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# B cell receptor accessory molecule CD79 gets involved in response against *Streptococcus agalactiae* infection and BCR signaling in Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

CD79, composed of two distinct chains called CD79a and CD79b, is a transmembrane protein that forms a B cell antigen receptor with membrane immunoglobulin, and generates a signal following antigen recognition by the B cell receptor. In this study, the *CD79a* (*OnCD79a*) and *CD79b* (*OnCD79b*) were cloned and identified from Nile tilapia (*Oreochromis niloticus*). The cDNA of ORF for *OnCD79a* and *OnCD79b* are 669 and 627 bp, coding 222 and 208 amino acids, respectively. The deduced protein analysis showed that both CD79a andCD79b contain an immunoreceptor tyrosine-based activation motif in their intracellular tails that used to propagate a signal in a B cell. Expression analysis revealed that both *CD79a* and *CD79b* expressed at high levels in immune tissues, such as anterior kidney and spleen, and in $IgM^+$ B cells. Upon *Streptococcus agalactiae* (*S. agalactiae*) infection, the expressions of *OnCD79a* and *OnCD79b* were significantly up-regulated in anterior kidney and spleen. The significant up-regulations of *OnCD79a* and *OnCD79b* were also detected in leukocytes after *in vitro* challenge with *S. agalactiae*. Further, stimulations of LPS and anti-OnIgM monoclonal antibody induced significant up-regulations of *OnCD79a* and *OnCD79b* in leukocytes. Taken together, the results of this study indicated that CD79 molecule, playing roles in BCR signaling, was likely to get involved in host defense against bacterial infection in Nile tilapia.

## 1. Introduction

B lymphocytes play an important role in humoral immunity of the adaptive immune system by secreting antibodies. The response of B cells to pathogens and other foreign substances is mediated through the cell surface B cell receptor (BCR) partly [1,2]. BCR complex is the key molecule guiding B cell differentiation and responding to antigen stimulation, which is composed of membrane immunoglobulin (mIg) non-covalently associated with Igα (CD79a)/Igβ (CD79b) heterodimers. In human, CD79a and CD79b are coded by mb-1 and B29 genes [3–5], respectively. It is a multi-protein structure that pivotally to antigen recognition and signal transduction in B cells [6,7]. The mIg is responsible for binding antigen, and the cytoplasmic tail (CYT) of the mIg heavy chain is too short to transmit activating signals to the inside of the cell [8,9]. The CD79a/CD79b heterodimer becomes the signal transduction component of the BCR complex and is responsible for initiating complex intracellular signaling pathway, which govern the B cell immune response to exogenous antigen recognition and binding by mIgs [1,10].

Structurally, both CD79a and CD79b consist of a single Ig-like domain in the extracellular region, a transmembrane (TM) region and a CYT [11,12]. The CYT contains an immune-receptor tyrosine-based activation motif (ITAM), which contains two conserved sequence YxxL/I, separated by 6–9 amino acids (Y is tyrosine, L is leucine, I is isoleucine and x denoting any amino acid) [11,13–15]. This motif plays an essential role in the communication between the BCR and two types of protein tyrosine kinases, including the sac family kinases (*Lyn*, *Blk*, and *Lck*) and the spleen tyrosine kinase (*Syk*). The activation of *Syk* triggers a signal transduction cascade resulting in cytoskeletal reorganization and changes in gene expression that controls cellular differentiation, proliferation and development [16–18]. Stimulation of the BCR results in transfer of the B cell from the G0 phase of the cell cycle to G1, and the transmembrane CD79a and CD79b proteins provide the signaling function for the BCR [19].

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CD79 transcripts have been identified in mammals [20–22], avian [23,24] and several fish species, such as channel catfish (*Ictalurus punctatus*) [25,26] and rainbow trout (*Oncorhynchus mykiss*) [27]. Although several studies indicated that the CD79 might get involved in immune response against bacterial infection [28–30], the study on CD79 in teleost remains limited, especially its function in the BCR signaling pathway. Nile tilapia (*Oreochromis niloticus*) is one of important economical fishes and widely cultured in the world, especially in China. The pathogen bacterium *Streptococcus agalactiae* (*S. agalactiae*) has been reported to cause the mass mortality in Nile tilapia, resulting in huge economic loss [31,32]. Thus, it is highly essential to identification of critical immunological components and understanding their functions in defense mechanism against the bacterial disease. Considering the important role of CD79 in immunomodulation, the molecular characterization and expression of CD79 from Nile tilapia were identified and cloned. Their constitutive mRNAs expression in different tissues (especially in anterior kidney and spleen) were detected. In addition, the expression patterns of *OnCD79* upon *S. agalactiae* infection *in vivo* and *in vitro* were investigated. The constitutive transcriptions in the $IgM^+$ cells from spleen and anterior kidney were analyzed. The critical role of CD79 in BCR activation under the inductive effect of lipopolysaccharide (LPS) and the specific anti-OnIgM monoclonal antibody was also studied in this paper, respectively. These results would provide valuable information for better understanding the role of CD79 in BCR signaling and host defense against bacterial infection in Nile tilapia.

