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Genomic characterization and expression analysis of complement component 8α and 8β in rock bream (*Oplegnathus fasciatus*)

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ABSTRACT

The complement component 8α and 8β are glycoproteins that mediate formation of the membrane attack complex (MAC) on the surface of target cells. Full-length complement C8α (*Rb-C8α*) and C8β (*Rb-C8β*) sequences were identified from a cDNA library of rock bream (*Oplegnathus fasciatus*), and their genomic sequences were obtained by screening and sequencing of a bacterial artificial chromosome (BAC) genomic DNA library of rock bream. The *Rb-C8α* gene contains 64 bp of 5′-UTR, open reading frame (ORF) of 1794 bp, which encodes a polypeptide of 598 amino acids, 212 bp of 3′-UTR. The *Rb-C8β* gene contains 5′-UTR of 27 bp, open reading frame (ORF) of 1761 bp, which encodes a polypeptide of 587 amino acids, 3′-UTR of 164 bp. *Rb-C8α* consists of 11 exons interrupted by 10 introns and *Rb-C8β* consists of 12 exons interrupted by 11 introns. Sequence analysis revealed that both *Rb-C8α* and *Rb-C8β* contain thrombospondin type-1, a low-density lipoprotein receptor domain class A, membrane attack complex/perforin (MACPF) domain and epidermal growth factor like domain. The promoter regions of both genes contain important putative transcription factor binding sites including those for NF-κB, SP-1, C/EBP, AP-1, and OCT-1. *Rb-C8α* and *Rb-C8β* showed the highest amino acid identity of 62% and 83% to rainbow trout C8α and Japanese flounder C8β respectively. Quantitative real-time PCR analysis confirmed that *Rb-C8α* and *Rb-C8β* were constitutively expressed in all examined tissues, isolated from healthy rock bream, with highest expression occurring in liver. Pathogen challenge, including *Edwardsiella tarda*, *Streptococcus iniae*, and rock bream iridovirus led to up regulation of *Rb-C8α* and *Rb-C8β* in liver. Positive regulations upon bacterial and viral challenges, and high degree of evolutionary relationship to respective orthologues, confirmed that *Rb-C8α* and *Rb-C8β* important immune genes, likely involved in the complement system lytic pathway of rock bream.

1. Introduction

Innate immune system is the ancient and principal line of defense, present in all animals from invertebrates to vertebrates. It comprises of different pathogen recognition systems and effector systems. The complement system has been primarily viewed as a supportive first line of defense against microbial pathogens as a component of innate immunity (Law and Reid, 1988; Nakao et al., 2011). The complement system, acts as important effect or mechanism to link both innate and adaptive immunity. It is composed of more than 35 soluble plasma proteins that play key roles in complement-mediated killing of pathogens through the lytic pathway. Three distinct pathways, namely, the classical, lectin and alternative pathways, are responsible for target recognition and formation of a protease complex for C3 activation. All three pathways converge into a final common pathway, known as cytolytic pathway. The cytolytic pathway is initiated by the cleavage of C5 into anaphylatoxin C5a and C5b by the C5 convertases (Nakao et al., 2011).

Membrane attack complex/perforin (MACPF) domain is one of the common domains identified in many different functional proteins. They exhibit limited sequential similarity but contain a signature MACPF motif (Y/W–G–T/S–H–F/Y–X_6–G–G) (Ponting, 1999). MACPF domain containing protein superfamily is one of

