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Expression and functional analysis of polymeric immunoglobulin receptor in Nile tilapia (*Oreochromis niloticus*)

Shuo Liu, Meng Chen, Fangfang Yan, Enxu Zhou, Bingxi Li, Shengli Fu, Xiaoxue Yin, Zheng Guo, Jianmin Ye*

Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, Guangdong Provincial Engineering Technology Research Center for Environmentally-Friendly Aquaculture, College of Life Sciences, South China Normal University, Guangdong 510631, PR China

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ABSTRACT

Polymeric immunoglobulin receptor (pIgR) plays important roles in transport of polymeric immunoglobulins across mucosal epithelial cells and prevention of access of pathogens into the organisms. In this study, a *pIgR* ortholog (*OnpIgR*) was cloned and identified from Nile tilapia (*Oreochromis niloticus*). The open reading frame (ORF) of *OnpIgR* is 1023 bp, encoding a predicted protein of 341 amino acids. The deduced amino acid sequence of OnpIgR is highly homologous to other teleost's pIgRs, containing two Ig-like domains (ILDs), a transmembrane region and an intracellular region. Expression analysis revealed that *OnpIgR* was exhibited in all tested tissues including liver, spleen, skin and intestines, and highly expressed in the liver. Immunofluorescence detection indicated that the natural pIgR expressed on the membrane of the epithelial cells. After challenge of bacterial pathogen (*Streptococcus agalactiae*), *OnpIgR* expression was significantly up-regulated in intestines and skin, as well as intestinal epithelial cells. In addition, recombinant OnIFN-γ induced the expression of pIgR in the intestinal epithelial cells. Moreover, recombinant OnpIgR protein was able to bind both *S. agalactiae* and *A. hydrophila in vitro*, and interact with (r)OnIgM and (r)OnIgT. Taken together, the results indicated that OnpIgR, involving in the polymeric immunoglobulin transport, might play an important role in mucosal immunity against bacterial infection.

1. Introduction

Polymeric immunoglobulin receptor (pIgR), a key immune defense component in mammals, transports dIgA or pentameric IgM across mucosal epithelial cells, which plays an important role in prevention of access of pathogens into the organisms and neutralization of toxic agents (Brandtzaeg et al., 1997; Kaetzel and Mostov, 2005; Norderhaug et al., 1999). The pIgR is conserved in structure, containing an extracellular region composed of tandem Ig-like domains, a transmembrane region and a cytoplasmic tail (Mostov et al., 1984). Mammalian pIgR extracellular region has five Ig-like domains (D1-D5). Bovine and rabbit also have three Ig-like domains (D1, D4, and D5), created by alternative splicing (Kulseth et al., 1995; Kühn and Kraehenbuhl, 1981; Kühn et al., 1983; Mostov et al., 1980). In addition, chicken pIgR consists of four Ig-like domains, corresponding to mammalian D1 and D3–5.

The synthesis of pIgR and pathway of pIgR-mediated transport of immunoglobulins across polarized epithelial cells have been exhaustively characterized in mammals (Kaetzel, 2005). In humans, the pIgR is synthesized by epithelial cells lining mucous membranes and exocrine glands epithelial cells (Kaetzel and Mostov, 2005). The pIgR protein firstly process in rough endoplasmic reticulum as a transmembrane glycoprotein, and then secrete to basolateral surface of the epithelial cell (Kaetzel and Mostov, 2005; Mostov et al., 2003). At this surface, pIgR binds the secretory immunoglobulins which is produced locally by plasma cells, and then the pIgR-Ig complex is endocytosed and delivered to apical membrane (Shimada et al., 1999). Finally, the pIgR is proteolytically cleaved the extracellular domain, known as the secretory component (SC), which protects antibody from proteolytic degradation, mediates interactions and neutralization with host and bacterial factors (Shimada et al., 1999). In human intestinal and airway mucosae, pIgR expression depends not only on the microbes (Johansen et al., 2004), but also on transcriptional enhancement by proinflammatory and immunoregulatory cytokines, such as TNF, IFN-γ, IL-1 and IL-4 (Blanch et al., 1999; Schjerven et al., 2000, 2003), which provides an immunoregulatory link between enhanced Immunoglobulin production and export during low-grade inflammation or

* Corresponding author at. College of Life Sciences, South China Normal University, No.55 West Zhongshan Avenue, Guangzhou, Guangdong Province 510631, PR China.
E-mail address: jmye@m.scnu.edu.cn (J. Ye).

