body weight) twice a day. Based on the Standards of Linxia Salmon and National Trout Elite Breeding and Protection Farm (Linxia, Gansu, China, Approved by Department of Agriculture, China, 2009), water temperature and dissolved oxygen levels were adapted to ~16 °C and ~7 mg/L, respectively.

2.4. Preliminary tests

Two preliminary studies were performed to evaluate the temperature and infection concentration of *V. anguillarum*. The first preliminary study showed that the bacteria resulted in death at 20 °C rather than 16 °C. Therefore, trout were acclimated at 20 °C for another 14 days and then challenged with *V. anguillarum at* 10^7, 10^8 or 10^9 CFU/mL. The second preliminary study evaluate the infection concentration of *V. anguillarum*. Within each *V. anguillarum* concentration, 90 trout were equally distributed into three tanks. The 90 uninfected trout (saline-challenged, 0.9% NaCl) were also equally distributed into three tanks as control group. The mortality was monitored from 0 to 120 h post challenge (Fig. 1). Based on the two preliminary studies, *V. anguillarum* with 10^7 CFU/mL at 20 °C showed moderate mortality and then we selected this concentration for further studies.

2.5. V. anguillarum challenge experiment

Trout were divided into two groups as control group and challenge group (trout in challenge group were further divided into sensitive group and resistant group). In control group, 90 individuals were randomly assigned into triplicate tanks, with 30 individuals in each biological replicate. Likewise, the challenge group had three replicated tanks (biological replicate #1, #2 and #3) and each tank contained 30 individuals. In challenge group, trout was challenged by intraperitoneal injection of 200 μL *V. anguillarum* at 10^7 CFU/mL, while the same volume of physiological saline (saline-challenged, 0.9% NaCl) was used in control group. Within biological replicate #1 of challenge group, we took the first three dead trout and pooled them as sample #1 of sensitive group. We also took the three eventually surviving trout and pooled them as sample #1 of resistant group. Likewise, we had the same sampling protocol within biological replicate #2 and #3 of challenge group, thus collecting the sample #2 and #3 of sensitive group or resistant group. In control group, three trout were randomly sampled and pooled within each biological replicate. Prior to sampling, trout was firstly anesthetized by MS-222 (35–45 mg/L (ppm)). The small portions of brain, spleen, kidney and other tissues were isolated immediately and stored at −80 °C in RNase-free tubes for further RNA-Seq analysis.

2.6. RNA-Seq analysis

Total RNA was extracted by TRIzol method (Invitrogen, CA, USA) and digested with RNase-free DNase I (Takara, Shiga, Japan) to remove genomic DNA. RNA concentration and integrity were evaluated by NanoDrop ND-1000 (NanoDrop Technologies, Delaware, USA) and 1% agarose gel electrophoresis. A total of 27 libraries (3 tissues x 3 replicated samples x 3 treatment groups) were constructed via TruSeq™ RNA Sample Prep Kit (Illumina, California, USA). The libraries were sequenced with Illumina HiSeq 4000 platform (OEbiotech, Shanghai, China) to generate the 150 bp paired-end raw reads. The clean reads for further analysis were obtained from the raw reads with the process of Trimmomatic Software [53].

The clean reads were aligned to the reference genome (GCA_002163495.1) with HISAT, a highly efficient spliced alignment program for mapping RNA-seq reads [54]. The multiple mapped reads were removed and the count numbers of unique mapped reads and FPKM (Fragments Per kb Per Million Reads) were retrieved and normalized with previous references [55–58]. Based on DEGSeq R package, statistical analysis of transcripts were conducted with the parameters of "p-value" < 0.05 and $|\log2(\text{fold change})| > 1$ [59]. Differentially expressed genes (DEGs) were assigned to Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, focused on signaling pathways associated with immune response [60–62].

2.7. Statistical analysis

MetaboAnalyst is widely used for analyses of metabolomic and transcriptomics data [63–65]. Based on previous studies in teleosts, animals, plants and microorganisms, and the online protocols of MetaboAnalyst (https://www.xialab.ca/tools.xhtml), the original data (RNA-Seq) were normalized by sum, cube root transformation and pareto scaling, thus obtaining a belt data distribution for further statistical analysis via MetaboAnalyst [63–68]. The normalized RNA-Seq data were further analyzed by principal components analysis (PCA), heatmap, correlation coefficients and variable importance in projection (VIP) (Figs. 3–6, S1, Tables S2–S6). The SPSS 16.0 and GraphPad Prism 8.0 were used for statistical analysis. One-way analysis of variance (ANOVA) was used to test the effect of *V. anguillarum* challenge on gene transcript levels of trout in control, resistant and sensitive groups. If the significant differences were observed among different groups, differences of means were further tested via Tukey's Multiple Comparison test with p-value < 0.05. Results are expressed as mean ± standard error (means ± S.E.).

