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Short communication

Molecular cloning and characterization of a galectin-1 homolog in orange-spotted grouper, *Epinephelus coioides*

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ABSTRACT

As a member of animal lectin family, galectin has the functions of pathogen recognition, anti-bacteria and anti-virus. In the present study, a galectin-1 homolog (EcGel-1) from grouper (*Epinephelus coioides*) was cloned and its possible role in fish immunity was analyzed. The full length cDNA of EcGel-1 is 504 bp, including a 408 bp open reading frame (ORF) which encodes 135 amino acids with a molecular mass of 15.19 kDa. Quantitative real-time PCR analysis indicated that EcGel-1 was constitutively expressed in all analyzed tissues of healthy grouper. The expression of EcGel-1 in the spleen of grouper was differentially up-regulated challenged with Singapore grouper iridovirus (SGIV), poly (I:C), and LPS. EcGel-1 was abundantly distributed in the cytoplasm in GS cells. Recombinant EcGel-1(rEcGel-1) protein can make chicken erythrocyte aggregation, and combine with gram negative bacteria and gram positive bacteria in the presence of 2-Mercaptoethanol (β-ME). Taken together, the results showed that EcGel-1 may be an important molecule involved in pathogen recognition and pathogen elimination in the innate immunity of grouper.

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1. Introduction

The immune system of vertebrate is mainly consists of innate and adaptive immunity. Fish belongs to the lowest vertebrate. In its process of resistance to the pathogen infection, the innate immunity plays a vital role. Recognition of pathogens is mediated by a series of pattern recognition receptors (PRRs), which identified pathogen-associated molecular patterns (PAMPs) shared by a wide range of microorganisms, thereby successfully protecting invertebrates and vertebrates from invasion [1].

Galectins constitute an evolutionary conserved family of β-galactoside-binding proteins, and are increasingly being proposed to play salient roles in innate immunity [2,3]. They are broadly distributed in nature from lower invertebrates to mammals [4–6]. Based on their distinct molecular structures, galectins can be divided into three categories [7–9]: (1) the prototype, contains a single carbohydrate recognition domain (CRD), including galectin-1, 2, 5, 7, 10, 13, 14; (2) the chimeric type, contains a CRD and a collagen repetitive structure domain. Galectin-3 is the only member; (3) type tandem repeats, two CRD, including Galectin-4, 6, 8, 9, 12. They are usually in the form of bivalent or mutivalent.

Galectin-1 exists as functional monomer or homodimer bound by non covalent. Each kind of form has different functions. The dimerization of galectins-1 is critical for their function in mediating cell-cell or cell-ECM interactions [3]. A major feature of galectin-1 is

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that at low concentration (Kd~7 μM), it can spontaneously disintegrate into monomer, and still can combined with sugars, but the combining ability is much lower than that of dimer [10]. It is the first member to be found in the family of galectins of human [11]. It is expressed in multiple types of cells, such as thymic epithelial cells, endothelial cells, dendritic cells, macrophages, fibroblasts cells and bone marrow stromal cells [12–15]. In addition, galectin-1 is a kind of typical cytoplasm protein, while on the cell surface, extracellular matrix, cytoplasm and nuclear can also find its expressions [16]. Unlike other lectins, Galectin-1 is Ca^{2+} ion independent and shows specific affinity with β-galactoside [5].

Host or animal galectins are critical recognition molecules. On one hand, they can recognize the living bacteria, promoting the establishment of symbiosis relationship of mutual benefit between the host and parasitic microorganism; On the other hand, they can activate the innate immune response by identifying pathogenic microbes. Moreover, they can also mediate the downstream response, such as aggregation, curing, induce phagocytosis, activate complement, activate platelets, strengthen the natural killer cell (NKC) activity and so on [17–20]. The immune functions of galectin-1 were concentrated in mammalian model organism, while less were reported in bony fishes. At present, galectin-1 genes have been cloned and their functions involved immunity have been investigated in some bony fishes, such as bass, flounder, medaka, salmon, rainbow trout and pufferfish [21–25].

The orange-spotted grouper, *Epinephelus coioides*, is a valuable and popular seafood fish, and one of the major mariculture species in China. However, in recent years, with rapid development of marine farming activities, outbreaks of viral diseases have affected grouper aquaculture industry causing heavy economic losses [26,27]. In our previous studies, C-type lectin sequences of grouper (*E. coioides*) have been identified and their functions involved immunity have been investigated [28,29]. However, the study on the immune function of galectin-1 in grouper is poorly understood.

In the present study, a galectin-1 homolog (EcGel-1) from grouper (*Epinephelus coioides*) was cloned and its possible role in fish immunity was analyzed. The results showed that EcGel-1 may be an important molecule involved in pathogen recognition and pathogen elimination in the innate immunity of grouper.

