Identification and expression analysis of IL-2 receptors in large yellow croaker (Larimichthys crocea)

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Interleukin-2 (IL-2) signals influence various lymphocyte subsets during differentiation, immune responses and homeostasis. IL-2 acts on different cells by binding to its receptors (IL-2R), which consists of three subunits, IL-2Ra (CD25), IL-2Rβ (CD122), and the common gamma chain or γc (CD132). In the present study, three IL-2 receptor subunits, designated as LcCD25-like (LcCD25L), LcIL-2Rβ and Lcγc, were characterized in large yellow croaker (Larimichthys crocea). The LcCD25L, like other teleost CD25L or IL-2/IL-15Ra, contains only one sush domain at N-terminus. The syneity of CD25L from different teleost are conserved. The deduced protein of LcIL-2Rβ and Lcγc exhibits a typical class I cytokine receptors architecture, including a cytokine-binding homology domain (CHD) consisting of two fibronectin type-III (FNIII) domains (D1 and D2) and a conserved WSXWS motif in D2 domain. These three IL-2 receptor subunits were constitutively expressed in all tissues and primary immune-related cells examined. The LcCD25L was highly expressed in blood, while LcIL-2Rβ and Lcγc were highly expressed in spleen and gill. For immune-related cells, LcCD25L and LcIL-2Rβ were highly expressed in PKLs, while the Lcγc exhibited the highest expression in PKMs. These three IL-2 receptor subunits could be dramatically induced by T cell mitogen PHA in PKLs, which mainly composed of T and B lymphocytes. The results presented indicated that large yellow croaker IL-2R might exercise function on lymphocytes, especially on activated T cells.

Introduction

Interleukin 2 (IL-2), a four α-helix bundle cytokine, plays a crucial role in promoting proliferation, differentiation and survival of T cell, as well as the proliferation and antibody production of activated B cells [1–3]. IL-2 can increase the cytolytic activity and cytokine synthesis of both NK and CD8\textsuperscript{+} T cells [4]. The generation, maintenance and regulation of CD8\textsuperscript{+} memory T cells also relies on the function of IL-2 [5]. IL-2 performs its biological functions by binding to three kinds of receptors, the low-affinity, intermediate-affinity and high-affinity IL-2Rs, which are composed of IL-2Ra, IL-2Rβ plus γc, or all three subunits, respectively [6]. IL-2Ra, also designated as CD25, is a proprietary receptor subunit specific for IL-2, while IL-2Rβ is shared by IL-15 and IL-2. Combined with other subunits, the γc can form different receptors for γc cytokine family including IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 [7].

In mammals, CD25 is structurally related to the IL-15Ra. Both of them have extracellular sushi domain followed by a transmembrane domain and a short cytoplasmic domain [8]. The sushi domain is essential for the ligand binding [9,10]. IL-2Rβ and γc are typical class I cytokine receptors consisting of an extracellular cytokine-binding homology domain (CHD) followed by transmembrane and cytoplasmic domain [11]. The CHD consists of two fibronectin type-III (FNIII) domains, D1 and D2, connected by a linker. The D1 domain contains four conserved cysteine residues forming two intramolecular disulfide bonds, and the D2 has a conserved WSXWS motif [11]. Both D1 and D2 are composed of seven β-strands. Unlike receptors for certain growth factors such as insulin and EGF, the cytoplasmic region of IL-2Rβ and γc have no intrinsic protein kinases activity but two proline-rich motifs which involved in JAKs docking. IL-2Rβ is associated with JAK1 and γc is associated with JAK3 [12].