3. Results

3.1. Identification of the trout sstr and igf2bp genes

The bovine rhodopsin and human SSTRs were used as the reference

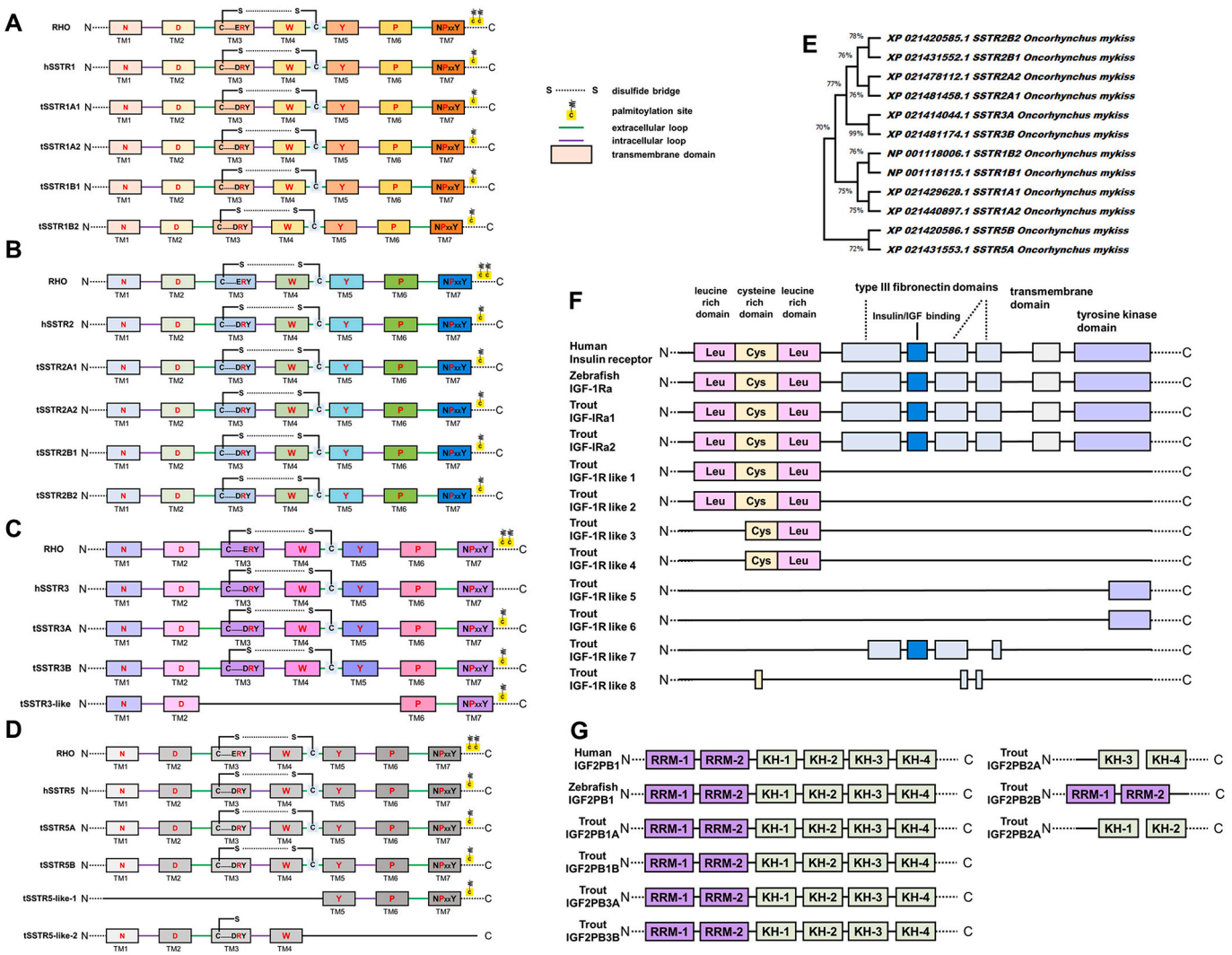

Fig. 2. Identification of the functional genes produced by 4R in GHRH-GH-SST-IGF axis. Twelve *sstr* genes were identified as functional genes (A–D). The twelve *sstr* genes were further classified into 4 subtypes based on phylogenetic analysis (E). Two annotated *igfr* genes, which is previously identified by Alzaid et al. [12] (F) and four *igf2bp* (G) genes were identified as functional genes. Details of figs. A–D are shown in Figs. S2–S5 and Table S1. Details of phylogenetic analysis of SSTRs in teleosts are shown in Fig. S7. The conserved GPCRs structures include seven transmembrane domains (TMs) with most conserved residues in each TM (highlight in red), the highly conserved disulfide bride between TM III and extracellular loop II, potentially conserved palmitoylation sites at the C terminus and the highly conserved motifs of E/DRY of TM III and NPxxY of TM VII. In figures A–D, each box represents one TM, N and C represents N and C terminus, respectively. The representative structures of IGFRs includes two leucine rich domains, one cysteine rich domains, the type III fibronectin domains, the domain associated with insulin/IGF binding, TM and tyrosine kinase domain. The typical IGF2BPs structures were involved in two RNA recognition motifs (RRM) and four hnRNP-K homology domains (KH). Details of figures are displayed in Figs. S2–S5.
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sequences to identify the trout SSTRs [69–73]. Twelve *sstr* genes showed functional G protein coupled receptors (GPCRs) structures (Fig. 2A–D, Figs. S2–S4, Table S1). These structures include the seven transmembrane domains (TM) with most conserved residues in each TM, the highly conserved disulfide bride between TM III and extracellular loop II, potentially conserved palmitoylation sites at the C terminus and the highly conserved motifs of E/DRY of TM III and NPxxY of TM VII (Fig. 2A–D, Figs. S2–S4, Table S1).

Based on previous studies in salmonid IGF1Rs [12], two annotated IGFR sequences (IGF-IRa1 and IGF-IRa2) were identified in our studies. These two IGFR sequences contained the representative structures of insulin/IGF receptors, such as the two leucine rich domains, one cysteine rich domains, the type III fibronectin domains, domain associated with insulin/IGF binding, TM and tyrosine kinase domain (Fig. 2F, Table S1). Likewise, we also identified 4 *igf2bps* genes in trout based on previous studies in human and zebrafish (Fig. 2G, Fig. S5, Table S1) [74–77].

3.2. Differentially expressed genes of GHRH-SST-GH-IGF axis

Compared to control group, trout of sensitive group or resistant group showed up-regulated *igf2bp* subtypes and *igfbp1a1* in brain, kidney or spleen, while genes of *igf* subtypes (excepting for the brain *igf1b* in sensitive group) and *ghr* were down-regulated after *V. anguillarum* challenge (Fig. 3A, B, 3D, 3E, 3G, 3H). Apart from brain *igfbp3a1* in sensitive group, other *igfpbs* subtypes showed down-regulation after *V. anguillarum* challenge, including brain *igfbp2a1*, *igfbp4* subtypes, *igfbp5b2*, *igfbp6b1* (Fig. 3A and B), and kidney and spleen *igfbp4* and *igfbp5* subtypes (Fig. 3D, E, 3G, 3H). Compared to resistant group, trout in sensitive group showed down-regulated *sstr1a2, sstr3a, igf1a subtypes*, *igfbp4 subtypes* and *igfbp5b2* (Fig. 3C, F, 3I).

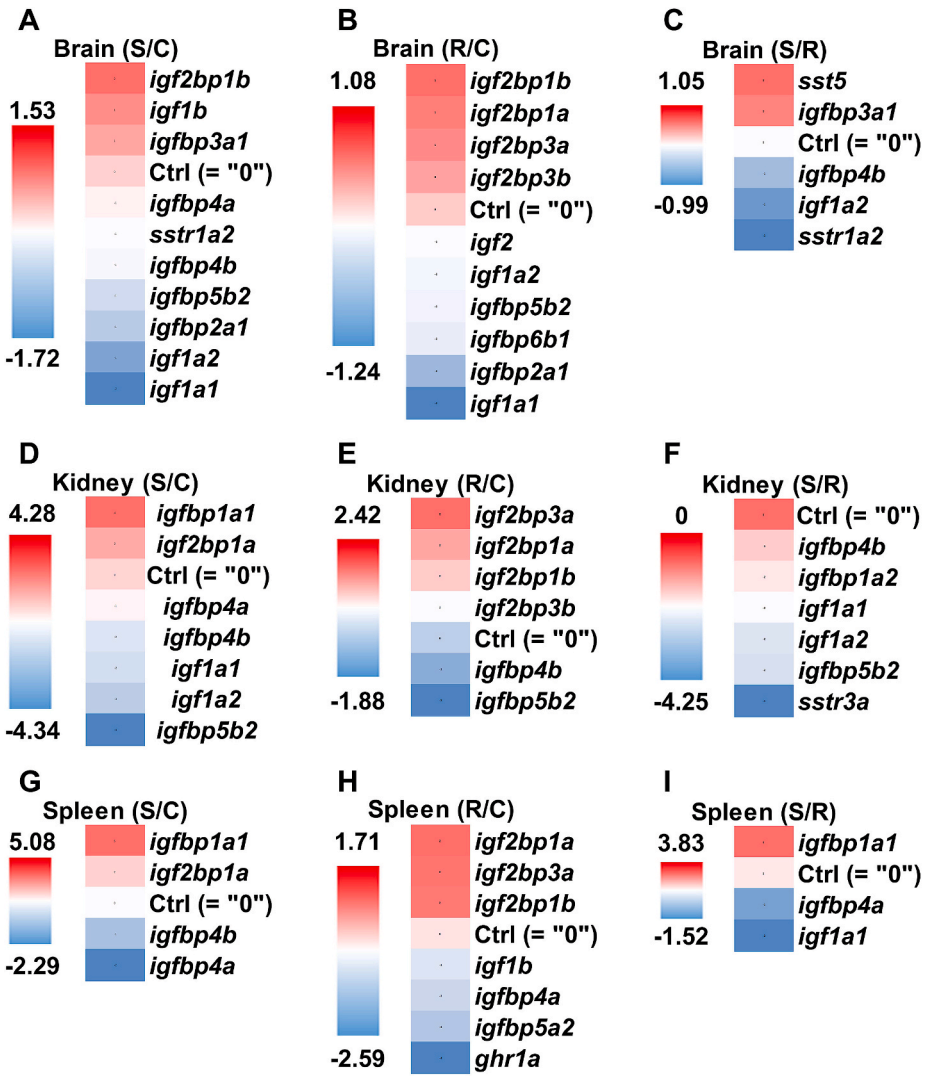

Fig. 3. Overview of differentially expressed genes of GHRH-SST-GH-IGF axis after *V. anguillarum* challenge. Differentially expressed genes in Brian (A–C), kidney (D–F) and Spleen (G–I) are shown. The C, S and R represent control group, sensitive group, and resistant group, respectively. Red color indicates enhanced transcription whereas cyan color represents repressed transcription. The numbers above and below the color scale represent the maximum and minimum values of log2 (Fold Change). Details of figures are displayed in Table S2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
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3.3. Principle component analysis and heatmap

The separated PCA plots were generated by principle component 1 and 2 (PC1 and PC2) in brain (Fig. 4A) and peripheral immune tissues (Fig. 4B), indicating that trout exerts group-specific gene transcriptions in response to *V. anguillarum* challenge in GHRH-SST-GH-IGF axis. In brain, control group and sensitive group were clustered closely (Fig. 4A), while the three groups were more distantly related in peripheral immune tissues (Fig. 4B). Heatmaps showed the transcriptional profiles of genes in GHRH-SST-GH-IGF axis could be further classified into six clusters in brain and five clusters in kidney and spleen (Fig. 4C and D).

3.4. Identification of the key gene(s) resulting in group-specific responses in brain

The loading plot exerts how strongly each variable influences a principal component. In the loading plot, variables located further away from the center exhibit stronger influences and variables contributing similar information (or with high similarity) are grouped together. We observed that genes including *sst* subtypes, *igf1a* subtypes, *igfbp3b1*, and *igfbp4* subtypes strongly influenced the group-specific brain gene transcriptions in response to *V. anguillarum* challenge (Fig. 5A, genes with high similarity were marketed by colorful circles).

Analyses of correlation coefficients and VIP could effectively identify the genes that most heavily drove the group-specific brain gene transcriptions. Variables with higher VIP scores and correlation coefficients were considered significant and discriminatory. Results of correlation coefficients and VIP analyses (Fig. 5C and D) supported the results of loading plot (colorful circles in Fig. 5A correspond to colorful bars in Fig. 5B and C), indicating that *sst* subtypes, *igf1a* subtypes, *igfbp3b1*, and *igfbp4* subtypes displayed significant effects on group-specific brain gene transcriptions of trout in response to *V. anguillarum* challenge. Based on the results of Fig. 5A–C, the relative expressions of the representative genes are shown in Fig. 5D–K.

