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Characterization and expression analysis of laboratory of genetics and physiology 2 gene in sea perch, *Lateolabrax japonicus*

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LGP2 (laboratory of genetics and physiology 2) as a key component of the retinoic acid-inducible gene I (*RIG-I*)-like receptors (RLRs), plays a predominant role in modulating RLRs-mediated cellular antiviral signaling during viral infection. In the present study, we cloned the *LGP2* gene from the sea perch (*Lateolabrax japonicus*) (*LjLGP2*), an economically important farmed fish. The complete cDNA sequence of *LjLGP2* was 2790 nt and encoded a polypeptide of 682 amino acids which contains four main structural domains: one DEAD/DEAH box helicase domain, one conserved restriction domain of bacterial type III restriction enzyme, one helicase superfamily c-terminal domain and one C-terminal domain of *RIG-I*, similar to most vertebrate *LGP2*. Subcellular localization analysis showed that *LjLGP2* spanned the entire cytosol. The *LjLGP2* mRNA was widespread expressed in the tested 10 tissues of healthy fish and significantly up-regulated post NNV infection. Furthermore, time course analysis showed that *LjLGP2* transcripts significantly increased in the spleen, kidney and liver tissues after NNV infection. *LjLGP2* mRNA expression was rapidly and significantly up-regulated in LJB cells after poly I:C stimulation and NNV infection. The present results suggest that *LjLGP2* may be involved in recognization of NNV and play a role in antiviral innate immune against NNV in sea perch.

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1. Introduction

Innate immunity establishes the first line of defense against pathogenic microorganisms. The host senses pathogenic microorganisms invasion by recognition of pathogen-associated molecular patterns (PAMP) with pattern recognition receptors (PRR), including Toll-like receptor (TLR), *RIG-I*-like receptors (RLRs) and NOD-like receptors (NLRs) [1]. RLRs mainly sense cytoplasmic viral RNA and play a key role in regulating the inflammatory response against infectious viruses [2]. The RLRs family contains three members: *RIG-I*, *MDA5* (Melanoma differentiation associated gene 5), and *LGP2*. All three RLRs contain a DExD/H-box helicase domain and a C-terminal domain. *RIG-I* and *MDA5* further contain N-terminal tandem caspase activation and recruitment domain (CARD) which *LGP2* lacks [3]. The CARD within these RLRs are mainly responsible for downstream signaling by interacting with its adaptor protein *MAVS* (Mitochondrial antiviral signaling protein) to activate the transcription factors NF-κB and *IRFs*, and then induce production of type I IFNs and pro-inflammatory cytokines.

Abbreviations: LGP2, laboratory of genetics and physiology 2; RIG-I, retinoic acid-inducible gene I; RLR, retinoic acid-inducible gene I-like receptors; MAVS, mitochondrial antiviral signaling protein; PAMP, pathogen-associated molecular patterns; PRR, pattern recognition receptors; TLR, Toll-like receptor; NLRs, NOD-like receptors; MDA5, melanoma differentiation associated gene 5; CARD, N-terminal tandem caspase activation and recruitment domain; 5′ppp-ssRNA, 5′-triphosphate single-stranded RNAs; NNV, nervous necrosis virus; SMART, simple modular architecture research tool; RGNNV, redspotted grouper nervous necrosis virus; $TCID_{50}$, 50% tissue culture infective dose; DEXDc, DEAD/DEAH box helicase domain; ResIII, conserved restriction domain of bacterial type III restriction enzyme; HELICc, helicase superfamily c-terminal domain; RIG-I CTD, C-terminal domain of RIG-I.

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Consequently these factors initiate innate immune responses and acquired immune responses, enhancing the host's antiviral ability [3]. *RIG-I* senses 5′-triphosphate single-stranded RNAs (5′ppp-ssRNA) and short dsRNA (<1 kb), while *MDA5* is activated by long dsRNA (>3 kb) including dsRNA replication intermediates of positive-sense RNA viruses, the genomes of dsRNA viruses and polyinosinic:polycytidylic acid (poly I:C) [4]. *LGP2* recognizes 5′ppp-ssRNAs and dsRNAs [5].

Although the regulation mechanisms of *RIG-I* has already been well understood in RLR signaling, less is known about the *MDA5* and *LGP2*. Especially, many studies have reported that *LGP2* had negative and positive regulatory roles in innate antiviral immunity [6], little is known about the precise roles and molecular mechanism of *LGP2* mediating opposing roles in antiviral immunity. At present, the cDNA sequence of *LGP2* gene has been obtained from a few teleost fish, such as the olive flounder, *Paralichthys olivaceus* [7]; atlantic cod, *Gadus morhua* [8]; common carp, *Cyprinus carpio* [9], rainbow trout, *Oncorhynchus mykiss* [10]; fugu, *Takifugu rubripes* [11]; and grass carp, *Ctenopharyngodon idella* [12]. Previous works showed that *LGP2* transcripts increased significantly after viral infection or poly I:C stimulation in these species [7,10], indicating that *LGP2* was pivotal for antiviral innate immune defense.