The human CD25 has a short cytoplasmic tail thus incapable of recruiting intracytoplasmic signaling molecules, but could greatly enhance the affinity between IL-2R complex and ligand [13,14]. It has been proved that IL-2 functions through high-affinity receptor, while these IL-2R subunits did not pre-formed a stable heterotrimer [15]. Once IL-2 recognized and bound to CD25, its structure changed slightly. Then the IL-2/CD25 complex associated with IL-2Rβ and finally the γc was recruited [13]. The cytoplasmic tails of both IL-2Rβ and γc contain signaling motifs responsible for transducing signals to the cytoplasm [16,17]. After the IL-2/IL-2R signaling complex formed, the cytoplasmic domain

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of IL-2Rβ and γc got close to each other and resulted in the activation of JAK1 and JAK3 [18–20]. Then the activated JAK1 and JAK3 phosphorylated key residues in IL-2Rβ and caused the activation of JAK-STAT pathway, phosphoinositide 3-kinase (PI3K)-mTOR pathway and the mitogen-activated protein kinase (MAPK) pathway [7,21], which are related to cell growth and survival. JAK-STAT pathway also regulates the expression of genes essential for cell function and differentiation [21–23].

Recently, CD25L, IL-2Rβ and γc have been characterized in several fish species [24–30]. In pufferfish (Tetraodon nigroviridis), the recombinant CD25L can associate with both IL-2 and IL-15 in a dose-dependent manner with a higher affinity to IL-15 [29]. The grass carp (Ctenopharyngodon idella) IL-2, CII-2, could bind to the CD25L homologue with high affinity [31]. Thus, it is proposed that the fish CD25L gene might be related to an ancestral molecule giving rise to both mammalian IL-2Ra and IL-15Ra [24,29]. Two IL-2Rβ homologues have been characterized in trout (Oncorhynchus mykiss) [24,26], while only one was found in flounder [26]. In trout, zebrafish (Danio rerio) and grass carp, two γc genes have been identified [24,25,27,28]. And it was proved that zebrafish γc could mediate signal pathway which played key role in early lymphopoiesis [27].

In the present study, we report the characterization and expression analysis of three IL-2 receptor subunits in large yellow croaker (Larimichthys crocea), which designated as LcCD25-like (LcCD2SL), LcIL-2Rβ and Lcγc. They were differentially expressed in different tissues and primary immune-related cells. Their expression could be modulated by Poly(I:C) or Vibrio alginolyticus (V. alginolyticus) stimulation in vitro, or by PHA, Con-A, Poly(I:C), inactivated V. alginolyticus stimulation in primary head kidney lymphocytes in vitro.

2. Materials and methods

2.1. Fish and challenge experiments

Large yellow croakers (weight: 102 ± 15.7 g, length: 21 ± 1.7 cm) were purchased from a mariculture farm in Ningsde, Fujian, China. Fish were reared with fed pelleted food twice a day in floating cages at 23 °C in recirculating seawater. The challenge experiments were performed after acclimated for 7 days. The V. alginolyticus was suspended in PBS at a concentration of 1 × 106 colony-forming units/mL. Poly(I:C) was resolved in PBS at 1 mg/mL. Then fish were randomly assigned into three groups (30 individuals/group), and intraperitoneally injected with V. alginolyticus, Poly(I:C) or PBS at a dose of 0.2 mL per 100 g body weight. The spleen and head kidney were collected from 5 fish at 3, 6, 12, 24, 48, and 72 h after injection.