3.5. Identification of the key gene(s) resulting in group-specific responses in peripheral immune tissues

The loading plot exerts that genes including *ghr1a*, *igf1a* subtypes, *igfbp5* subtypes, *igfbp6a* subtypes and *igf2bp* subtypes strongly influenced the group-specific gene transcriptions in peripheral immune tissues of trout in response to *V. anguillarum* challenge (Fig. 6A, genes with high similarity were marketed by colorful circles). Results of correlation coefficients and VIP analyses (Fig. 6C and D) further supported the results of loading plot. The relative expressions of representative genes in peripheral immune tissues are shown in Fig. 6D - K.

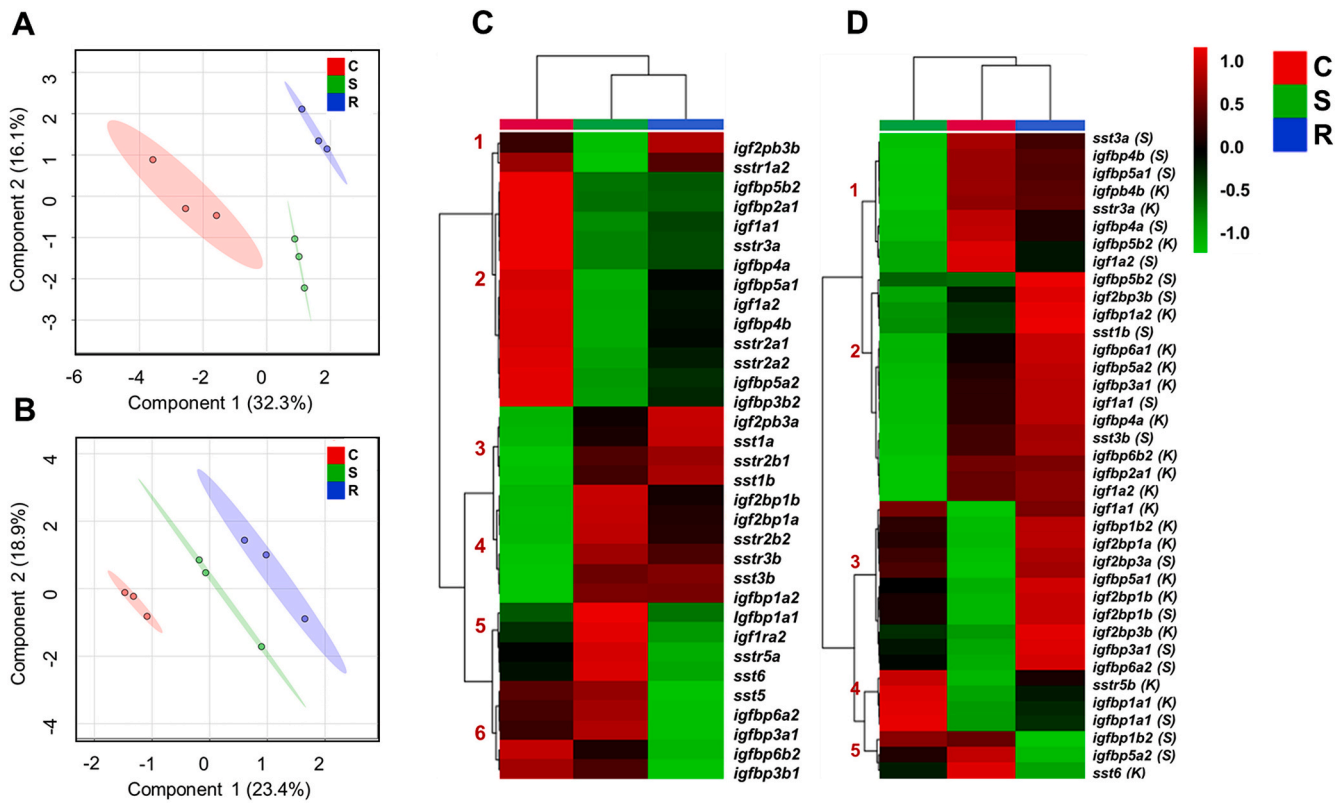

Fig. 4. Principal component analysis (A, brain; B, peripheral immune tissues) and heatmap (C, brain; D, peripheral immune tissues) of genes in GHRH-GH-SST-IGF axis after *V. anguillarum* challenge. The C, S and R represent control group, sensitive group, and resistant group, respectively. In the heatmap, red color indicates up-regulation and green color indicates down-regulation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
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4. Discussion

4.1. Novel sstr and igf2bp paralogs

In this study, we identified 12 *sstr* and 4 *igf2bp* paralogs in rainbow trout (Fig. 2 and Figs. S2–S5), which is consistent with previous studies showing ssWGD results in significant genetic expansions of *akirin and hox* genes in Salmonids [78,79]. We also showed two trout IGFR subtypes (IGF-IRa1 and IGF-IRa2), which are previously identified by Alzaid et al. [12], contained conserved functional domains with the orthologs of human and zebrafish. Given that previous studies have already investigated the expanded paralogs of GH and IGF systems [4,35,39–45,80], we provided new insight into the characterization and evaluation of the complete repertoire of GHRH-SST-GH-IGF axis in rainbow trout. We also revealed that *sst*, *igfpbs* and *igf2bps* genes probably play important roles in regulating the crosstalk between immune and growth systems.

4.2. V. anguillarum infection affected the transcriptions of genes which were involved in growth regulation

With a finite set of energetic resources, teleosts need to effectively allocate the energy into different physiological processes. Consequently, an energy trade-off is exhibited between immunity and growth [4,81,82]. For example, previous studies showed that activation of the immune system results in reallocation of energy away from growth in trout [4,83]. IGFs are important in regulating cellular growth, proliferation and apoptosis [84,85], while IGFPB1s inhibit the IGFs functions [60,86]. In this study, *V. anguillarum* infection resulted in decreased *ghr1a*, *igf1a1* and *igf1a2* expressions in trout (Fig. 3). Compared to trout of resistant group, trout in sensitive group showed significantly up-regulated *igfbp1a1* and down-regulated *igf1a1* and *igf1a2* after *V. anguillarum* challenge (Fig. 3). The GO analysis showed the GO terms associated with growth and apoptotic regulations were significantly changed by *V. anguillarum* infection, with the involvement of *igf1a1* and *igf1a2* (Fig. S6). Based on our results and previous studies [4], we proposed the physiological function of IGF1 was inhibited by *V. anguillarum* infection due to the energy trade-off between growth performance and immunological competence.

4.3. V. anguillarum infection affected the transcriptions of genes which were involved in neuro-endocrine-immune network

4.3.1. The SST system

In addition to the canonical functions of suppressing growth, recent studies showed mammalian SSTs modulate immune systems and stressful responses (regulated by hypothalamus-pituitary-adrenal (HPA) axis) [87,88]. In teleosts, the stressful responses (regulated by hypothalamus-pituitary-interrenal (HPI) axis) and immune systems are closely connected by neuro-endocrine-immune network [3]. Long-term activation of the HPI axis results in energy cost, thus attenuating immune competence and endocrine homeostasis [3,83]. In this study, we revealed several brain *sst* genes significantly contributed to the discriminatory features in PCA (Figs. 4A and 5A - 5E)). These results indicated that the SSTs have conserved physiological functions in regulating neuro-endocrine-immune system in vertebrates.

We also observed the differently expressed *sstr* genes after *V. anguillarum* infection (Figs. 3 and 5A - 5C), showing that SSTR systems regulate the neuro-endocrine-immune network in trout, which was consistent with previous studies in mammals [88–90]. SST-SSTR system