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infection (Brandtzaeg et al., 1992).

Up to now, the pIgR in teleost has been characterized in a few species, such as fugu (*Takifugu rubripes*) (Hamuro et al., 2007), carp (*Cyprinus carpio*) (Rombout et al., 2008), orange-spotted grouper (*Epinephelus coioides*) (Feng et al., 2009), rainbow trout (*Oncorhynchus mykiss*) (Zhang et al., 2010), Atlantic salmon (*Salmo salar*) (Tadiso et al., 2011) and dojo loach *(Misgurnus anguillicaudatus)* (Yu et al., 2017). The fish pIgR comprises two Ig-like domains (D1-D2) which are homologous to mammalian D1 and D5 by phylogenic sequence analysis, respectively (Feng et al., 2009). Studies on the function of teleosts pIgR showed that its domains associate with polymeric IgM and IgT/IgZ (Hamuro et al., 2007; Sunyer, 2013), and transport Igs to intestines, skin and gill mucus (Xu et al., 2013a, 2013b, 2016a, 2016b; Zhang et al., 2010). Further, *pIgR* expression in fish was induced by Gram-negative and Gram-positive bacteria and also teleost pIgR could interact with pathogenic bacteria (Kelly et al., 2017; Yang et al., 2017). Similar to the mammals, the epithelial cells in teleost express pIgR and play a key role in the pathway of pIgR-mediated transport of immunoglobulins to mucus (Hamuro et al., 2007; Rombout et al., 2008). However, there is rarely known about the expression pattern of pIgR in teleost epithelial cells upon challenges, as well as the regulation of cytokines in the pIgR expression.

Nile tilapia (*Oreochromis niloticus*) is one of economically important cultured fish in the world. However, bacterial pathogens (*Streptococcus agalactiae* and *Aeromonas hydrophila*) have been reported recently leading to serious economic losses in tilapia industry (Tang et al., 2014; Wang et al., 2017a, 2017b). Considering the crucial role of pIgR in mucus defense, the cDNA of Nile tilapia *pIgR* was cloned in this study based on the predict sequence of *O. niloticus pIgR* mRNA and expressed the recombinant pIgR protein using an *Escherichia coli* expression system. The mRNA expression level of *pIgR* was investigated in skin and intestines upon bacterial (*S. agalactiae*) infection. In addition, the distribution pattern of OnpIgR in skin epithelial cells was addressed and the *pIgR* mRNA expression pattern and natural pIgR in intestinal epithelial cells was assessed. Concurrently, the regulation of (r)OnIFN-γ on pIgR expression was detected in Nile tilapia. Moreover, the binding capability of the (r)OnpIgR with bacteria and Igs was examined. These studies will provide a light on understanding of pIgR functions in the mucosal defense of Nile tilapia.

2. Materials and methods

2.1. Fish rearing, injection and sample collection

Healthy Nile tilapia (*O. niloticus*), weighting about 80 ± 10 g, was obtained from Guangdong Tilapia Breeding Farm (Guangdong, China) and maintained in the automatic filtering aquaculture system under 28 ± 2 °C with acclimated for three weeks prior to treatments (Ding et al., 2016; Mu et al., 2017). All animal protocols were reviewed and approved by the University Animal Care and Use Committee of the South China Normal University.

Three fish were dissected for tissue sampling to investigate *OnpIgR* expression in healthy Nile tilapia. The tissue samples including head kidney, intestines, gill, thymus, skin, muscle, spleen and liver were collected and frozen immediately by liquid nitrogen, followed by storage at −80 °C.