2.2. Cloning of full-length cDNA of LcIL-2Rαs gene

To obtain the full-length cDNA of LcCD2SL and Lcγc, PCR was performed with specific primers designed according to the large yellow croaker genome database (JRPUI00000000) [32]. The resulted PCR products were cloned into pMD18-T Simple vector (TaKaRa, China) and sequenced. For LcIL-2Rβ, the 5'- and 3'-RACE PCR were performed using 5'-Full RACE Kits with TAP and 3'-Full RACE Core Sets (TaKaRa, China) according to the manufacturer's instructions. For 5'-RACE, 2.5 μg of total RNA was dephosphorylated with calf intestine phosphatase, digested by tobacco acid pyrophosphate (TAP) to remove the 5'-cap structure, and ligated to 5'-RACE Adaptor at 5'-end using T4 RNA ligase. Then the ligated RNA was transcribed into cDNA with Random 9-mer Primer and used as template for the subsequent Outer PCR reaction with 5'-RACE Outer Primer and IL-2Rβ-5’RACE Outer-R Primer (Table 1). Inner PCR reaction was performed with 5'-RACE Inner Primer and IL-2Rβ-5’RACE Inner-R primer using Outer PCR product as template. The resulted PCR product was cloned into pMD18-T Simple vector and sequenced. For 3'-RACE, 1 μg of total RNA was transcribed into cDNA with 3'-RACE Adaptor and used as template for the subsequent Outer PCR reaction with 3'-RACE Outer Primer and IL-2Rβ-3’RACE Outer-F primer. Inner PCR reaction was performed with 3'-RACE Inner Primer and IL-2Rβ-3’RACE Inner-F primer using Outer PCR product as template. The resulted PCR product was cloned into pMD18-T Simple vector and sequenced. All the sequences were assembled and the full-length cDNA of LcIL-2Rβ was obtained. To confirm the integrity of the LcIL-2Rβ cDNA sequences, PCR were performed with IL-2Rβ-cDNA-F and IL-2Rβ-cDNA-R primers using the first strand cDNA as a template, then the resulted PCR product was cloned into pMD18-T Simple vector and sequenced. All the primers used in this study were listed in Table 1.

2.3. Bioinformatics analysis of LcIL-2Rs

Sequence similarity analysis was performed using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Signal peptide was predicted using SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Putative glycosylation sites were predicted by NetGlyce 1.0 Server (http://www.cbs.dtu.dk/services/NetGlyce/). Multiple sequence alignment was performed with Clustal Omega Program (https://www.ebi.ac.uk/Tools/msa/clustalo/). For phylogenetic tree construction, amino acid sequences of IL-2/IL-15R subunits without signal peptide from different species are aligned with mafft software (v7.453) [33]. The phylogenetic tree was constructed with mayabayes method using phylosuite software (v1.2.1) based on the multiple alignment result [34]. To analyze the genomic structure or gene synteny, cDNA and genomic DNA sequences of IL-2R/IL-15R subunits without signal peptide from different species are aligned with maft software (v7.453) [33]. The phylogenetic tree was constructed with mayabayes method using phylosuite software (v1.2.1) based on the multiple alignment result [34]. To analyze the genomic structure or gene synteny, cDNA and genomic DNA sequences of IL-2R/IL-15R subunits were mined from GenBank. The genomic DNA organization of each gene was analyzed by Splign program (https://www.ncbi.nlm.nih.gov/sutils/splign/splign.cgi?textpage=online&klevel=afom) through aligning the cDNA sequence with its corresponding genomic DNA sequence. Gene synteny at the IL-2R/IL-15R subunits loci on chromosome was analyzed based on the NCBI database.

2.4. Primary immune-related cells

The primary head kidney lymphocytes (PKLs), primary head kidney macrophages (PKMs) and primary head kidney granulocytes (PKGs) were obtained as reported previously [35]. For LcIL-2Rs induction, PKLs were stimulated with inactivated V. alginolyticus (2 × 104 CFU/mL) or Poly(I:C) at a final concentration of 50 μg/mL, or with PHA or Con-A (Sigma, USA) at a final concentration of 10 μg/mL. Then cells were harvested at 4, 8 and 24 h post-stimulation for total RNA extraction. The control group was stimulated with an equal volume of PBS.