## 2. Materials and methods

### 2.1. Experimental animals, pathogenic infection and challenge experiment

Nile tilapia (*Oreochromis niloticus*), about 100 ± 10 g, were obtained from Guangdong Province Tilapia Breeding farm (Guangdong, China) and acclimatized in 300 L with a semi-automatic circulating water system and a light and dark period of 12 h:12 h at 28 ± 2 °C [33–35]. Fresh water exchange was approximately 10% per day. All animal procedures were reviewed and approved by the University Animal Care and Use Committee of the South China Normal University.

*S. agalactiae* (ZQ1901), a gift from the Guangdong Ocean University (Guangdong, China) [36,37], was inoculated into brain heart infusion agar (BHI) at 37 °C with shaking at 180 rpm until reaching an optical density (O.D.) to 1.0 at 600 nm. The pathogenic bacteria was collected by centrifuge at 5000 rpm for 10 min and re-suspended in phosphate buffered solution (PBS) for subsequent challenge experiments.

The challenge experiment was performed by injecting with 0.1 mL PBS (the control group), or with live *S. agalactiae* 100 μL re-suspended in sterile PBS at a final concentration of approximately $1 \times 10^7$ CFU/mL [34,35]. At 0, 12, 24, 48, 72 and 120 h post-infection, anterior kidney (AK) and spleen (SP) were collected from the challenged group and control group (n = 5). At the same time, the tissues including peripheral blood (BL), AK, SP, posterior kidney (PK), liver (LI), muscle (MU), skin (SK), brain (BR), heart (HE), thymus (TY), gill (GI) and intestine (IN) were collected from the health fish (n = 3). All these tissues were frozen by liquid nitrogen immediately, followed by storage at −80 °C.

### 2.2. Cloning and sequence analysis of OnCD79

Total RNA was extracted from the anterior kidney of health tilapia using Trizol (Invitrogen, USA) according to the protocol [34]. The quality and quantity of RNA of each sample was determined by Nanodrop 2000 assay (Thermo, USA). All extracted RNA samples with A260/A280 ratio between 1.8 and 2.0 were used for cDNA synthesis. And the ordinary templates for PCR were generated by Prime script™ II 1st cDNA Synthesis Kit (TaKaRa, Japan) following the manufacture's protocol (1 μg total RNA per 20 μL reaction). Two gene specific primers, showed in the Table 1, were designed based on the website, National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/, according to the predicted potential open reading frame (ORF) (GenBank Accession: CD79a, XP_003458832.1; CD79b, XP_003453421.1) by software Primer 5.0 (USA). PCR products were purified and ligated into the pMD18-T simple vector (TaKaRa, Japan). After transformed into the competent *E. coli* DH5α cells (Tiangen, China), two positive clones for each product were sequenced at Invitrogen (Shanghai, China).

**Table 1**
Primers used in this study.

| Primers | Sequence (5'-3') | Application |
|---|---|---|
| CD79a-F | ATGAAAATGGGGATGGTTGTAATAT | ORF sequence |
| CD79a-R | TTATGGTTTCTCCAGCTGGATTTCT | |
| CD79b-F | ATGCGCTGGATGCTAGCTGGATGCT | |
| CD79b-R | TCACTCCCACGGGGGCCTCAGCTCCC | |
| qCD79a-F | CATCATAACAAAACTCAGGAGG | qRT-PCR |
| qCD79a-R | GTAGACACGCAGGTAGGTTCCAT | |
| qCD79b-F | TGTGCCCATTTACTGTTCATCCTC | |
| qCD79b-R | CGCCACTGTGCCTCATTTGT | |
| β actin-F | AACAACCACACACCACACATTTC | |
| β actin-R | TGTCTCCTTCATCGTTCCAGTTT | |

The cDNA sequence of *CD79* was analyzed using the BLAST at the website, National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast), and the deduced amino acid sequence was analyzed using the Expert Protein Analysis System (http://www.expasy.org/). The molecular mass (MM) and theoretical isoelectric point (PI) of CD79 was calculated by the Prot-Param tool (http://www.expasy.ch/tools/protparam.html). The putative signal peptide cleavage site was identified using the SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Domains in OnCD79a and OnCD79b amino acid sequences were detected using the simple modular architecture research tool (SMART) program (http://www.smart.emblheidelbergde/) and multiple alignments analysis of each protein were performed using the DNAMAN software 7.0. A neighbor-joining phylogenetic tree was constructed using MEGA 6.0 program with 1000 bootstraps [28,38].

### 2.3. Tissue expression analysis of CD79 mRNA by quantitative real-time PCR

The tissue distribution analysis of *CD79a* and *CD79b* genes were conducted in three healthy tilapia tissues by real-time quantitative PCR detecting system (qPCR), including BL, AK, PK, SP, LI, MU, SK, BR, HE, TY, GI and IN. β actin (shown in Table 1) was selected as an internal control to verify the successful reverse transcription and to calibrate the cDNA template. The specific primers for *CD79a* and *CD79b* were designed to amplify a product of 100–200 bp. qPCR was performed in a total volume of 20 μL containing 10 μL of 2 × TaKaRa Ex Taq™SYBR premix (TaKaRa, Japan), 3 μL of the 1:10 diluted cDNA (15 ng), 2 μL of each primer (2 μM) and 3 μL sterile water. The qPCR parameters included a denaturing step at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and a dissociation cycle (30 s at 95 °C, 1 min 60 °C and 30 s at 95 °C) by ABI 7500 Sequence Detection System (Applied Biosystems, USA). Each sample was run in three biological replicates. Melting curve analysis of the amplified products was performed at the end of each PCR to confirm that a single PCR product was generated. For each mRNA, gene expression was corrected by the endogenous control β actin expression in each sample and expressed as $2^{-\Delta Ct}$, where ΔCt is determined by subtracting the β actin Ct value from the target Ct as previously described [39].

