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Negative regulation of the antiviral response by grouper LGP2 against fish viruses

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ABSTRACT

Laboratory of genetics and physiology 2 (LGP2), a member of RIG-I like receptor (RLR) family, plays crucial roles in modulating cellular antiviral response during viral infection. However, the detailed roles of LGP2 in different virus infection were controversial up to now. Here, we cloned a LGP2 gene from orange-spotted grouper (EcLGP2) and investigated its roles in response to grouper virus infection. EcLGP2 encoded a 678-aa protein which shared 83% identity to sea perch (*Lateolabrax japonicas*). Amino acid alignment showed that EcLGP2 contained three conserved domains, including a DEAD/DEAH box helicase domain, a helicase superfamily C-terminal domain and a C-terminal domain of RIG-I. In healthy grouper, the transcript of EcLGP2 could be predominantly detected in kidney, gill, fin, spleen and skin. Subcellular localization analysis showed that EcLGP2 distributed throughout the cytoplasm in grouper cells. Notably, the intracellular distribution of EcLGP2 was altered at the late stage of Singapore grouper iridovirus (SGIV) infection, but remained unchanged during red-spotted grouper nervous necrosis virus (RGNNV) infection. Moreover, overexpression of EcLGP2 *in vitro* significantly enhanced the viral replication of SGIV and RGNNV, evidenced by the acceleration of CPE occurrence and the up-regulation of the viral gene transcription or protein synthesis. Further studies indicated that overexpression of EcLGP2 decreased the expression level of interferon related molecules or effectors, including IRF3, IRF7, ISG15, IFP35, MXI, MXII, and MDA5, suggesting that the negative feedback of interferon immune response by EcLGP2 might contribute to the enhancement of RGNNV infection. Moreover, the expression levels of pro-inflammation cytokines, including IL-8 and TNFα were significantly decreased, but that of IL-6 was increased by the ectopic expression of EcLGP2. Thus, our results will contribute greatly to understanding the roles of fish LGP2 in innate immune response during iridovirus and nodavirus infection.

1. Introduction

During the co-evolution of host-pathogens interaction, pattern recognition receptors (PRRs) are identified to be directly responsible for sensing the presence of pathogens and activating innate immune responses. Currently, PRRs were subdivided into four major families, including Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and C-type lectin receptors (CLRs) [1,2]. Among these PRRS, the RLR family was now found to be composed of RIG-I, melanoma differentiation associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) [3]. All the RLRs contained a central DEAD box helicase/ATPase domain, and a C-terminal regulatory domain, while only RIG-I and MDA5 contained two N-terminal caspase recruitment

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domains (CARDs). They were demonstrated to localize in the cytoplasm and recognize the genomic RNA of dsRNA viruses and dsRNA generated during ssRNA virus infection [2]. Subsequently, the intracellular signaling components were activated and ultimately culminating in the expression of a variety of antiviral immune molecules and proinflammatory cytokines which orchestrated the host response to virus infection [4].

As an important member of RLR family, LGP2 was demonstrated to exert critical roles during virus infection. LGP2 was originally identified as a negative regulator of RLR signaling [5,6]. Overexpression of LGP2 has been shown to result in down-regulating IFN production in cells after virus infection. Furthermore, LGP2-deficient mice exhibit enhanced resistance to lethal vesicular stomatitis virus (VSV) infection [7,8]. In contrast, recent findings also demonstrated that LGP2 was essential for type I IFN production in response to picornaviridae infection. Moreover, LGP2 facilitated viral RNA recognition by RIG-I and MDA5 through its ATPase domain [9,10]. For DNA virus, the levels of IFNβ, CXCL10 and CCL5 were induced by vaccinia virus Ankara (MVA) infection but the immune response was impaired in LGP2-deficient cells compared to wild-type cells [11]. Except the critical regulatory roles in IFN immune response, LGP2 was also found to not only promote an essential pro-survival signal in response to antigen stimulation to confer CD8(+) T cell-number expansion and effector functions against divergent RNA viruses [12], but also play a key role in conferring tumor cell survival following cytotoxic stress induced by ionizing radiation [13]. In fish, overexpression of Japanese flounder LGP2 significantly delayed the cytopathic effects of viral hemorrhagic septicemia virus (VHSV), or hirame rhabdovirus (HIRRV). Moreover, overexpression of LGP2 increased the mRNA expression levels of type I IFN and IFN-inducible genes (MX and ISG15) in response to viral infections [14,15]. Thus, LGP2 was proposed to exert crucial but controversial roles during different virus infections.

Groupers, *Epinephelus* spp. have attracted much attention due to the considerable economic value and the sex-reversal phenotype in the specific developmental stage [16]. In grouper industry, the outbreak of viral disease, especially evoked by red-spotted grouper nervous necrosis virus (RGNNV) and Singapore grouper iridovirus (SGIV) have caused heavy economic losses in recent years [17,18]. Our previous studies uncovered that several immune signaling molecules played different immune regulatory roles in RGNNV and SGIV infection [19–24]. Interferon stimulated genes and the related transcript factors, including ISG15 and IRF3, were demonstrated to exert crucial roles in RGNNV infection [20,22]. Recent studies indicated that another member of RLR, grouper MDA5 was involved in both RGNNV and SGIV infection [25]. Given that LGP2 played different roles during various virus infections, whether grouper LGP2 functioned as antiviral molecules during iridovirus or nodavirus infection still remained unknown.