| Primer name | Sequence (5’-3’) |
|-------------|------------------|
| For gene cloning |                   |
| CD25L-cDNA-F | ATGGATCTGCGCTCCTCCCTC |
| CD25L-cDNA-R | CATCGACGATCCTGATGTTG |
| IL-2R-5’RACE Outer-R | CTTAATGCA CAGATCTCT CGTGT |
| IL-2R-5’RACE Inner-R | GAAACAGGAA CACTCTGTA GTACCG |
| IL-2R-3’RACE Outer-F | CCAACACAGGAGCGTATCGACCC |
| IL-2R-3’RACE Inner-F | CACCTCTCAGGGAGGAAATCTAAG |
| IL-2R-cDNA-F | ATGACATCTGCTGCCCGTG |
| IL-2R-cDNA-R | TCAGTATGTATGCTGATTG |
| γc-cDNA-F | CCGGATCATCGGCACTTGGC |
| γc-cDNA-R | CTCGAGCAAGAAATCCTGTC |
| For real-time PCR |                  |
| CD25L-5’-RACE | TGATCCCGAGATGCGACACCC |
| CD25L-5’-RACE | TGATGGTTCGTCATGCTGTTT |
| IL-2R-5’-RACE | GGAACATCTGGAGAGGTG |
| IL-2R-5’-RACE | GTGGAAACCCGTCTGCTACG |
| γc-3’-RACE | TCCCGTATAGCACGCTGAGT |
| γc-3’-RACE | ACACAAGAACCCGCGGTCC |
| β-actin-3’-RACE | CACCTGACAGACTCTGATG |
| β-actin-3’-RACE | AGTGAAGTTGGTCTGTTG |

Table 1

Oligonucleotide primers used in this study.
### 2.5. Expression analysis of LcIL-2Rs by real-time PCR

To determine the tissue expression patterns of LcIL-2Rs, various tissues (spleen, head kidney, intestine, gill, heart, liver, skin, stomach, blood, muscle and brain) and primary immune-related cells (PKLs, PKMs and PKGs) were collected from three healthy fish. Then the same tissue from each fish were pooled for total RNA extraction, which were reversely-transcribed into first-strand cDNA as described previously [36] and used as template in gene expression analysis. Similarly, primary immune-related cells isolated from 3 fish from were used to extract total RNA and prepared cDNA template. Real-time PCR was performed with gene-specific primers (Table 1) on the Mester cycycler egra- dient realplex® (Eppendorf, Germany) using SYBR® Premix Ex Taq™ (TakaRa, China). The Lcβ-actin was amplified as an internal control with specific primers (Table 1). The expression level of LcIL-2Rs was normalized by the Lcβ-actin using the 2-ΔΔCT method as described, and expressed as the ratio to the LcIL-2Rs expression level in the liver [36,37]. Each experiment was repeated three times.

To further understand the modulation of LcIL-2Rs expression upon *V. alginolyticus* or Poly(I:C) stimulation in vivo, total RNA were extracted from head kidney and spleen at 3, 6, 12, 24, 48 and 72 h post-stimulation. Real-time PCR were then performed using primers described above. The relative expression levels of LcIL-2Rs were normalized by Lcβ-actin and expressed as fold changes by comparing the normalized gene expression level of *V. alginolyticus*- or Poly(I:C)-stimulation fish with that of the PBS-stimulation fish at the same time point [38]. Each experiment was repeated three times.

### 2.6. Statistical analysis

All data were analyzed using GraphPad Prism 7 software and expressed as mean ± standard error of the mean (SEM) of three independent experiments. P value was determined by a two-tailed Student t-test using IBM SPSS Statistics 20. *P < 0.05, **P < 0.01.

### 3. Results

#### 3.1. Sequence analysis of LcCD25L

A CD25L homolog (XM 010748639.3) in large yellow croaker (*L. crocea*) has been cloned. Its full-length cDNA contained an ORF of 696 bp, which encoded a protein of 231 amino acids (aa) (Supplemental Fig. 1A). The precursor LcCD25L contained a 23-aa signal peptide at N-terminus, an extracellular region with three putative N-glycosylation sites (Table 2), a transmembrane domain and a short intracellular region (Supplemental Fig. 1A). The calculated molecular weight of mature LcCD25L was 22.9 kDa and its theoretical protein isoelectric point (PI) was 6.36. A sushi domain presented at position 42–99 aa, with four conserved cysteine residues which may form two disulfide bonds (C30–C73 and C57–C67) (Supplemental Fig. 2A).