### 2.4. Cell sorting and qPCR analysis of sorted cells

Isolated leukocytes from anterior kidney and spleen were re-suspended in PBS and incubated for 1 h on ice with anti-OnIgM

monoclonal antibody (1 mg/mL, 1:2000 dilution) [38,40] labeled with Alexa Fluor 647 (Thermo, USA), respectively. Following two washing steps, cells were re-suspended in PBS and IgM positive ($IgM^+$) and negative ($IgM^-$) cells were sorted by a BD FACS Aria III (BD, USA) flow cytometry, using first their forward scatter (FSC, relating to size) and side scatter (SSC, relating to complexity) to exclude the granulocyte gate and then on the basis of the fluorescence emitted by the sample. $IgM^+$ and $IgM^-$ cells were then collected in different tubes for RNA isolation.

Total cellular RNA was isolated from $IgM^+$ and $IgM^-$ sorted populations using 1 mL Trizol (Invitrogen, USA) as described above. To evaluate the levels of transcription of the different genes, qPCR was performed with ABI 7500 System (Applied Biosystems, USA), using SYBR Green PCR core Reagents and specific primers (shown in Table 1). The efficiency of the amplification was determined for each primer pair using serial 10-fold dilutions of cDNA. Each sample was measured in triplicate under the conditions as described above. The expression levels were calculated as the method described in Materials and methods 2.3.

### 2.5. Time-course analysis of CD79 in response to S. agalactiae challenge in vivo

Spleen and anterior kidney were selected to analyze the gene temporal expression profile of *CD79a* and *CD79b* in *S. agalactiae* challenge, and RNA extraction, cDNA synthesis and expression analysis were performed according to the above of methods by qPCR. The pairs of specific primers, showed in Table 1 were used here. The data were presented as relative mRNA expression levels (means ± SD, n = 5), which denoted the n-fold differences relative to the untreated samples by $2^{-\triangle\triangle CT}$ method [41].

### 2.6. Isolation and stimulation of anterior kidney leukocytes in vitro

The leukocytes from anterior kidney were separated by Histopaque® 1077 (Sigma, USA) as our previous works (n = 5) [34,38]. One hundred microliters cells with a final concentration of $1 \times 10^7$ cells/mL were dispensed into each well in a 96-well culture micro plate (Thermo, USA) and incubated at 25 °C after treatment with inactivated *S. agalactiae* ($1 \times 10^7$ CFU/mL), 40 μg/mL LPS (Sigma, USA) [38] or 10 μg/mL anti-On IgM monoclonal antibody [38,40] for 0, 3, 6, 12, 24 and 48 h, respectively. The untreated cells were served as control. The cells were washed once with cold PBS, centrifuged at 600 × *g* at 4 °C for 5 min, added 1 mL Trizol (Invitrogen, USA) with repeated pipetting and then stored at −80 °C for expression analysis. The relative expressions of OnCD79 were calculated by $2^{-\triangle\triangle CT}$ method [41].

### 2.7. Statistical analysis

Data handling and analyses were performed using Microsoft Office Excel 2010. Statistical analyses were performed using a Statistical Package for Social Sciences (SPSS) 17.0 software by one-way ANOVA and represented as mean ± standard deviation. The statistical significance was defined as $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. The graphic representations in this study were made by Sigma Plot 10.0 software.

## 3. Results

### 3.1. Nile tilapia CD79 cDNA, amino acid sequences and phylogenetic cluster analysis

The ORF of CD79a cloned in this study was 669 bp in length, which predicted to encode 222 amino acids with a signal peptide of 21 resides (Fig. 1). The theoretical MW was 25.16 kDa and PI was 6.14. Comparatively, the ORF of CD79b was 627 bp, encoding 208 amino acids with a predicted signal peptide of 19 resides (Fig. 1). The theoretical MW and PI of CD 79b were 23.84 kDa and 5.33, respectively. According to the functional domain diagrams predicted by SMART, the mature CD79a contains the extracellular Ig domain (residue 29 to 121), the TM region (residue 138–160), and one CYT containing the ITAM motif (residue 177 to 198). Comparatively, The CD79b consists of the extracellular Ig domain (residue 30 to 121), the TM domain (residue121 to 139) and the ITAM domain (residue 175 to 197).

The deduced amino acids of OnCD79 were aligned with other known CD79 sequences, which showed that OnCD79 genes were homologous to other teleost species and mammals (Fig. 1). In order to analyze the phylogeny of CD79, a phylogenetic tree was constructed with other species (Fig. 2). The non-fish vertebrate classes converged within one of the main branches, and the teleost independently clustered as another main one.

### 3.2. Tissue distribution of CD79a and CD79b in healthy tilapia

The mRNA expression patterns of *OnCD79a* and *OnCD79b* in healthy fish tissues were detected by qPCR. It indicated that the *CD79a* and *CD79b* expressions shared a similar distribution, with a wide tissue distribution and a high expression level in immune organs (Fig. 3). The main expression of *CD79* in immune tissues, such as spleen, anterior kidney and blood, showed obvious tissue specific variation of *OnCD79* (Fig. 3). The most predominating expressions of both *OnCD79a* and *OnCD79b* were detected in the spleen.