In the present study, a LGP2 homolog from marine fish, orange-spotted grouper (*Epinephelus coioides*) (EcLGP2), was cloned and its roles during fish virus infection were studied. Our results will contribute greatly to understanding the functions of fish LGP2 in response to DNA virus and RNA virus infection.

2. Material and methods

2.1. Fish, cells and viruses

Orange-spotted groupers, *E. Coioides* (50–60 g) used in this study were purchased from a marine fish farm, Hainan Province, China. Groupers were kept in a laboratory recirculating seawater system for 2–3 weeks before use. Grouper spleen (GS) cells were propagated and grown in Leibovitz's L15 medium containing 10% fetal bovine serum (FBS) at 25 °C [20]. The stocks of SGIV and RGNNV were kept at −80 °C until used.

2.2. Cloning of EcLGP2 and sequence analysis

According to the EST sequences of EcLGP2 from grouper spleen transcriptome [26], RACE assay was performed and the full length cDNA of EcLGP2 was obtained using the primers listed in Table 1. Sequence analysis were carried out using BLAST program in NCBI database. The conserved domains were predicted using SMART program. Multiple sequences alignment was performed using ClustalX1.83 software and edited using GeneDoc program. The phylogenetic analysis was performed using Mega 4.0 software.

2.3. Expression patterns for EcLGP2 in grouper

The tissue distribution pattern of EcLGP2 in orange-spotted grouper was examined by quantitative real-time PCR (qRT-PCR). In brief, the total RNA was extracted from different tissues from 3 healthy groupers, including head kidney, heart, liver, spleen, intestine, muscle, brain, skin, gill, stomach and kidney, as described previously [22]. Then the expression level of EcLGP2 was determined as described in the following section.

To evaluate the actions of EcLGP2 in innate immunity, groupers were injected with poly I:C or SGIV, as described previously [24]. Briefly, poly I:C or SGIV-injected groupers were collected at

Table 1
Primers used in this study.

Name	Sequence (5′–3′)
EcLGP2-5′NGSP1	TGAGTTTAGGAACTACTACAAAGTTGGT
EcLGP2-5′NGSP2	TTCGAGGACTGGGAGCCTGGGCGT
EcLGP2-3′GSP1	GATCTGTGGCAATGGTCGTTCACCCT
EcLGP2-3′GSP2	GTCCTTGTGGGTGTGGTGACACTCAT
EcLGP2-Flag-F	CAGGGTACCGAATGGCAGATTTTAAGCTGTTTGAA
EcLGP2-Flag-R	CATGAATTCGTCGAAGAGGTCAGGGAAGTTGT
pEGFP-EcLGP2-F	TAACTCGAGCTATGGCAGATTTTAAGCTGTTTGAA
pEGFP-EcLGP2-R	ACTGGTACCGTCGAAGAGGTCAGGGAAGTTGT
EcLGP2-RT-F	TGGTGGTACGCTATGGACTGC
EcLGP2-RT-R	TTGTAGCTCAGTTATCTTTGTGCGA
Actin- RT-F	TACGAGCTGCCTGACGGACA
Actin- RT-R	GGCTGTGATCTCCTTCTGCA
SGIV-MCP-RT-F	GCACGCTTCTCTCACCTTCA
SGIV-MCP-RT-R	AACGGCAACGGGAGCACTA
SGIV-VP19-RT-F	TCCAAGGGAGAAACTGTAAG
SGIV-VP19-RT-R	GGGGTAAGCGTGAAGACT
SGIV-ICP18-RT-F	ATCGGATCTACGTGGTTGG
SGIV-ICP18-RT-R	CCGTCGTCGGTGTCTATTC
SGIV-VP49-RT-F	CCCGCAATGAACTCGCCAAAACT
SGIV-VP49-RT-R	CCGTGACGTACTGCCAAGCCTGA
RGNNV-RdRp-RT-F	GTGTCCGGAGAGGTTAAGGATG
RGNNV-RdRp-RT-R	CTTGAATTGATCAACGGTGAACA
RGNNV-CP-RT-F	CAACTGACAACGATCACACCTTC
RGNNV-CP-RT-R	CAATCGAACACTCCAGCGACA
EcIL6-RT-F	CTCTACACTCAACGCGTACATGC
EcIL6-RT-R	TCATCTTCAAACTGCTTTTCGTG
EcIRF3-RT-F	ATGGTTTAGATGTGGGGGTGTCGGG
EcIRF3-RT-R	GAGGCAGAAGAACAGGGAGCACGGA
EcIRF7-RT-F	CAACACCGGATACAACCAAG
EcIRF7-RT-R	GTTCTCAACTGCTACATAGGGC
EcIL8-RT-F	GCCGTCAGTGAAGGGAGTCTAG
EcIL8-RT-R	ATCGCAGTGGGAGTTTGCA
EcTNFa-RT-F	GTGTCCTGCTGTTTGCTTGGTA
EcTNFa-RT-R	CAGTGTCCGACTTGATTAGTGCTT
EcIL-1β-RT–PF	AACCTCATCATCGCCACACA
EcIL-1β-RT-PR	AGTTGCCTCACAACCGAACAC
EcISG15-RT-F	CCTATGACATCAAAGCTGACGAGAC
EcISG15-RT-R	GTGCTGTTGGCAGTGACGTTGTAGT
EcMDA5-RT-F	ACCTGGCTCTCAGAATTACGAACA
EcMDA5-RT-R	TCTGCTCCTGGTGGTATTCGTTC