The genomic organization of IL-15Ra/CD25L from different species are not conserved. The LcCD25L and human (*Homo sapiens*) IL-15Ra both consist of 7 exons and 6 introns. Tropical clawed frog (*Xenopus tropicalis*) IL-15Ra1 consists of 10 exons and 9 introns, but chicken (*Gallus gallus*) IL-15Ra and trout CD25L consist of 5 exons and 4 introns (Supplemental Fig. 3A). Syntenic analysis showed that genes such as PFKFB3 and RMB17 in the vicinity of LcCD25L locus on the chromosome of large yellow croaker were conserved in teleost and tetrapod except chicken. Human CD25 and IL-15Ra are linked together and neighbored to PFKFB3 and RMB17. Unlike human, chicken CD25 and IL-15Ra are not closely adjacent (Supplemental Fig. 3B). Interestingly, two IL-15Ra receptor homolog genes have been found in the corresponding genome loci of tropical clawed frog, while only one CD25L existed in the corresponding genome locus of teleost.

#### 3.2. Sequence analysis of LcIL-2Rβ

The full-length cDNA of large yellow croaker IL-2Rβ homolog (*L. crocea*) contained an ORF of 1653 bp which encoded a 550-aa protein (Supplemental Fig. 1B). The precursor of LcIL-2Rβ contained a N-terminal signal peptide of 20-aa and a mature peptide consisting of an extracellular region with six putative N-glycosylation sites (Table 2), a transmembrane domain and an intracellular region (Supplemental Fig. 1B). The calculated molecular mass of mature LcIL-2Rβ is 59.2 kDa and its theoretical PI is 6.16. The extracellular region of LcIL-2Rβ contains a CHD domain consisting of two FNIII like domains (D1 and D2) and conserved proline residues at the linker region between the D1 and D2. Each FNIII like domain contains seven predicted β-strand. Six conserved cysteine residues implicated in the formation of three disulfide bonds in mammals (C1–C2, C3–C6 and C4–C5) were seen in the LcIL-2Rβ D1 domain (Supplemental Fig. 2B). A conserved WSXWS motif responsible for ligand binding exists in the D2 domain. A conserved Box 2 motif as well as four tyrosine residues important for IL-2 and IL-15 signaling transduction are present in the cytoplasmic region of LcIL-2Rβ (Supplemental Fig. 2B).

The genomic organization of IL-2Rβ from different species are conserved except salmon (*Salmo salar*) and trout IL-2Rβ. Most IL-2Rβ, including human IL-2Rβ, were consisted of 9 exons and 8 introns, while salmon IL-2Rβ consists of 8 exons/7 introns and trout IL-2Rβ consists of 7 exons/6 introns (Supplemental Fig. 4A). Syntenic analysis showed that C14TNP6 were adjacent to IL-2Rβ locus on the chromosome of most species, while on the other end the gene adjacent to IL-2Rβ locus in teleost was TEX33 and TMPRSS6 in tetrapod (Supplemental Fig. 4B).

| Features | Human IL-2Ra | Human IL-15Ra | Large yellow croaker CD25L | Human IL-2Rβ | Large yellow croaker IL-2Rβ | Human γc | Large yellow croaker γc |
|---|---|---|---|---|---|---|---|
| Extracellular domain | Two sushi domain C24–C68 | One sushi domain C30–C53 | One sushi domain C30–C53 | Two FNIII domain C16–C46 | Two FNIII domain C25–C46 | Two FNIII domain C30–C53 | Two FNIII domain C30–C53 |
| Intramolecular disulfide | C50–C53 | C59–C53 | C59–C57 | C59–C105 | C46–C86 | C59–C105 | C182–C231 |
| N-linked glycosylation sit | N137 | N40, N138, N142 | N32, N43, N71, N142 | N23, N32, N43, N71, N142 | N23, N32, N43, N71, N142 | N24, N31, N84, N115, N149, N208 | N24, N31, N84, N115, N149, N208 |
| Functional motif | WSXWS motif, Box1 and 2 motif | WSXWS motif, Box2 motif | WSXWS motif, Box1 and 2 motif | WSXWS motif, Box1 and 2 motif | WSXWS motif, Box1 and 2 motif | WSXWS motif, Box1 and 2 motif | WSXWS motif, Box1 and 2 motif |