### 3.3. Transcription of CD79a and CD79b in sorted $IgM^+$ lymphocytes

To examine the transcriptional heterogeneity of *CD79a* and *CD79b* in $IgM^+$ and $IgM^-$ cell populations of anterior kidney and spleen, the $IgM^+$ and $IgM^-$ lymphocytes were sorted out by flow cytometry. The sort effectiveness was detected after cell sorting, which indicated that the cell sorted was successful with a purity of 95.8% and 99%, as shown in Fig. 4A, for the $IgM^+$ and $IgM^-$ lymphocytes, respectively.

The qPCR analysis of *OnCD79a* and *OnCD79b* showed that both genes were expressed in $IgM^+$ and $IgM^-$ lymphocytes, while the transcriptional levels of $IgM^+$ cells were significantly higher than those of $IgM^-$ cells in anterior kidney and spleen (Fig. 4B). Compared with the total leukocytes, both expressions of *CD79a* and *CD79b* of lymphocytes ($IgM^+$ or $IgM^-$) were significantly higher than those of leukocytes from both tissues (Fig. 4B).

### 3.4. Expression pattern of OnCD79 upon S. agalactiae infection in vivo

In order to investigate the effect of bacterial infection on expression patterns of *OnCD79a* and *OnCD79b* in spleen and anterior kidney, tilapias were infected with *S. agalactiae in vivo*. Tissues were collected and the transcriptional levels of *CD79*a and *CD79b* were analyzed at time points post-infection (0, 12, 24, 48, 72, and 120 h p.i.) (Fig. 5). Upon *S. agalactiae* infection, the transcriptional expressions of *OnCD79a* and *OnCD79b* were significantly up-regulated ($p < 0.05$) in both spleen and anterior kidney. In spleen, the *CD79a* expression was significantly up-regulated (13.2-fold) at 24 h p.i. and the *CD79 b* at 48 h p.i. (6.9-fold) (Fig. 5A). In anterior kidney, the *CD79a* transcription reached its peak at 24 h p.i. with 21.6-fold and the *CD79b* attained the maximum at 12 h p.i. (5.45-fold) (Fig. 5B).

### 3.5. Expressions of OnCD79a and OnCD79b after stimulation in vitro

To explore effects of stimuli on *OnCD79a and OnCD79b* expressions *in vitro*, anterior kidney leukocytes isolated from tilapia were stimulated with inactivated *S. agalactiae*, LPS and anti-OnIgM monoclonal antibody. As shown in Fig. 6, both expressions of *OnCD79a and OnCD79b* were significantly up-regulated in anterior kidney leukocytes after the stimulations of *S. agalactiae*, LPS and anti-OnIgM monoclonal antibody.

