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Intelectin gene from the grass carp *Ctenopharyngodon idella*: cDNA cloning, tissue expression, and immunohistochemical localization

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Abstract

The cDNA encoding grass carp intelectin was isolated from a head kidney cDNA library, and termed gcIntL. The deduced amino acid sequence of gcIntL consists of 318 amino acids, and about 55% identical and 74% similar to human intelectin, which is a new type of lectin recognizing galactofuranose, and plays a role in the recognition of bacteria-specific components in animal hosts. The gcIntL gene consists of seven exons and six introns, spacing over approximately 3 kb of genomic sequence. Phylogenetic analysis clearly demonstrated that the gcIntL formed a clade with *Danio rerio* intelectin and 35 kDa serum lectin. By real-time quantitative RT-PCR analysis, gcIntL transcripts were significantly induced in head kidney, trunk kidney, spleen, and intestine from LPS-stimulated fish. RT-PCR and Western blotting analysis demonstrated that the mRNA and protein of gcIntL gene have the same expression pattern, and both were detected in brain, gill, intestine, head kidney, trunk kidney, spleen, and heart. Furthermore, gcIntL protein could be detected in gill, intestine, trunk kidney, head kidney, spleen, heart, and brain including medulla oblongata and optic lobe, as determined by immunohistochemistry. This is the first report of intelectin expression pattern in fish, and of recombinant gcIntL and polyclonal antibody against gcIntL.

Keywords: Intelectin; Grass carp; Expression; Organ distribution

1. Introduction

Animal lectins are classified into two major groups, calcium dependent lectins (C-type lectins) and calcium-independent galactose-specific lectins (galectins) [1,2]. Among them, galactose-binding lectins play roles in cell differentiation [3], apoptosis [4], recognition of tumor antigens [5], and uptake of galactosylated glycoproteins [6]. In host defense, the recognition of bacterial components is important for induction of immune responses. Galactose-binding lectins such as intelectin also have a role in recognition of bacteria [7].

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Mammalian intelectin gene (intelectin 1) was first reported in mouse, and was specifically expressed in intestinal Paneth cells [8]. Pemberton et al. [9] reported intelectin 2 in the BALB/c and 129/SvEv strains, which plays an important role in the innate immune response to parasite infection. Human intelectin genes have two different transcripts, and human intelectin gene (HL-1) is a new type of lectin recognizing galactofuranosyl residues of pathogens, and plays a role in the recognition of bacteria-specific components [7]. The second human intelectin variant, HL-2, was found to be expressed specifically in the small intestine [10].

While searching for genes involved in the fish immune response, a clone similar to human intelectin was obtained in grass carp *Ctenopharyngodon idella*. The nucleotide sequence of the intelectin gene and its organization were then determined, and its expression at levels of transcripts and protein were examined. The distribution of the intelectin was also analyzed in several organs by immunohistochemical localization.

2. Materials and methods

2.1. Fish challenge experiment and cDNA library construction

Two grass carp, about 100 g each in body weight, were acclimatized in an aerated fresh water tank at room temperature under natural photoperiod for a week before being stimulated by intraperitoneal injection of 150 μg *Escherichia coli* lipopolysaccharide in 300 μl phosphate-buffered saline (PBS)/100 g fish (LPS, Sigma).

Three days after the injection, about 1 g head kidney from each fish was used to prepare total RNA by using Trizol Reagent (Invitrogen, USA) according to the manufacturer's instructions. The mRNA was then isolated from the total RNA using the polyAtract Isolation System (Promega). To construct a cDNA library with full-length insertion, the SMART cDNA library construction kit (CLONTECH) was used following the manufacturer's instructions with minor modification. The synthesized ds cDNA by Long-Distance PCR were directly ligated into a pGEM®-T Easy Vector (Promega) and transformed into *E. coli* DH5α cells. The clones were picked out randomly, and stored at −80 °C for further analysis.

2.2. Sequence and phylogenetic analysis

The size of the cDNA insert was checked after PCR amplification using $M13^+$ and $M13^-$ primers that were targeted to the cloning site of the vector. The cDNAs larger than 1000 bp were sequenced using the dideoxy chain-termination method on an automatic DNA sequencer (ABI Applied Biosystems Model 377). Homology search was performed by the BLASTN, BLASTX, and TBLASTX programs at web servers of the National Center for Biotechnology Information. One clone was found to be similar to human intelectin 2 and was further analyzed.

Protein prediction was performed using software at the ExPASy Molecular Biology Server (http://expasy.pku.edu.cn). The putative ORFs were analyzed for the presence of signal peptides using the algorithms signalP 3.0. The conserved domain was identified by the Pfam HMM search (http://expasy.pku.edu.cn). Multiple alignments were generated by the CLUSTAL 1.8 program within DNASTAR. Identities among the full-length intelectin from different species were determined using the MEGALIGN program in DNASTAR. A phylogenetic tree was constructed based on the deduced amino acid sequences using the Neighbor-Joining (NJ) algorithm in MEGA version 2.1, with its reliability assessed by 1000 bootstrap repetitions.

2.3. Sequencing the genomic DNA

The genomic DNA was purified from the spleen of the grass carp using Wizard® Genomic DNA Purification Kit (Promega). Based on the cDNA full-length sequence, primers were designed to obtain the full-length genomic sequence of grass carp intelectin, termed gcIntL. Initially, PCR was performed using the primer pairs gcIntLF1/gcIntLR1, gcIntLF2/gcIntLR2 and gcIntLF3/gcIntLR3 for amplification of the internal region of gcIntL gene. The annealing temperature was 57 °C, 52 °C, and 54 °C, respectively. The gene-specific primers for cloning the 5′ end and 3′ end of gcIntL gene were gcIntLR and gcIntLF. The PCR was initially performed with gene-specific primers and the adapter primer AP1, followed by a second PCR with the same gene-specific primers and the adapter primer AP2. The PCR cycling conditions were one cycle of 3 min at 94 °C, five cycles of 30 s at 94 °C, 30 s at 64 °C, and 90 s