2. Materials and methods

2.1. Fish and cell lines

Juvenile orange-spotted grouper, *E. coioides* (10–12 cm) were purchased from a mari-culture farm at Wenchang, Hainan province, China. Fish were cultured in the pool with the laboratory recirculating seawater at 25–30 °C for two weeks and were not fed for three days before the experiment. The fish were anesthetized using “hypothermic anaesthesia” by pouring ice into fish bucket before anatomy. Tissues containing liver, spleen, kidney, brain intestine, heart, skin, muscle, stomach, head kidney and gill were dissected out and fast frozen in liquid nitrogen, then stored at −80 °C till used.

Grouper spleen (GS) cells were cultured in Leibovitz's L15 medium containing 10% fetal bovine serum (Invitrogen, USA) at 25 °C [30].

2.2. Cloning of E. coioides galectin-1(EcGlec-1) full-length cDNA by RACE-PCR

RNA was extracted from the frozen liver sample of grouper using SV Total RNA Isolation System (Promega) based on the manufacturer's recommendations. The quality of total RNA was evaluated by agarose electrophoresis. The total liver RNA was applied to synthesize the first-strand cDNA, which was to be as templates for 3′ RACE and 5′ RACE using the SMART™ RACE cDNA amplification kit (Clontech, USA). Primers were designed according to the identified EST sequences from the transcriptome libraries provided by our laboratory [31]. Among them, primers Glec-1-5′-224, Glec-1-3′-64 (Table 1) and UPM (offered by the kit) were performed for the first round PCR. The products of the first round PCR were diluted 50 times and subsequently performed as the template for the nested PCR, which was conducted with the specific primers Glec-1-5′-304, Glec-1-3′-198 (Table 1) and NUP (offered by the kit). The resulting fragments of 3′ and 5′ RACE were detected on 1% agarose gels, purified by the AxyPrep DNA gel extraction kit (AxyGEN) and sequenced.

2.3. Bioinformatics analysis

The BLAST programs on the NCBI (http://blast.ncbi.nlm.nih.gov/Blast) were chose to analyze the cDNA and predicted amino acid sequence of EcGlec-1. The cDNA was translated using the EditSeq 7.1. SMART software was performed to analyze the signal sequence and CRD domain predictions. Multiple-sequence alignment of the reported protein sequences of galectin-1 and homologies among CRD domains were carried out by the ClustalX 2.0. A phylogenetic tree was constructed using the MEGA 6.0 software, numbers at branch nodes represent the boot-strap majority consensus values of 1000 replicates.

2.4. Analysis of tissue-specific distribution of EcGlec-1

Total RNA was isolated from healthy grouper liver, spleen, kidney, brain, intestine, heart, skin, muscle, stomach, head kidney and gill, respectively, using SV Total RNA Isolation System (Promega) according to the manufacturer's protocol. Six independent samples were employed in this experiment to eliminate the individual differences. To assess the quality of RNA extracted as is mentioned above, electrophoresis was applied on 1% agarose gel. The RNA was prepared for cDNA synthesis using ReverTra Ace qPCR RT Kit (TOYOBO, Japan).

Expression distribution of EcGlec-1 in each tissues were investigated by qRT-PCR using primers Glec-1-s-rt and Glec-1-r-rt (Table 1). Primers Actin-F and Actin-R (Table 1) was used to amplify β-actin as the reference for internal standardization. qRT-PCR amplification was performed on Roche LightCycler 480 Real-time PCR system (Roche, Switzerland) using the 2 × SYBR Green Real-time PCR Mix (TOYOBO, Japan).

2.5. Immune challenge of orange-spotted grouper

Grouper of 10–12 cm (n = 180) were divided into four groups. The fishes in the experimental group were intraperitoneal injected (i.p.) 100 μl individually with polyinosine-polycytidylic acid (poly(I:C), Sigma, USA) (10 μg/ml), lipopolysaccharides from *Escherichia coli* 055:B5 (LPS, Sigma, USA) (10 μg/ml) and SGIV at a concentration of 10^5 TCID50/ml. The group of untreated groupers was employed as the control. Spleens of six fish in each group were collected for quantitative real-time PCR (qRT-PCR) at 3, 6, 12, 24, 48, 72, 96 and 120 h.

Total RNA was isolated from different tissues of grouper, using SV Total RNA Isolation System (Promega) according to the manufacturer's protocol. The quality of total RNA was assessed by electrophoresis on 1% agarose gel. Total RNA was reverse transcribed to synthesize the first-strand cDNA by ReverTra Ace kit (TOYOBO, Japan) according to the manufacturer's instructions.

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