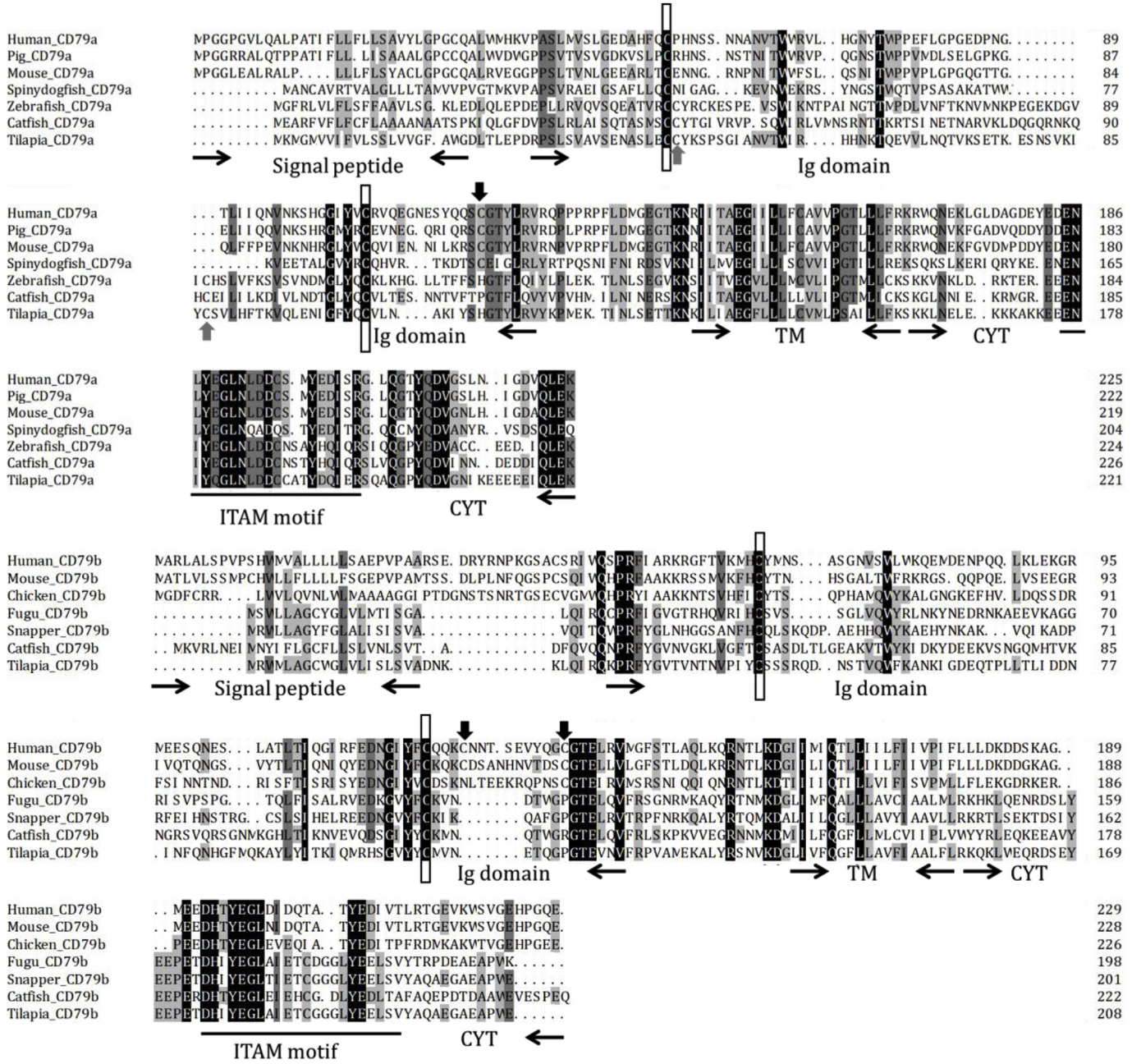


**Fig. 1.** Multiple alignment of the deduced amino acid sequences of CD79a and CD79b among different species. It was produced using DNAMAN software 7.0. The signal peptide and Ig domain, TM, and CYT boundaries are indicated under the sequences. The two conservative cysteines in the Ig domain were boxed, while the unconserved were indicated with a gray arrowhead (↑) below the sequences and black arrowhead (↓) above the sequences. Human_CD79a (AAC60653.1), Pig_CD79a (NP_001129434.1), Mouse_CD79a (NP_031681.2), Spinydogfish_CD79a (CCO61977.1), Zebrafish_CD79a (NP_001313399), Catfish_CD79a (ABM53182.1), Snapper_CD79a (AJG39040.1), Human_CD79b (EAW94232.1), Mouse_CD79b (CAJ18566.1), Chicken_CD79b (NP_001006328.2), Fugu_CD79b (XP_003961066.1), Channel Catfish_CD79b (ABM53183.1), Snapper_CD79b (AJG39039.1).

Challenged with *S. agalactiae*, *CD79a* expression significantly increased at 3 h (2.6-fold), and *CD79b* was significantly up-regulated with a 3.4-fold increase at 6 h post-stimulation (Fig. 6A). The LPS stimulation induced *CD79a* expression with a 7.1-fold increase at 12 h, and *CD79b* with 3.7-fold rise at 3 h post-challenge (Fig. 6B). When stimulated with anti-OnIgM monoclonal antibody, the expression of *CD79a* increased significantly and reached the maximum at 6 h (11.1-fold) and *CD79 b* at 3 h (2.3-fold) post-stimulation (Fig. 6C), indicating the potential role of OnCD79 in signal transduction of BCR activation.

## 4. Discussion

CD79 is a transmembrane protein that forms a complex with BCR and generates a signal following recognition of antigen by the BCR, which plays a critical role in B cell differentiation, proliferation, and effector functions [7–9]. In the current study, CD79 from Nile tilapia was identified and characterized, indicating that CD79 was likely to get involved in host defense against bacterial infection and play a role in BCR signaling.

The analysis of structure domains revealed that OnCD79 was conservative, similar as other known CD79 molecules [5,26,28–30,42], mainly in an extracellular Ig domain with conserved cysteine residues

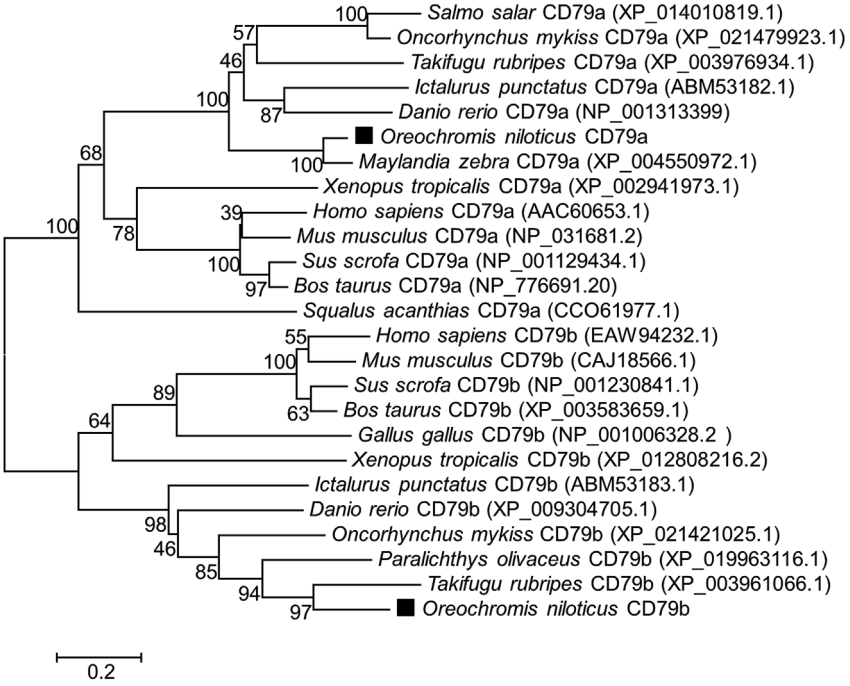


**Fig. 2.** Phylogenetic tree of the CD79a and CD79b among different species. The phylogram was constructed by the neighbor joining methods using MEGA 6.0 and the bootstrap value was set at 1000. The tetraspanins amino acid sequence of all species was obtained from the NCBI database.