Supplemental Table 2: Characteristics of IL-2/15 receptor subunits.
3.3. Sequence analysis of γc

We have cloned and identified a γc homolog (XM_010755342.3) in large yellow croaker (Lcγc). Its full-length cDNA contained an ORF of 1044 bp and encoded a 347 aa protein (Supplemental Fig. 1C). The precursor of Lcγc protein contained a 17-aa signal peptide at N-terminus and a mature peptide consisting of an extracellular region with seven putative N-glycosylation sites (Table 2), a transmembrane domain and an intracellular region (Supplemental Fig. 1C). The molecular mass of mature Lcγc is 37.2 kDa with a theoretical pI of 6.09. A CHD, which consists of two FNIII like domains (D1 and D2 domain) and conserved proline residues at the linker region between the D1 and D2 was present in the extracellular region of Lcγc. Four conserved cysteine residues implicated in the formation of two disulfide bonds in mammals (C1–C2 and C3–C4) were seen in the D1 domain. A WSXWS motif and two conserved cysteine residues are present in the D2 domain. In the cytoplasmic region of Lcγc, a conserved Box 1 motif important for signaling of γc cytokine family is also present.

The genomic organization of γc from different species are conserved except fugu γc. The fugu γc gene is consisted of 9 exons and 8 introns, while γc from other species were consists of 8 exons and 7 introns (Supplemental Fig. 5A). Syntenic analysis showed that SNX12 in the vicinity of Lcγc, which plays a role in cytokine signaling, is conserved in the genome of large yellow croaker.
of γc locus on the chromosome of large yellow croaker were overall coincident with those of other teleost γc except zebrafish. SNX12 were also found in the tetrapod γc locus on the chromosome but far away (Supplemental Fig. 5B).

3.4. Phylogenetic analysis of LcIL-2Rs

The LcCD25L, LcIL-2Rβ and Lcγc share 27.70–35.00%, 31.09–46.73% and 29.06–56.94% amino acid sequence identities with their corresponding homologs from teleost species according to multiple alignment analysis. Whereas they shown a relative low identities ranging from 19.17% to 23.47% (for LcCD25L), 18.51% to 20.04% (for LcIL-2Rβ) and 25.32% to 28.33% (for Lcγc), with their tetrapod homologs, respectively. The phylogenetic tree showed that LcCD25L grouped closely with teleost CD25L molecules and formed a clade with IL-15Rα and CD25 from mammals, Aves and amphibian. It is noteworthy that tropical clawed frog IL-15Rα2 grouped with chicken and mammals CD25, while its IL-15Rα1 form a branch separate from major clades of CD25/CD25L/IL-15Rα from other vertebrate. The LcIL-2Rβ and Lcγc were clustered with IL-2Rβ and γc molecules from other species, respectively (Fig. 1).

3.5. Expression of LcIL-2Rs in different tissues and primary immune-related cells

The real-time PCR results showed that all LcIL-2Rs were constitutively expressed in blood, gill, heart, spleen, brain, muscle, intestine, skin, stomach, head kidney and liver at expression level from the high-
est to the lowest (Fig. 2A). The LcIL-2Rβ showed high expression level in spleen followed by gill, heart and blood (Fig. 2C). High levels of Lcγc expression were detected in spleen, gill and head kidney (Fig. 2E). All LcIL-2Rs exhibited a lowest expression in liver.

For primary immune-related cells, the LcCD25L and LcIL-2Rβ exhibited highest expression in PKLs (Fig. 2B and D), while the highest expression of Lcγc was detected in PKMs (Fig. 2F).