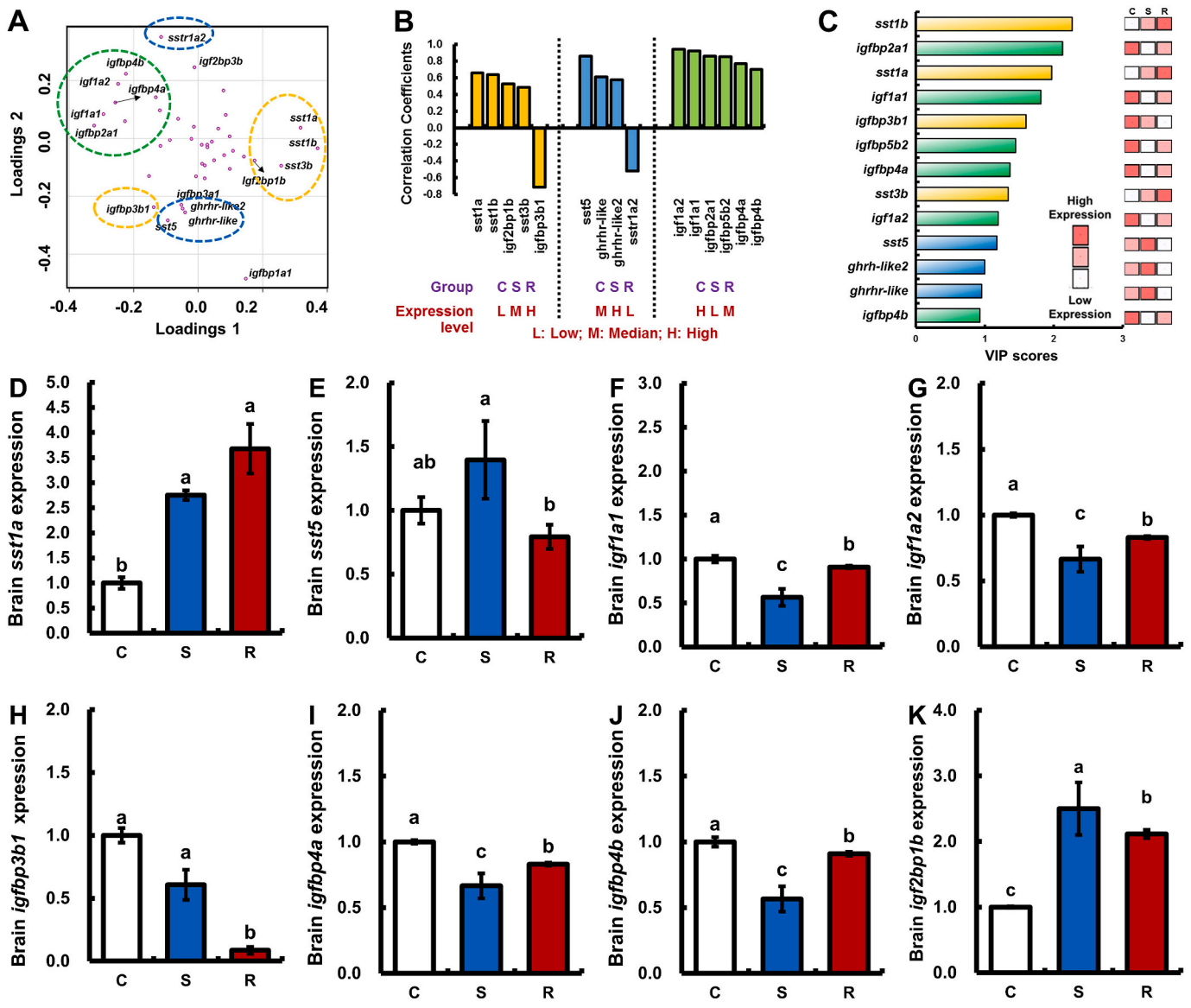

Fig. 5. Identification of the key gene(s) strongly involved in group-specific responses of GHRH-SST-GH-SST axis in brain. The key genes were identified by loading plot of the PLS-DA (A), correlation coefficients analysis (B) and variable important in projection (VIP) analysis (C). In the loading plot, variables located further away from the center exhibit stronger influences and variables contributing similar information (or with high similarity) are grouped together. Variables with higher VIP scores (VIP score > 1.1, [112]) and correlation coefficients were considered significant and discriminatory. The colorful circles in Fig. 5A correspond to colorful bars in Fig. 5B and 5C. The relative expressions of the representative genes are shown (D - K, different letters indicate significant differences among groups (one-way analysis ANOVA followed by Tukey's multiple comparison test with $p < 0.05$). The C, S, and R represent control group, sensitive group, and resistant group, respectively. Details of figures are shown in Tables S2–S4.
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is mainly expressed in central nervous system (CNS) in mammals [73, 91], while we observed significantly down-regulated kidney *sstr5a* (Fig. 3F). Previous studies showed SSTRs activate the IGF-induced apoptosis via intracellular signaling transduction, rather than the extracellularly endocrine axis of SST-GH-IGF axis [88]. These evidences indicated that the peripheral SSTRs of trout were probably associated with the regulating of immune responses. In order to investigate the intracellular signaling pathways that are associated with immune responses, our further studies will concentrate on pharmacological characteristics of trout SST-SSTR systems.

4.3.2. The IGFBPs system

In blood circulatory systems and/or local tissues, IGF molecules are present in a complex with IGFBPs [92]. Compared to the IGFR, IGFBPs exhibit an equal or greater affinity to the IGFs, thus serving as key carriers and governors in regulating the functions of IGFs [46,93]. There already exists several studies showing that IGFBPs regulate immune responses in both teleosts and mammals [5–7]. For example, previous studies showed *igfbp1a1* is involved in cytokines regulation in trout in response to *A. salmonicida* infection [4]. In this study, we observed that trout in sensitive group exhibited up-regulated spleen and kidney *igfbp1a1* after *V. anguillarum* challenge (Fig. 3). We also observed group-specific transcriptions of *igfbp4* and *igfbp5* genes. Although trout of resistant group and sensitive group both exhibited down-regulated *igfbp4a, igfbp4b* or *igfbp5b2,* trout of sensitive group exerted greater down-regulations when compared to the trout of resistant group. In mammals, IGFBP4 and IGFBP5 exert opposite actions in modulating IGF-regulated growth and development [84,94–96]. However, studies in Salmonidae fishes are more consistent, indicating that both *igfbp4* and *igfbp5* promote fish growth [15,26,97]. Trout in sensitive group had to

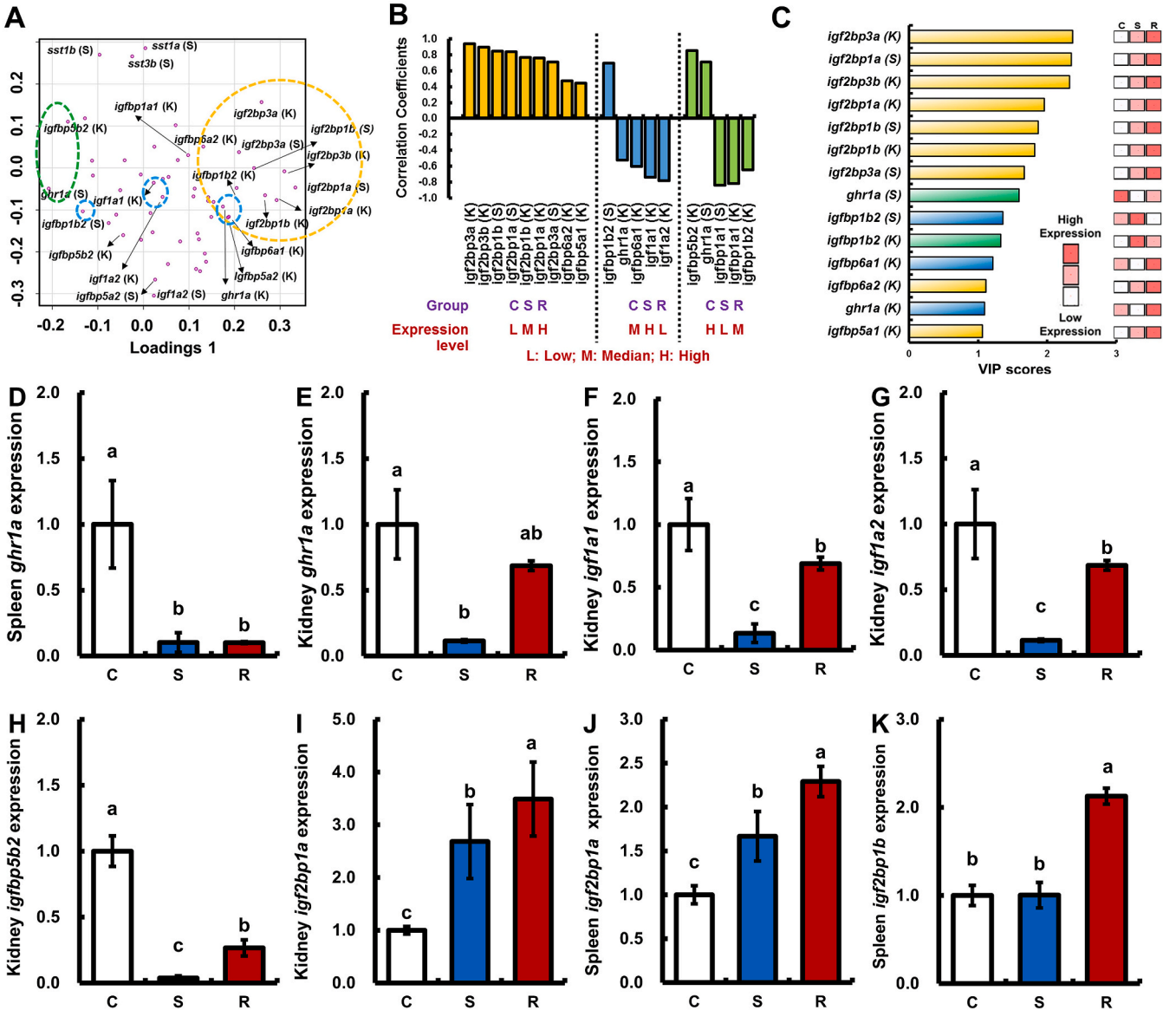

Fig. 6. Identification of the gene(s) strongly involved in group-specific responses of GHRH-SST-GH-SST axis in peripheral immune tissues. The key genes were identified by loading plot of the PLS-DA (A), correlation coefficients analysis (B) and variable important in projection (VIP) analysis (C). In the loading plot, variables located further away from the center exhibit stronger influences and variables contributing similar information (or with high similarity) are grouped together. Variables with higher VIP scores (VIP score > 1.1, [112]) and correlation coefficients were considered significant and discriminatory. The colorful circles in Fig. 6A correspond to colorful bars in Fig. 6B and 6C. The relative expressions of the representative genes are shown (D - K, different letters indicate significant differences among groups (one-way analysis ANOVA followed by Tukey's multiple comparison test with $p < 0.05$). The C, S, and R represent control group, sensitive group, and resistant group, respectively. Details of figures are shown in Table S2, S5 and S6.
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allocate more energy to immune responses, thus exhibiting growth retardation with greater down-regulated *igfbp4a, igfbp4b* or *igfbp5b2.*