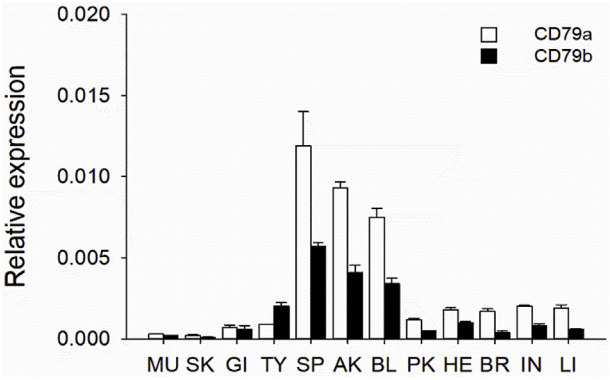


**Fig. 3.** Tissue distributions of *OnCD79a* (A) and *OnCD79b* (B) in healthy Nile tilapia. Each sample had three replicates (n = 3). BL, peripheral blood; TY, thymus; SP, spleen; AK, posterior kidney; PK, terminal kidney; GI, gill; BR, brain; HE, heart; IN, intestine; SK, skin; MU, muscle; LI, Liver. Data are shown as the mean gene expression relative to the expression of endogenous control $\beta$ actin ± SD.

and intracellular ITAM motif. There were two conservative cysteines located in the Ig domain of CD79 that can form the disulfide bond (Fig. 1). However, sequencing shows that tilapia CD79a and CD79b, as well as group, pufferfish and channel catfish CD79 molecules [26,29,43], lack the conserved cysteines found between the Ig domain and TM that form the interchain disulfide bond in mammals [9,44]. There are two additional cysteine residues in elsewhere were found in OnCD79a, but OnCD79b sequences lack these cysteine residues as the study on Chinese sucker [30], which may indicate that the interchain disulfide bond is not necessary for heterodimerization of teleost fish CD79s [30]. In addition, the high sequence conservativeness of the CYT among mammals reflected the presence of a functionally critical conserved motif [15]. The ITAM region with conservative (D/E(x)7D/ExxYxxL(x)6-8YxxI/L in teleost CD79a and CD79b indicated that teleost fish CD79s probably play a similar role as mammals in antigen presentation and signal transduction [18,21].

The tissue distribution of *OnCD79* in healthy fish showed that the *OnCD79* expression had a wide distribution. The high expression of *CD79* was detected in immune organs, such as anterior kidney and spleen, with the highest expression of *OnCD79b* in anterior kidney and *OnCD79b* in spleen (Fig. 3). These findings were consistent with the report in Chinese sucker and group [29,30]. Being the major immune tissues, anterior kidney and spleen contain lots of B cells and get involved in adaptive immune [45]. In teleost, the spleen is a major secondary lymphoid organ, which captures the invading antigens to act in the initiation of the adaptive immune response [46,47]. Further, anterior kidney is a key immune organ and the major site of hematopoiesis including B cells [48,49]. However, the finding of tissue distribution of *CD79* in humphead snapper was different from the pattern of the current study in Nile tilapia [28]. The variations in the CD79 tissue expression between these two fish species might be due that these species are living in environment at different salinities (tilapia, a freshwater fish vs. snapper, a marine fish), which resulted in the experimental fish with different physiological status in the various studies [28,30].

The CD79a/CD79b heterodimer is the signal transduction component of the BCR complex, and BCR on $IgM^+$ B cell is responsible for initiating complex intracellular signaling pathways [9]. The analysis of *CD79a* and *CD79b* in $IgM^+$ cells from anterior kidney and spleen showed that transcriptions of *OnCD79a* and *OnCD79b* were highly expressed in $IgM^+$ cells and much less expressed in other leukocytes (Fig. 4). CD79 expressed in $IgM^-$ cells may be due to the existence of IgT cells [50,51], which needs CD79 to form the receptor on the cell membrane surface to transmit the signal. The expression of *CD79a* was higher than that of *CD79b* in both $IgM^+$ cells from anterior kidney and spleen. What's more, this phenomenon may indicate that *CD79a* plays a more important role than *CD79b* in BCR signal transduction. In mouse, studies demonstrated that mutations in CD79a resulted in a complete block in B-cell development, but CD79b mutation partially blocked the B-cell development [52]. However, the functions of CD79a and CD79b in teleost B cell activation and differentiation need to be further studied.