3.6. Modulation of the expression of LcIL-2Rs in response to Poly(I:C) or V. alginolyticus

The expression of LcIL-2Rs in response to Poly(I:C) or V. alginolyticus were detected in spleen and head kidney of large yellow croaker. Upon Poly(I:C) stimulation, the expression of LcCD25L, LcIL-2Rβ and Lcγc increased since 3 h post-stimulation (hps), then reached peak at 12 hps and decreased from 24 to 48 hps (Fig. 3A, C and E). After V. alginolyticus stimulation, the transcription level of LcCD25L peaked at 12 hps with 5.3- and 9.2-fold increase in spleen and head kidney, respectively (Fig. 3B). The transcription level of LcIL-2Rβ peaked at 6 hps with 2.4- and 2.9-fold increase in spleen and head kidney, respectively (Fig. 3D). While that of Lcγc was only slightly up-regulated at 12 hps with 1.3-fold increase in spleen and 1.4-fold increase in head kidney at 3 hps with significant difference (Fig. 3F).

3.7. Modulation of the expression of receptor subunits of LcIL-2 in PKLs

In PKLs, the LcCD25L expression peaked at 24 hps of PHA (3.9-fold), Con-A (1.7-fold), Poly(I:C) (2.8-fold) and inactivated V. alginolyticus (1.6-fold) (Fig. 4A). The transcription of LcIL-2Rβ was up-regulated by all stimulants, with 8.4-fold increase by PHA, 3.8-fold increase by Con-A, 1.64-fold increase by Poly(I:C) and 2.8-fold increase by V. alginolyticus at 24 hps (Fig. 4B). The transcripts of Lcγc increased by PHA (up to 3.0-fold), Con-A (1.3-fold) and V. alginolyticus (1.5 fold) at 24 hps but is refractory to Poly(I:C) (Fig. 4C).

4. Discussion

In mammals, the IL-2 and IL-15 receptor complexes both possess three subunits, the shared IL-2β and γ chains, and an unique α receptor chain called CD25 for IL-2 and IL-15Rα for IL-15 [8,39]. Both CD25
and IL-15Ra possess ‘sushi’ domain, but CD25 contains two while IL-15Ra contains one [8]. The genes encoding CD25 and IL-15Ra have similar intron-exon structures. They are closely linked on human chromosome 10 or mouse chromosome 2 [8]. In tropical clawed frog, two IL-15Ra homologous genes present at the same locus harboring mammalian CD25 and IL-15Ra. Interestingly, phylogenetic analysis shown that tropical clawed frog IL-15Ra2 grouped with chicken and mammals CD25. While its IL-15Ra1 form a branch separate from major clades of CD25/CD25L/IL-15Ra from other vertebrate. However, there is only one IL-15Ra-like gene, defined as CD25L, was identified at the same locus of bony fish currently [24,29]. Thus, we proposed that these tropical clawed frog IL-15Ra homologs might arise through a random gene duplication from teleost CD25L, and diverged into unique IL-2Rs and IL-15Ra in mammals. Moreover, the recombinant puffer fish CD25L protein could bind to both IL-2 and IL-15, but its preference to IL-15 is five times higher than that to IL-2 [29]. Grass carp CD25L could bind to CD12C with high affinity. But once the key residues of CD12C involved in the contact region were mutated, the binding affinity decreased with 20–25 times [31]. Thus, these results suggest that teleost CD25L could be utilized in both IL-2 and IL-15 signal transduction, and further supported that teleost CD25L served as an ancestral molecule which tropical tetrapod CD25 and IL-15Ra homologs are derived.

Mammalian IL-2Rβ and γc exhibit intermediate-affinity with IL-2, but they are indispensable for both IL-2 and IL-15 signal transduction [8]. Both of the LcLIL-2Rβ and Lcγc have a long intracellular domain as their mammalian counterparts, but the conserved motifs are somewhat different. The Box1 and Box2, which play crucial roles for the interaction of IL-2Rβ and γc with JAKs, are conserved in mammalian IL-2Rβ and γc [40]. But in large yellow croaker, LcLIL-2Rβ only has the conserved Box2 but lacks the Box1. The Lcγc together with other teleost γc have only one conserved Box1 motif. Despite of that, the zebrafish γc with only Box1 motif could still lead to a conserved signaling transduction via JAK3 to regulate early zebrafish lymphopoiesis [27]. Thus that deficiency of some motif of teleost IL-2Rβ and γc might not alter their signaling function.