4.3.3. *The IGF2BPs system*

Human and rat studies showed *IGF2BPs* are highly expressed during embryo and fetal stages, regulating cell functions including migration, proliferation and differentiation [74,98]. Indeed, IGF2BPs are pro-survival and anti-apoptotic factors [74]. Expressions of IGF2BPs are normally repressed in adult tissues but become reactivated in neoplasm and even cancer. Studies concentrated on physiological functions of teleost *igf2bps* were limited. Our studies showed that the up-regulated *igf2bp1s* and *igf2bp3s* in immune tissues strongly influenced the group-specific gene transcriptions of GHRH-SST-GH-IGF axis of trout in response to *V. anguillarum* infection (Figs. 3, 4B and 6). These results suggested *V. anguillarum* infection probably resulted in abnormally cellular functions such as hyperplasia and neoplasia, which is consistent with previous study in turbot (*Scophthalmus maximus*), in which *V. anguillarum* infection activates the cancer associated signaling pathways [99]. We also observed that the differently expressed genes in responses to *V. anguillarum* infection were enriched in cancer and/or apoptosis associated signaling pathways (Fig. S6). All these results were consistent with previous studies [100–104], showing bacterial infection has been linked to chronic inflammation and dyshomeostasis of cellular proliferation, differentiation and apoptosis.

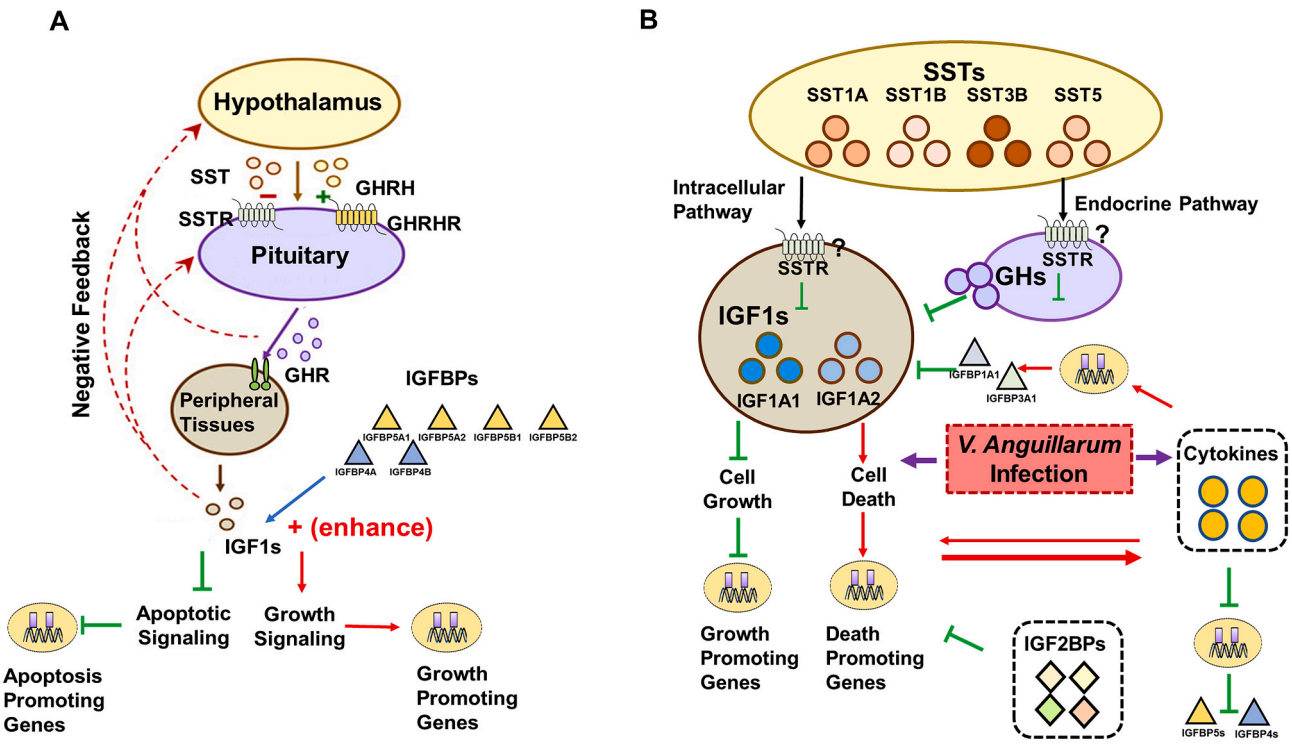

Fig. 7. A proposed model to describe the endocrine-immunity network-modulated crosstalk between cell survival and programmed death. In normal conditions (A), trout IGFs systems (IGF1s, IGFBP4s and IGFBP5s), which are modulated by GHRH-SST-GH-IGF axis, predominately stimulate the cellular growth and proliferation and inhibit the apoptosis, thus promoting the growth and development. In bacterial infection conditions (B), IGF1s- and IGFBP3A1-regulated programmed cell death is predominated despite the pro-survival factors, such as IGF2BPs, up-regulation. The endocrine-immune network probably contributed to the pathophysiology: 1, *V. anguillarum* infection and the resulting inflammation cause the programmed death (apoptosis and/or necrosis) of the infected cells, preventing bacteria further proliferation. The dead cells further release inflammatory cytokines and these cytokines stimulate the transcriptions of IGFPB1A1 to prevent IGF1s functions. 2, SSTs could also suppress the IGF1s functions via endocrine inhibition of SST-GH-IGF axis and/or directly intracellular signaling pathway. These proposed mechanisms are based on previous studies on IGF systems-regulated cell proliferation, differentiation or apoptosis [91–94], and interactions among bacterial infection, inflammation and the programmed cell death [64–66].
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4.4. A potential model which describes the IGF- and IGFBPs-regulated crosstalk between cell growth and programmed death

Previous studies showed that the IGF systems regulate the interactions among bacterial infection, inflammation and the programmed cell death [105–111]. Based on these evidences, we proposed a hypothetical model to describe the IGF- and IGFBPs-regulated crosstalk between cell growth and programmed death. In normal conditions (Fig. 7A), trout IGFs systems (IGF1s, IGFBP4s and IGFBP5s) promoted the growth and development by predominately stimulating the cellular growth and proliferation, and inhibiting cellular apoptosis. In bacterial infection conditions (Fig. 7B), although the pro-survival factors (IGF2BPs) were induced, the IGFBP1s and IGFBP3A1 predominately resulted in programmed cell death. The endocrine-immune network probably contributed to the pathophysiological processes: 1, *V. anguillarum* infection and the resulting inflammation cause the programmed death (apoptosis and/or necrosis) of the infected cells, preventing bacteria further proliferation. The dead cells further release inflammatory cytokines, and these cytokines could suppress the IGF1s functions. 2, the SSTs could further suppress the IGF1s functions via inhibiting the SST-GH-IGF axis.

5. Conclusion

In this study, we showed ssWGD has expanded the gene copies of trout GHRH-SST-GH-IGF axis. Based on *V. anguillarum* challenge experiment, we further revealed that the gene transcriptional profiles of *sst*, *igfpbs* and *igf2bps* were significantly changed by *V. anguillarum* infection. Gene enrichment analyses showed the differently expressed genes were enriched in functions associated with crosstalk between immune and growth regulations. Our results presented here could improve the understanding of the pleiotropic effects of GHRH-SST-GH-IGF axis in regulating crosstalk between growth and immunity in trout.