To investigate the immune responses of *OnpIgR*, thirty fish were randomly distributed into 2 groups of 15 fish each, one group was intraperitoneally injected with 100 μL (1×10^7 CFU/mL) live bacteria liquid *S. agalactiae* (ZQ1901), and one group injected with 100 μL PBS, respectively (Gan et al., 2014; Wang et al., 2012). Samples of skin and intestines of three fish were collected at 0, 6, 12 and 24 h post-injection, and all the tissue samples were gathered and frozen by liquid nitrogen for further use.

Three fish from each group were randomly sampled at 0, 6, 12 and 24 h post-injection. Samples of skin and intestines were gathered and frozen by liquid nitrogen for further use.

2.2. RNA extraction and cDNA synthesis

Total RNA from the samples was isolated by using Trizol Reagent (Invitrogen, USA) according to the manufacturer's protocol. The concentration and quality of the total RNA was assessed using a NanoDrop 2000 spectrophotometer (Thermo, USA) at 260 nm and 260/280 nm absorbance ratios. The cDNAs were synthesized by PrimerScript™ RT reagent kit (TaKaRa, Japan), then diluted 10-fold and stored at −80 °C for the use of quantitative real time PCR (qRT-PCR).

2.3. Cloning the OnpIgR sequence and bioinformatics analysis

Based on the predict sequence of *O. niloticus pIgR mRNA* (GenBank accession XM_019352968), the complete ORF primers pIgR-F1 and pIgR-R1 were designed by Primer Premier 5.0 and summarized in Supplementary Table 1. The target products were tested using a 1% agarose gel electrophoresis (BIOWEST, Spain), cloned into the pMD-18T vector (TaKaRa, Japan) and then sequenced by Invitrogen. In addition, the cDNA sequences of Nile tilapia IgM (GenBank accession KC708223.1) and IgT (GenBank accession KX214533.1) were also cloned.

The potential ORF was identified using the Finder program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The protein analysis and homology analyses of nucleotide sequences and amino acid sequence were conducted with ExPASy tools (http://expasy.org/tools/) and BLAST programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi), respectively. Multiple alignment of pIgR amino acid sequences were performed with the Clustalw2 program (http://www.ebi.ac.uk/Tools/clstalw2/) and the DNAMAN software. The structural features of protein sequence were analyzed with SMART online website (http://smart.embl-heidelberg.de/) and prediction of signal peptide was confirmed by SignalP (http://cbs.dtu.dk/services/SignalP/). Phylogenic trees were constructed using MEGA 6.0 software with 1000 bootstrap replications (Mu et al., 2018).

2.4. Quantitative real-time PCR analysis of pIgR mRNA expression

The analysis of *pIgR* mRNA expression in different healthy tissues and following bacterial injection were determined by qRT-PCR, which was implemented by the 7500 Real Time PCR System (Life Technologies, USA) with the Hieff™ QPCR SYBR Green Master Mix (YEASEN, China). The PCR reaction contained 10 μL 2 × Taq™SYBR premix, 3 μL of diluted cDNA, 2 μL of each primer (2 μM), 3 μL DEPC treated water (Invitrogen, USA) in a 20 μL final volume. The PCR program as follows: 95 °C for 3 min, 95 °C for 15 s, 60 °C for 1 min then go to step 2 and repeated for 40 cycles. The gene specific primers qpIgR-F and qpIgR-R (Supplementary Table 1) were designed to amplify pIgR fragment, while tilapia β-actin primers (Supplementary Table 1) were used to amplify actin fragment as an internal control to normalize the potential variations for RT-PCR. Each sample was examined in triplicate. The relative expression of *OnpIgR* mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), and the quantitative expression data were presented as the means ± standard deviation (SD).

2.5. Isolation and culture of primary skin and intestinal epithelial cells in vitro

Healthy Nile tilapias were not fed for 2 days prior to sampling, and fishes were anesthetized in diluted MS-222 (1:2500, Sigma, USA) (Xu et al., 2016a, 2016b). Tissues of intestines and skin were collected and transferred to a 50 mL conical sterilized centrifuge tube (Corning, USA) with PBS containing 280 μg/mL gentamicin (Amerso, USA), 30 μg/mL fungizone (Sigma, USA), 1% Penicillin/streptomycin (Hyclone, USA)