During the course of human IL-2 signal transduction, the activation of JAK1 and JAK3 induces a number of tyrosine phosphorylation in the IL-2Rβ chain. These phosphorylated tyrosine residues, especially Y328, Y392, and Y510 in human IL-2Rβ, play crucial roles in recruiting and activating adaptor molecules involved in MAPK, PI3K and JAK-STAT5 signal pathways [6,40]. The tyrosine residue Y328 and Y510 of human IL-2Rβ and γc are conserved among all known vertebrates, while Y392 was missed in avian and teleost IL-2Rβ. Thus teleost IL-2Rβ might mediate a conserved signal pathways as in mammals but with some difference. The exact signal pathways mediated by IL-2Rβ still remains to be determined in teleost. Although tyrosine residues were phosphorylated during receptor activation, the deletion of tyrosine in human γc show no deficit in IL-2R signal transduction [17]. Moreover, no one of the tyrosine residues was conserved among vertebrate γc. So the biological significance of tyrosine phosphorylation in γc remains to be determined also.

Mammalian CD25 is found mainly expressed on activated T cells and NK cells [41]. The CD25Ls in teleost, such as in dojo loach (Misgurnus anguillicaudatus), rock bream (Oplegnathus fasciatus), trout and large yellow croaker, were highly expressed in immune related tissues [42,43]. Mammalian IL-2Rβ is constitutively expressed by NK, NKT and memory CD8+ T cells [41]. In teleost, flounder IL-2Rβ and were found to be expressed in both T cells and B cells [26], and LcIL-2Rβ was highly expressed in PKLs, which mainly composed of T and B lymphocytes. Teleost γc, including Lcγc, are constitutively expressed in all tested tissues [24,25,27,28]. Also mammalian γc is constitutively expressed in lymphocytes, neutrophils and monocytes [12,44,45]. The Lcγc constitutively expressed in three isolated primary immune-related cells with high level in PKMs. Given the high expression of LcIL-2Rβ subunits in lymphocytes, as well as they could be induced by T cell mitogen, LcIL-2Rs might exercise function on lymphocytes, especially on activated T cells.

In summary, three IL-2 receptor subunits were identified and characterized in large yellow croaker. The LcCD25L is structurally and evolutionally related to both tetrapod CD25 and IL-15Ra. The LcLIL-2Rβ and Lcγc showed similar structure and conserved evolutionary relationship to their tetrapod counterparts. The encoding genes of these three IL-2 receptor were constitutively expressed in all detected tissues of healthy fish and primary immune-related cells. Their expression could be modulated by Poly(I:C) or bacterial stimulation in vivo, as well as by PHA, Con-A, Poly(I:C) or inactivated V. alginolyticus in PKLs in vitro. These results suggest that LcIL-2Rs may function in mediating immune response and T cell activation as their mammalian homologs. Further studies are still needed to fully elucidate the ligand specificity and downstream signaling pathway of LcIL-2Rs.

Ethics statement

The studies were carried out in strict accordance with the Regulations of the Administration of Affairs Concerning Experimental Animals

Fig. 4. Expression analysis of LcLIL-2Rs genes in PKLs after immune stimulation. The PKLs were stimulated with PHA, Con-A, Poly(I:C), inactivated V. alginolyticus or with PBS as control for 4, 8 and 24 hrs. The expression level was normalized to that of the LcIF-actin from the same sample, and expressed as fold change relative to the expression levels in control PKLs. Deviation bars represent the standard errors of the mean (± SEM) at each time point. *P < 0.05, **P < 0.01.