In order to examine the effect of pathogenic bacterial infection on expression patterns of *OnCD79a* and *OnCD79b* in immune tissues, tilapias were infected with *S. agalactiae in vivo*. Since anterior kidney and

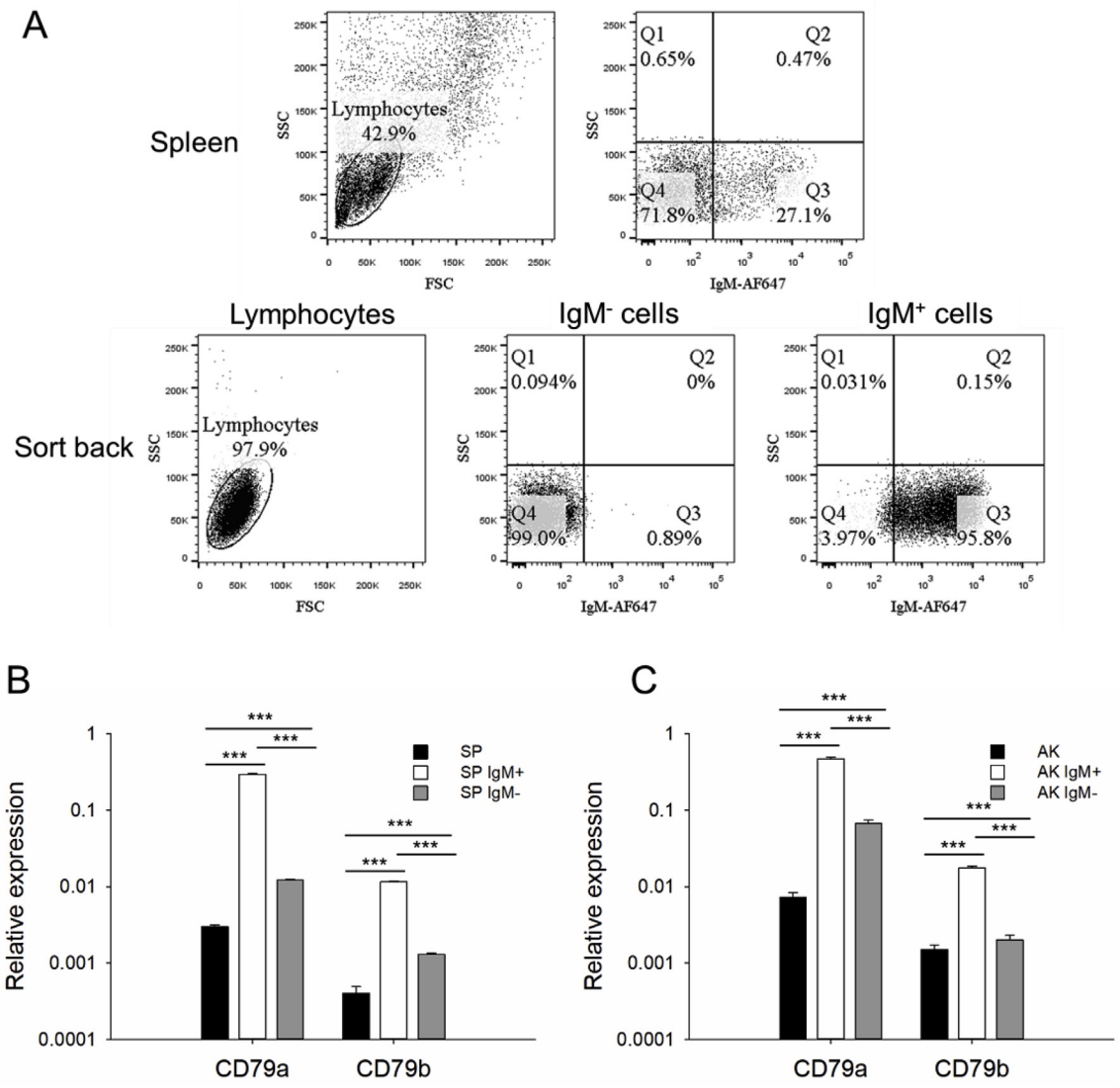


**Fig. 4.** Expressions of *OnCD79a* and *OnCD79b* transcripts in total leukocytes, sorted $IgM^+$ and $IgM^-$ cells from spleen and anterior kidney. The transcriptions were evaluated through qPCR in triplicates. Data from three independent experiments are shown as the mean gene expression relative to the expression of an endogenous control ($\beta$ actin) ± SD.

spleen were important immune tissues in teleost and main target organs attacked by *S. agalactiae* infection [28,38,40], these two organs were collected and analyzed in this study. Upon *S. agalactiae* infection, expressions of *OnCD79a* and *OnCD79b* were significantly up-regulated in both anterior kidney and spleen (Fig. 5). The fold-increase of *CD79a* expression was higher than that of *CD79b*, indicating that *CD79a* may exhibit a faster and stronger response to *S. agalactiae* infection than *CD79b* (Fig. 5). It is consistent with the finding in snapper as well [28]. In addition, after *S. agalactiae* stimulation *in vivo*, expressions of *OnCD79a* and *OnCD79b* were also significantly up-regulated in