CRediT authorship contribution statement

Zhi-Shuai Hou: Conceptualization, Project administration, Writing - original draft, Writing - review & editing. **Yuan-Ru Xin:** Methodology, Project administration, Resources, Investigation. **Chu Zeng:** Methodology, Project administration, Resources, Investigation. **Hong-Kui Zhao:** Methodology, Investigation. **Yuan Tian:** Methodology. **Ji-Fang Li:** Conceptualization, Project administration, Writing - original draft, Writing - review & editing. **Hai-Shen Wen:** Conceptualization, Project administration, Writing - original draft, Writing - review & editing.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2020.08.037.

References

[1] J.D. Veldhuis, A. Iranmanesh, D. Erickson, F. Roelfsema, C.Y. Bowers, Lifetime regulation of growth hormone (GH) secretion, in: G. Fink, D.W. Pfaff, J.W. Levine (Eds.), Handbook of Neuroendocrinology, Academic Press, New York, 2012, pp. 237–257.

[2] K. Gunawardane, T.H. Krarup, J.C. Sandahl, J.J. Lunde, Normal physiology of growth hormone in adults, in: L.J. De Groot, G. Chrousos, K. Dungan, K. R. Feingold, A. Grossman, J.M. Hershman, C. Koch, M. Korbonits, R. McLachlan, M. New, J. Purnell, R. Rebar, F. Singer, A. Vinik (Eds.), Endotext, MDText.com, Inc., South Dartmouth, MA, 2000.

[3] L. Tort, Stress and immune modulation in fish, Dev. Comp. Immunol. 35 (2011) 1366–1375.

[4] A. Alzaid, R. Castro, T. Wang, C.J. Secombes, P. Boudinot, D.J. Macqueen, S.A. M. Martin, Cross talk between growth and immunity: coupling of the IGF axis to conserved cytokine pathways in rainbow trout, Endocrinology 157 (2016) 1942–1955.

[5] J.C. O'Connor, R.H. McCusker, K. Strle, R.W. Johnson, R. Dantzer, K.W. Kelley, Regulation of IGF-I function by proinflammatory cytokines: at the interface of immunology and endocrinology, Cell, Immunol. 252 (2008) 91–110.

[6] T.J. Smith, Insulin-like growth factor-I regulation of immune function: a potential therapeutic target in autoimmune diseases? Pharmacol. Rev. 62 (2010) 199–236.

[7] N.J. Pooley, L. Tacchi, C.J. Secombes, S.A.M. Martin, Inflammatory responses in primary muscle cell cultures in Atlantic salmon (*Salmo salar*), BMC Genom. 14 (2013) 747.

[8] M. Wenger, N. Shved, G. Akgül, A. Caelers, A. Casanova, H. Segner, E. Eppler, Developmental oestrogen exposure differentially modulates IGF-I and TNF-α expression levels in immune organs of *Yersinia ruckeri*-challenged young adult rainbow trout (*Oncorhynchus mykiss*), Gen. Comp. Endocrinol. 205 (2014) 168–175.

[9] J.H. Kim, D.J. Macqueen, J.R. Winton, J.D. Hansen, H. Park, R.H. Devlin, Effect of growth rate on transcriptomic responses to immune stimulation in wild-type, domesticated, and GH-transgenic coho salmon, BMC Genom. 20 (2019) 1024.

[10] J. Pérez-Sánchez, P. Simó-Mirabet, F. Naya-Català, J.A. Martos-Sitcha, E. Perera, A. Bermejo-Nogales, L. Benedito-Palos, J.A. Calduch-Giner, Somatotropic axis regulation unravels the differential effects of nutritional and environmental factors in growth performance of marine farmed fishes, Front. Endocrinol. 9 (2018) 687.

[11] A. Alzaid, J.H. Kim, R.H. Devlin, S.A.M. Martin, D.J. Macqueen, Growth hormone transgenesis in coho salmon disrupts muscle immune function impacting cross-talk with growth systems, J. Exp. Biol. 221 (2018).

[12] A. Alzaid, S.A.M. Martin, D.J. Macqueen, The complete salmonid IGF-IR gene repertoire and its transcriptional response to disease, Sci. Rep. 6 (2016) 34806.

[13] Y.H. Choi, K.W. Kim, H.S. Han, T.J. Nam, B.J. Lee, Dietary Hizikia fusiformis glycoprotein-induced IGF-I and IGFBP-3 associated to somatic growth, polyunsaturated fatty acid metabolism, and immunity in juvenile olive flounder *Paralichthys olivaceus*, Comp. Biochem. Physiol. A Mol. Integr. Physiol. 167 (2014) 1–6.

[14] Y. Chen, Y. Zhou, X. Yang, Z. Cao, X. Chen, Q. Qin, C. Liu, Y. Sun, Insulin-like growth factor binding protein 3 gene of golden pompano (*TroIGFBP3*) promotes antimicrobial immune defense, Fish Shellfish Immunol. 103 (2020) 47–57.

[15] D. Garcia de la Serrana, D.J. Macqueen, Insulin-like growth factor-binding proteins of teleost fishes, Front. Endocrinol. 9 (2018) 80.

[16] K. Hirokawa, M. Utsuyama, Neuro-endocrine-immune network and its age-related changes, in: T. Fulop, C. Franceschi, K. Hirokawa, G. Pawelec (Eds.), Handbook on Immunosenescence, Springer, 2009, pp. 785–798.

[17] G.S.J. Pande, N.T. Suong, P. Bossier, T. Defoirdt, The catecholamine stress hormones norepinephrine and dopamine increase the virulence of pathogenic *Vibrio anguillarum* and *Vibrio campbellii*, EMS Microbiol. Immunol. 90 (2014) 761–769.

[18] Z. Zhou, L. Wang, X. Shi, H. Zhang, Y. Gao, M. Wang, P. Kong, L. Qiu, L. Song, The modulation of catecholamines to the immune response against bacteria *Vibrio anguillarum* challenge in scallop *Chlamys farreri*, Fish Shellfish Immunol. 31 (2011) 1065–1071.

[19] A.G. Maule, R.A. Tripp, S.L. Kaattari, C.B. Schreck, Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*), J. Endocrinol. 120 (1989) 135–142.

[20] F.E. Reyes-López, J. Aerts, E. Vallejos-Vidal, B. Ampe, K. Dierckens, L. Tort, P. Bossier, Modulation of innate immune-related genes and glucocorticoid synthesis in gnotobiotic full-sibling European sea bass (*Dicentrarchus labrax*) larvae challenged with *Vibrio anguillarum*, Front. Immunol. 9 (2018) 914.

[21] E. Faught, N. Aluru, M.M. Vijayan, The molecular stress response, in: C. B. Schreck, L. Tort, A.P. Farrell, C.J. Braune (Eds.), Fish Physiol., Elsevier, 2016, pp. 113–166.

[22] B. Sadoul, M.M. Vijayan, Stress and growth, in: C.B. Schreck, L. Tort, A.P. Farrell, C.J. Braune (Eds.), Fish Physiol., Elsevier, 2016, pp. 167–205.

[23] C.B. Schreck, L. Tort, The concept of stress in fish, in: C.B. Schreck, L. Tort, A. P. Farrell, C.J. Braune (Eds.), Fish Physiol., Elsevier, 2016, pp. 1–34.

[24] C. Berthelot, F. Brunet, D. Chalopin, A. Juanchich, M.P. Bernard, B. Noël, P. Bento, C. Da Silva, K. Labadie, A. Alberti, The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates, Nat. Commun. 5 (2014) 1–10.

[25] F.M. Lappin, R.L. Shaw, D.J. Macqueen, Targeted sequencing for high-resolution evolutionary analyses following genome duplication in salmonid fish: proof of concept for key components of the insulin-like growth factor axis, Mar. Genomics 30 (2016) 15–26.

[26] E.N. Fuentes, S.A.M. Martin, I.A. Johnston, D.J. Macqueen, Divergent regulation of insulin-like growth factor binding protein genes in cultured Atlantic salmon myotubes under different models of catabolism and anabolism, Gen. Comp. Endocrinol. 247 (2017) 53–65.

[27] D.J. Macqueen, I.A. Johnston, A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification, Proc. R. Soc. Lond. B Biol. Sci. 281 (2014) 20132881.

[28] P. Dehal, J.L. Boore, Two rounds of whole genome duplication in the ancestral vertebrate, PLoS Biol. 3 (2005) e314.

[29] S. Ohno, Evolution by Gene Duplication Springer, Springer-Verlag, Berlin, 1970, p. 160.

[30] D. Steinke, S. Hoegg, H. Brinkmann, A. Meyer, Three rounds (1R/2R/3R) of genome duplications and the evolution of the glycolytic pathway in vertebrates, BMC Biol. 4 (2006) 16.

[31] A. Sidow, Gen (om) e duplications in the evolution of early vertebrates, Curr. Opin. Genet. Dev. 6 (1996) 715–722.

[32] A.C. Sharman, P.W.H. Holland, Conservation, duplication, and divergence of developmental genes during chordate evolution, Neth. J. Zool. 46 (1995) 47–67.