Nervous necrosis virus (NNV) belonging to the family *Nodaviridae* has a bipartite genome of positive-sense RNA and has been identified as the mainly cause of high mortality of mass larval-stage marine and freshwater fish species [13]. Currently, more than 40 fish species including Anguilliformes, Gadiformes, Perciformes, Pleuroneetiformes and Tetraodontiformes, have been reported to be infected by NNV [14]. Sea perch (*Lateolabrax japonicus*) is an economically important cultured fish species in Asian, especially in Japan and China, and was also infected by NNV causing considerable economic losses [15]. However, the innate immune response against NNV is still unclear in sea perch. Considering the important roles of RLRs in recognization of virus RNA and inducing antiviral innate immune response. In this study, we clone the full-length cDNA sequence of *LGP2* from the economically important sea perch, and report the gene structure, phylogenetic analysis and subcellular localization of *LjLGP2*. Furthermore, we investigate the spatio-temporal expression *LjLGP2* gene in the tissues of healthy and NNV infected fish *in vivo*. Finally, we explored the temporary expression pattern of *LjLGP2* gene after poly I:C stimulation and NNV infection *in vitro*. Our results would enrich the knowledge of bony fish RLRs in antiviral immunity and provide a novel sight for the defense of NNV in sea perch.

2. Materials and methods

2.1. Cloning the full-length cDNA of LjLGP2

Total RNA was isolated from sea perch brain using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. 1 μg of total RNA was reverse transcribed into first-strand cDNA with PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Japan). Degenerate primers were designed to amplify the conserved domains of *LjLGP2* based on the published several bony fish *LGP2* cDNA sequences including *I. punctatus LGP2* mRNA (JQ008941), *C. idella* RNA helicase *LGP2* mRNA (FJ813483.2), *O. fasciatus* RNA helicase (*LGP2*) gene, complete CDS (KF267451.1), and *P. olivaceus LGP2* mRNA (HM070372.1). PCR reaction was performed with primers *LjLGP2*-1F and *LjLGP2*-1R (Table 1) according to the following program: 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 50 s, and elongation step at 72 °C for 10 min. Amplified PCR products were cloned into the PMD 19-T vector (Takara, Japan) and sequenced.

The 3′- and 5′-RACE-PCRs were performed as described before [16]. Briefly, the 3′ and 5′ ends of *LjLGP2* cDNA sequences were obtained using SMART™ RACE cDNA amplification kit (Clontech, USA) according to the manufacturer's instructions, respectively. All primers that were used are listed in Table 1. The purified PCR products were ligated with pRACE vector (Clontech, USA), and the confirmed clones were sequenced.

Table 1
Primers used for cloning and expression analysis.

Name	Sequence (5′–3′)
LjLGP2-1F	GACHGGWGGWGGAAAGAC
LjLGP2-1R	GRGGAGATKAGSAGGTTGAGD
LjLGP-3′-F	ATGACGCCCTGCTTATCAATGACACCC
LjLGP-5′-R	GGCAGTGGGTGCTTTTACGGGTTTT
Q*LjLGP2*-1F	CAAGAGTTCAGCAGCAGG
Q*LjLGP2*-1R	CACGGTGGTAGCAATCAG
β-actin-F	CAACTGGGATGACATGGAGAAG
β-actin-R	TTGGCTTTGGGGTTCAGG
LjLGP2-2F	ATGGCAGATTTTGGACTGTATG
LjLGP2-2R	ATCAAAGATGTCAGGGAAGTT

2.2. Bioinformatics analysis of LjLGP2

The *LjLGP2* sequence was analyzed as previous study [12]. Briefly, the *LjLGP2* sequence was analyzed using the BLAST program at NCBI (http://www.ncbi.nlm.nih.gov/blast) and the Expert Protein Analysis System (http://www.expasy.org/). Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/) was used to predict the protein domains, and the alignment of amino acid sequences was performed using the ClustalX 1.83 software. The phylogenetic tree was generated using the MEGA 6.06 package by the neighbor-joining method with bootstrap resampling (1000 pseudo-replicates).

2.3. Virus

NNV(RGNNV[redspotted grouper nervous necrosis virus], SBN147 strain) was isolated from diseased sea perch in Guangdong Province, China. RGNNV were propagated in sea perch brain (LJB) cells at 28 °C, and virus titers were detected by 50% tissue culture infective dose ($TCID_{50}$).

2.4. Fish

Healthy sea perch with average weight of ~50 g, procured from a fish farm in Zhuhai City, Guangdong Province, were acclimated at 28 °C for 1 week before process and fed with commercial feed twice a day.

2.5. In vivo NNV challenge and sample collection

For the viral challenge *in vivo*, 200 μl of RGNNV (3×10^7 $TCID_{50}$/ml) were injected into the abdominal muscles of healthy sea perch. The control fish were injected with 200 μl PBS.

For tissue distribution analyses, three individuals were sacrificed and tissues including heart, liver, intestines, gill, spleen, muscle, brain, kidney, thymus and eye were collected from 48 h post-injection sea perch. PBS-injection sea perch were used as control.

For the time-dependent assay, three individuals were sacrificed and tissues including kidney, spleen and liver were collected at 1, 2, 3, 4, 5 and 6 d after NNV injection. The kidney, spleen and liver tissues from healthy sea perch injected with PBS were used as control.

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