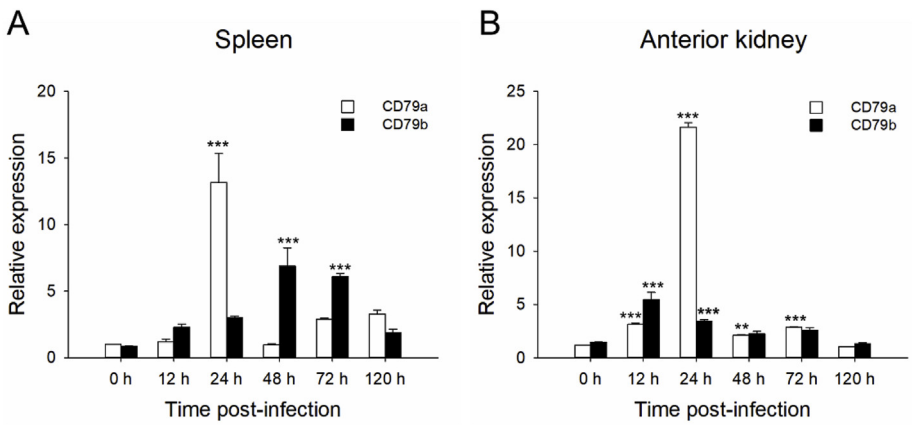


**Fig. 5.** Temporal mRNA expressions of *OnCD79a* and *OnCD79b* in the spleen (A) and anterior kidney (B) upon *S. agalactiae* challenge. At 0, 12, 24, 48, 72 and 120 h post-infection ($1 \times 10^7$ CFU/mL), five tilapias from each group were analyzed. Data are shown as the mean fold changes of CD79 gene expression in experiment relative to the expressions in control PBS. Significant difference was marked by asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

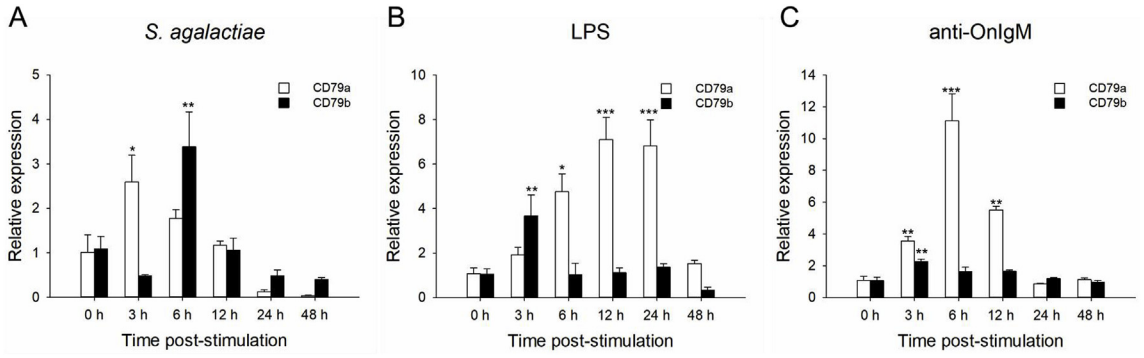


**Fig. 6.** Expressions of *OnCD79a* and *OnCD79b* transcripts in anterior kidney leukocytes. The leukocytes were stimulated with *S. agalactiae* ($1 \times 10^7$ CFU/mL), LPS (40 μg/mL) and anti-OnIgM monoclonal antibody (10 μg/mL), respectively. The data represented means ± SD (n = 5). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, and *β* actin was used as calibrator. Significant difference was marked by asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

leukocytes from anterior kidney (Fig. 6A). Therefore, the significant up-regulation of fish *CD79a* and *CD79b* transcripts induced by pathogenic bacterial infection indicated that CD79 was likely to get involved in host defense against bacterial infection in teleost [28,29].

LPS, a component of the outer membrane of gram-negative bacteria, is known to be a potent polyclonal activator for mammalian or teleost B leukocytes [45,53,54]. LPS is a multivalent antigen with an ability to effectively activate the BCR signaling pathway by cross-linking B cell antigen receptors. And the interaction of anti-OnIgM monoclonal antibody with membrane IgM would cause the cross-linking BCR to promote B cell activation [38]. In order to examine the effect of these two stimuli on *CD79* expression, leukocytes from anterior kidney were treated with LPS and anti-OnIgM monoclonal antibody *in vitro*. Upon both stimulations, *OnCD79a* and *OnCD79b* expressions were significantly up-regulated in leukocytes (Fig. 6B and C). Similar to the finding in bacterial infection *in vivo*, the fold of increase of *CD79a* expression was higher than that of *CD79b* in both *in vitro* stimulations (LPS 7.1 vs. 3.7, anti-IgM antibody 11.1 vs. 2.3, respectively). In addition, the fold of increase of *CD79a* expression upon anti-IgM antibody stimulation was greater than that upon LPS challenge (11.1 vs. 7.1) (Fig. 6B and C). The variation of *CD79* expression pattern upon stimulation with LPS or anti-OnIgM monoclonal antibody may be due that the way of two stimuli to cross-link BCR is different, resulting in the different patterns of CD79 expression in the signaling pathways. Thus, the *in vitro* study indicated that *CD79a* might play a crucial role in BCR signaling.

In conclusion, CD79 was identified and characterized from Nile tilapia that shared important structural domains with other species. The *OnCD79* transcription was highly expressed in immune tissues, such as anterior kidney and spleen, and $IgM^+$ B cells. Upon bacterial challenge, the expression of *OnCD79* was significantly up-regulated *in vivo* (anterior kidney and spleen) and *in vitro* (anterior kidney leukocytes). These findings indicated that the CD79 might get involved in host defense against bacterial infection. In addition, the significant up-regulation of *OnCD79* expression was detected in the anterior kidney leukocytes induction with mouse anti-OnIgM monoclonal antibody *in vitro*, revealing that CD79 might function as a B cell receptor accessory molecule and play a crucial role in B cell signaling pathway.

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