[33] O. Jaillon, J.M. Aury, F. Brunet, J.L. Petit, N. Stange-Thomann, E. Mauceli, L. Bouneau, C. Fischer, C. Ozouf-Costaz, A. Bernot, Genome duplication in the teleost fish Tetraodon nigroviridis reveals the early vertebrate proto-karyotype, Nature 431 (2004) 946–957.

[34] Y. Van de Peer, E. Mizrachi, K. Marchal, The evolutionary significance of polyploidy, Nat. Rev. Genet. 18 (2017) 411.

[35] D.J. Macqueen, D. Garcia de la serrana, I.A. Johnston, Evolution of ancient functions in the vertebrate insulin-like growth factor system uncovered by study of duplicated salmonid fish genomes, Mol. Biol. Evol. 30 (2013) 1060–1076.

[36] F.W. Allendorf, G.H. Thorgaard, Tetraploidy and the evolution of salmonid fishes, in: B.J. Turner (Ed.), Evolutionary Genetics of Fishes, Plenum Press, New York, 1984, pp. 1–53.

[37] S. Lien, B.F. Koop, S.R. Sandve, J.R. Miller, M.P. Kent, T. Nome, T.R. Hvidsten, J. S. Leong, D.R. Minkley, A. Zimin, The Atlantic salmon genome provides insights into rediploidization, Nature 533 (2016) 200–205.

[38] K.R. Von Schalburg, R. Yazawa, J. De Boer, K.P. Lubieniecki, B.C. Goh, C. A. Straub, M.R. Beetz-Sargent, A. Robb, W.S. Davidson, R.H. Devlin, Isolation, characterization and comparison of Atlantic and Chinook salmon growth hormone 1 and 2, BMC Genom. 9 (2008) 522.

[39] S.J. McKay, J. Trautner, M.J. Smith, B.F. Koop, R.H. Devlin, Evolution of duplicated growth hormone genes in autotetraploid salmonid fishes, Genome 47 (2004) 714–723.

[40] T. Akbarialmajough, R. Manaffar, E.H. Najdegerami, P. Bossier, New insight into the diversity of exon and intron 5 regions of *Oncorhynchus mykiss* growth hormone (GH) gene: sexual dimorphism in the GH gene, Rev. Aquacult. 12 (2020) 254–260.

[41] M.V. Pankova, A.D. Kukhlevsky, V.A. Brykov, Fish growth hormone genes: divergence of coding sequences in salmonid fishes, Russ. J. Genet. 53 (2017) 221–232.

[42] N.M. Very, J.D. Kittilson, L.A. Norbeck, M.A. Sheridan, Isolation, characterization, and distribution of two cDNAs encoding for growth hormone receptor in rainbow trout (*Oncorhynchus mykiss*), Comp. Biochem. Physiol. B Biochem. Mol. Biol. 140 (2005) 615–628.

[43] C.N. Walock, J.D. Kittilson, M.A. Sheridan, Characterization of a novel growth hormone receptor-encoding cDNA in rainbow trout and regulation of its expression by nutritional state, Gene 533 (2014) 286–294.

[44] F.M. Robertson, M.K. Gundappa, F. Grammes, T.R. Hvidsten, A.K. Redmond, S. Lien, S.A.M. Martin, P.W.H. Holland, S.R. Sandve, D.J. Macqueen, Lineage-specific rediploidization is a mechanism to explain time-lags between genome duplication and evolutionary diversification, Genome Biol. 18 (2017) 111.

[45] D.O. Daza, D. Larhammar, Evolution of the growth hormone, prolactin, prolactin 2 and somatolactin family, Gen. Comp. Endocrinol. 264 (2018) 94–112.

[46] D.O. Daza, G. Sundström, C.A. Bergqvist, C. Duan, D. Larhammar, Evolution of the insulin-like growth factor binding protein (IGFBP) family, Endocrinology 152 (2011) 2278–2289.

[47] B. Austin, D.A. Austin, in: B. Austin, D.A. Austin (Eds.), Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, Springer, 2012.

[48] H. Naka, J.H. Crosa, Genetic determinants of virulence in the marine fish pathogen *Vibrio anguillarum*, Fish Pathol. 46 (2011) 1–10.

[49] I. Frans, C.W. Michiels, P. Bossier, K.A. Willems, B. Lievens, H. Rediers, *Vibrio anguillarum* as a fish pathogen: virulence factors, diagnosis and prevention, J. Fish. Dis. 34 (2011) 643–661.

[50] X. Tang, L. Zhou, W. Zhan, Isolation and characterization of pathogenic *Listonella anguillarum* of diseased half-smooth tongue sole (*Cynoglossus semilaevis* Günther), J. Ocean Univ. China 7 (2008) 343–351.

[51] J. Xing, H. Xu, Y. Wang, X. Tang, X. Sheng, W. Zhan, Protective efficacy of six immunogenic recombinant proteins of *Vibrio anguillarum* and evaluation them as vaccine candidate for flounder (*Paralichthys olivaceus*), Microb. Pathog. 107 (2017) 155–163.

[52] J. Xing, H. Xu, Y. Wang, X. Tang, X. Sheng, W. Zhan, Identification of immunogenic proteins and evaluation of four recombinant proteins as potential vaccine antigens from *Vibrio anguillarum* in flounder (*Paralichthys olivaceus*), Vaccine 35 (2017) 3196–3203.

[53] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120.

[54] D. Kim, B. Langmead, S.L. Salzberg, HISAT: a fast spliced aligner with low memory requirements, Nat. Methods 12 (2015) 357.

[55] S. Anders, P.T. Pyl, W. Huber, HTSeq.a Python framework to work with high-throughput sequencing data, Bioinformatics 31 (2015) 166–169.

[56] A. Roberts, H. Pimentel, C. Trapnell, L. Pachter, Identification of novel transcripts in annotated genomes using RNA-Seq, Bioinformatics 27 (2011) 2325–2329.

[57] A. Roberts, C. Trapnell, J. Donaghey, J.L. Rinn, L. Pachter, Improving RNA-Seq expression estimates by correcting for fragment bias, Genome Biol. 12 (2011) R22.

[58] B. Li, C.N. Dewey, RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome, BMC Bioinf. 12 (2011) 323.

[59] S. Anders, W. Huber, Differential expression analysis for sequence count data, Genome Biol. 11 (2010) R106.

[60] Z.S. Hou, H.S. Wen, J.F. Li, F. He, Y. Li, X. Qi, Environmental hypoxia causes growth retardation, osteoclast differentiation and calcium dyshomeostasis in juvenile rainbow trout (*Oncorhynchus mykiss*), Sci. Total Environ. 705 (2020) 135272.

[61] M.D. Young, M.J. Wakefield, G.K. Smyth, A. Oshlack, Gene ontology analysis for RNA-seq: accounting for selection bias, Genome Biol. 11 (2010) R14.

[62] M. Kanehisa, M. Araki, S. Goto, M. Hattori, M. Hirakawa, M. Itoh, T. Katayama, S. Kawashima, S. Okuda, T. Tokimatsu, KEGG for linking genomes to life and the environment, Nucleic Acids Res. 36 (2007) D480–D484.

[63] H. Zhao, O. Soufan, J. Xia, R. Tang, L. Li, D. Li, Transcriptome and physiological analysis reveal alterations in muscle metabolisms and immune responses of grass carp (*Ctenopharyngodon idellus*) cultured at different stocking densities, Aquaculture 503 (2019) 186–197.

[64] J. Chong, D.S. Wishart, J. Xia, Using metaboanalyst 4.0 for comprehensive and integrative metabolomics data analysis, Curr. Protoc. Bioinformatics. 68 (2019) e86.

[65] J. Chong, J. Xia, MetaboAnalystR: an R package for flexible and reproducible analysis of metabolomics data, Bioinformatics 34 (2018) 4313–4314.

[66] R. Terrado, V. Campbell, D.A. Caron, K.B. Heidelberg, Changes in gene expression of *Prymnesium parvum* induced by nitrogen and phosphorus limitation, Front. Microbiol. 6 (2015) 631.

[67] Y. Zhang, K. Chen, S.A. Sloan, M.L. Bennett, A.R. Scholze, S. O'Keeffe, H. P. Phatnani, P. Guarnieri, C. Caneda, N. Ruderisch, An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex, J. Neurosci. 34 (2014) 11929–11947.

[68] S. Mager, U. Ludewig, Massive loss of DNA methylation in nitrogen-, but not in phosphorus-deficient *Zea mays* roots is poorly correlated with gene expression differences, Front. Plant Sci. 9 (2018) 497.

[69] G.E. Rovati, V. Capra, R.R. Neubig, The highly conserved DRY motif of class AG protein-coupled receptors: beyond the ground state, Mol. Pharmacol. 71 (2007) 959–964.

[70] O. Fritze, S. Filipek, V. Kuksa, K. Palczewski, K.P. Hofmann, O.P. Ernst, Role of the conserved NPxxY (x) 5, 6F motif in the rhodopsin ground state and during activation, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 2290–2295.

[71] Y.-X. Tao, The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology, Endocr. Rev. 31 (2010) 506–543.

[72] V.D. Corleto, S. Nasoni, F. Panzuto, S. Cassetta, G. Delle F, Somatostatin receptor subtypes: basic pharmacology and tissue distribution, Digest. liver dis. 36 (2004) S8–S16.

[73] T. Reisine, G.I. Bell, Molecular biology of somatostatin receptors, Endocr. Rev. 16 (1995) 427–442.

[74] J.L. Bell, K. Wächter, B. Mühleck, N. Pazaitis, M. Köhn, M. Lederer, S. Hüttelmaier, Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? Cell. Mol. Life Sci. 70 (2013) 2657–2675.

[75] J. Nielsen, J. Christiansen, J. Lykke-Andersen, A.H. Johnsen, U.M. Wewer, F. C. Nielsen, A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development, Mol. Cell Biol. 19 (1999) 1262–1270.

[76] S. Xie, D. Niu, Y. Du, K. Wei, H.D. Nguyen, J. Li, Expression and characterization of insulin-like growth factor II mRNA binding protein in the razor clam *Sinonovacula constricta*, Aquac. Fish. 2 (2017) 247–255.

[77] C. Duan, J. Ding, Q. Li, W. Tsai, K. Pozios, Insulin-like growth factor binding protein 2 is a growth inhibitory protein conserved in zebrafish, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 15274–15279.

[78] D.J. Macqueen, B.K. Kristjánsson, I.A. Johnston, Salmonid genomes have a remarkably expanded *akirin* family, coexpressed with genes from conserved pathways governing skeletal muscle growth and catabolism, Physiol. Genom. 42 (2010) 134–148.

[79] S. Mungpakdee, H.C. Seo, A.R. Angotzi, X. Dong, A. Akalin, D. Chourrout, Differential evolution of the 13 Atlantic salmon *Hox* clusters, Mol. Biol. Evol. 25 (2008) 1333–1343.

[80] B.N. Madison, S. Tavakoli, S. Kramer, N.J. Bernier, Chronic cortisol and the regulation of food intake and the endocrine growth axis in rainbow trout, J. Endocrinol. 226 (2015) 103–119.

[81] W.M. Rauw, Immune response from a resource allocation perspective, Front. Genet. 3 (2012) 267.

[82] B.C. Sheldon, S. Verhulst, Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology, Trends Ecol. Evol. 11 (1996) 317–321.

[83] Z.S. Hou, H.S. Wen, J.F. Li, F. He, Y. Li, X. Qi, Effects of long-term crowding stress on neuro-endocrine-immune network of rainbow trout (*Oncorhynchus mykiss*), Fish Shellfish Immunol. 95 (2019) 180–189.

[84] C. Duan, H. Ren, S. Gao, Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation, Gen. Comp. Endocrinol. 167 (2010) 344–351.

[85] J.I. Jones, D.R. Clemmons, Insulin-like growth factors and their binding proteins: biological actions, Endocr. Rev. 16 (1995) 3–34.

[86] H. Kamei, Y. Ding, S. Kajimura, M. Wells, P. Chiang, C. Duan, Role of IGF signaling in catch-up growth and accelerated temporal development in zebrafish embryos in response to oxygen availability, Development 138 (2011) 777–786.

[87] A. Stengel, Y.F. Taché, Activation of brain somatostatin signaling suppresses CRF receptor-mediated stress response, Front. Neurosci. 11 (2017) 231.

[88] M. Chalabi, C. Duluc, P. Caron, D. Vezzosi, J. Guillermet-Guibert, S. Pyronnet, C. Bousquet, Somatostatin analogs: does pharmacology impact antitumor efficacy? Trends Endocrinol. Metabol. 25 (2014) 115–127.

[89] S.A. Nanda, C. Qi, P.H. Roseboom, N.H. Kalin, Predator stress induces behavioral inhibition and amygdala somatostatin receptor 2 gene expression, Gene Brain Behav. 7 (2008) 639–648.

[90] A. Faron-Górecka, M. Kuśmider, M. Kolasa, D. Żurawek, K. Szafran-Pilch, P. Gruca, P. Pabian, J. Solich, M. Papp, M. Dziedzicka-Wasylewska, Chronic mild stress alters the somatostatin receptors in the rat brain, Psychopharmacology 233 (2016) 255–266.

[91] L.N. Møller, C.E. Stidsen, B. Hartmann, J.J. Holst, Somatostatin receptors, Biochim. Biophys. Acta Biomembr. 1616 (2003) 1–84.

[92] C. Duan, Q. Xu, Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions, Gen. Comp. Endocrinol. 142 (2005) 44–52.

[93] J.B. Allard, C. Duan, IGF-binding proteins: why do they exist and why are there so many? Front. Endocrinol. 9 (2018) 117.

[94] Y. Ning, A.G.P. Schuller, S. Bradshaw, P. Rotwein, T. Ludwig, J. Frystyk, J. E. Pintar, Diminished growth and enhanced glucose metabolism in triple knockout mice containing mutations of insulin-like growth factor binding protein-3,-4, and-5, Mol. Endocrinol. 20 (2006) 2173–2186.

[95] Y. Ning, A.G.P. Schuller, C.A. Conover, J.E. Pintar, Insulin-like growth factor (IGF) binding protein-4 is both a positive and negative regulator of IGF activity *in vivo*, Mol. Endocrinol. 22 (2008) 1213–1225.

[96] C. Duan, J.B. Allard, Insulin-like growth factor binding protein-5 in physiology and disease, Front. Endocrinol. 11 (2020) 100.

[97] L. Karthik, G. Kumar, T. Keswani, A. Bhattacharyya, S.S. Chandar, K.V.B. Rao, Protease inhibitors from marine actinobacteria as a potential source for antimalarial compound, PloS One 9 (2014).

[98] J. Brouwer-Visser, G.S. Huang, IGF2 signaling and regulation in cancer, Cytokine Growth Factor Rev. 26 (2015) 371–377.

[99] K. Zhang, X. Liu, M. Han, Y. Liu, X. Wang, H. Yu, J. Liu, Q. Zhang, Functional differentiation of three phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) in response to Vibrio anguillarum infection in turbot (*Scophthalmus maximus*), Fish Shellfish Immunol. 92 (2019) 450–459.

[100] Y. Zhang, X. Wang, H. Li, C. Ni, Z. Du, F. Yan, Human oral microbiota and its modulation for oral health, Biomed. Pharmacother. 99 (2018) 883–893.

[101] J. Parsonnet, Bacterial infection as a cause of cancer, Environ. Health Perspect. 103 (1995) 263–268.

[102] J.M. Grizzle, A.E. Goodwin, Neoplasms and related disorders, in: J.F. Leatherland, P.T.F. Woo (Eds.), Fish Diseases and Disorders, CABI, Wallingford, 2010, pp. 19–84.

[103] E.D. Lombardini, G.C. Hard, J.C. Harshbarger, Neoplasms of the urinary tract in fish, Vet. Pathol. 51 (2014) 1000–1012.

[104] T.M. Karpiński, Role of oral microbiota in cancer development, Microorganisms 7 (2019) 20.

[105] P. Delafontaine, Y. Song, Y. Li, Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 435–444.

[106] A. Kasprzak, A. Adamek, The insulin-like growth factor (IGF) signaling axis and hepatitis C virus-associated carcinogenesis, Int. J. Oncol. 41 (2012) 1919–1931.

[107] W.F. Blum, A. Alherbish, A. Alsagheir, A. El Awwa, W. Kaplan, E. Koledova, M. O. Savage, The growth hormone–insulin-like growth factor-I axis in the diagnosis and treatment of growth disorders, Endocr. Connect. 7 (2018) R212–R222.

[108] R. Kooijman, Regulation of apoptosis by insulin-like growth factor (IGF)-I, cytokine growth factor rev 17 (2006) 305–323.

[109] S. Nagata, M. Tanaka, Programmed cell death and the immune system, Nat. Rev. Immunol. 17 (2017) 333.

[110] E.H. Kim, S.W. Wong, J. Martinez, Programmed Necrosis and Disease: we interrupt your regular programming to bring you necroinflammation, Cell Death Differ. 26 (2019) 25–40.

[111] M. Lancellotti, R.F.C. Pereira, G.G. Cury, L.M. Hollanda, Pathogenic and opportunistic respiratory bacteria-induced apoptosis, Braz. J. Infect. Dis. 13 (2009) 226–231.

[112] J.A. Martos-Sitcha, P. Simó-Mirabet, V. de Las Heras, J.À. Calduch-Giner, J. Pérez-Sánchez, Tissue-specific orchestration of gilthead sea bream resilience to hypoxia and high stocking density, Front. Physiol. 10 (2019) 840.