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the largest families of pore-forming molecules in the complement system. Complement component C5, C6, C7, C8 and C9 proteins belong to MACPF family and they considered as components of the cytolytic pathway and known as terminal complement components (TCC). Among them, the C8 has most complex subunit arrangement comprising of three genetically distinct proteins (C8α, C8β, and C8γ). They are arranged as a disulfide-linked C8α–γ heterodimer that is noncovalently associated with C8β (Lebioda and Sodetz, 2005). Moreover, C8α and C8β are homologous to each other and to C6, C7 and C9. All proteins contain a variable number of N- and C-terminal modules and a central 40 kDa MACPF domain (Hobart et al., 1995). All of them possess thrombospondin (TSP), low density lipoprotein receptor (LDL-R), and epidermal growth factor precursor (EGFP) domains as N- and C-terminal motifs, respectively (diScipio et al., 1988; Hobart et al., 1995). Therefore, they are identified as structurally related proteins which differ in size, and complexity (Li et al., 2007; Volanakis and Frank, 1998). The membrane attack complex (MAC) is assembled by sequential binding of C5b with C6, C7, and C8 and incorporation of an alternative number (12–18) of the C9 molecules into a transmembrane pore (Muller-Eberhard, 1986). A study on carp serum complement revealed that different TCC molecules were interacted with molar ration of 1:1 except C9. The molar ratio of C9 found to be 1:4 with other TCC proteins (Nakao et al., 1996). Up-to-date several lytic pathway genes have been characterized at molecular levels. In fish, complement C6, C7, C8β, and C9 in trout (Chondrou et al., 2006; Kazantzi et al., 2003; Tomlinson et al., 1993; Zarkadis et al., 2005), C8β and C9 in Japanese flounder (Katagiri et al., 1999), C8β in carp (Nakao et al., 1996; Uemura et al., 1996) and C9 with its promoter region predicted via web-based software in grass carp (Li et al., 2007) and puffer fish (Yeo et al., 1997) have been studied at molecular levels. However, no study was carried out in fish to characterize C8α and no comprehensive pathogenically challenge study had been conducted for C8β in teleost. Therefore, study of expression behavior fish C8 homologous is important to understand their function in fish immunity.

The complement system is conserved in both vertebrates and invertebrates as a family of proteins. It has been identified in one of the most primitive metazoans such as Cnidaria in the history (Dishaw et al., 2005). Furthermore, the complement system has been recorded in some protostomes, such as arthropods (Zhu et al., 2005) and mollusks (Castillo et al., 2009), and anti-microbial activity of complement component 3 was demonstrated in horseshoe crabs (Ariki et al., 2008), sea urchin (Smith et al., 1999), and ascidians (Nonaka and Azumi, 1999). The complement system of primitive vertebrate like Cyclostomata and invertebrates are mostly similar and do not possess the antibody-recognizing activation cascade (the classical pathway-like) and a cytolytic pathway (Kimura et al., 2009). However, the complement system in fish is fully recognized with all three of the C3-activation pathways, and the cytolytic pathway, involved in same functions as recognized in mammalian complement, such as formation of transmembrane pore, opsonization, and anaphylatoxic leukocyte stimulation (Boshra et al., 2006).

Rock bream is one of the most economically important marine fish species in South Korea, and it has been recorded coastal water especially in coral beds of the Pacific and Indian Ocean. In recent years, mariculture sources of rock bream have experienced an alarming increase in prevalence and virulence of pathogenic infections, which have resulted in substantial economic losses (Park, 2009; Zenke and Kim, 2008). Therefore understanding of expression behavior of TCC genes are expedient in developing fish immunity. In this study, sequences of *Rb-C8α*, and *Rb-C8β*, from rock bream were identified using GS-FLX-based transcriptome sequencing techniques and characterized at the transcriptional and genomic levels. The evolutionary relationship was also determined by using molecular evolutionary genetic analysis (MEGA) neighbor joining tree method (v5.0). Further, tissue-specific expression and induced expression analysis were carried out to determine the normal expression profile and immune modulations of *Rb-C8α* and *Rb-C8β*, using pathogenic bacteria, and virus.

2. Materials and methods

2.1. Sequence identification from cDNA and genomic DNA libraries of the rock bream fish

We have established a rock bream cDNA shotgun sequence transcriptome database based upon data from a next-generation sequencing technology, the GS-FLX titanium system (DNA Link, Republic of Korea). In the first step, full-length cDNA sequences with highest homology to known complement component 8α and 8β were identified by a BLAST search on NCBI (Altschul et al., 1990).

Secondly, a bacterial artificial chromosome (BAC) library of rock bream was constructed (Lucigen® Co., Middleton, WI, USA) using randomly sheared genomic DNA from rock bream. The pSMART BAC vector was used to construct a library comprising 20 super pools, 16 row pools and 24 column pools spanning the entire rock bream genome. Screening of the BAC library was carried out for *Rb-C8α* and *Rb-C8β* using PCR-based screening method following the manufacturer's instructions. Positive clones were isolated, and purified with a Qiagen Plasmid Midi Kit (Hilden, Germany) and sequenced by Pyro-sequencing (GS-FLX 454). *Rb-C8α* and *Rb-C8β* genomic sequence were identified by cDNA alignment using the Spidey (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/) mRNA to genomic alignment program on NCBI).

2.2. In silico analysis of rock bream Rb-C8 homologues

Full-length cDNA sequences of *Rb-C8α* and *Rb-C8β* were analyzed by DNAssist (v2.2) and BLAST-P (Altschul et al., 1990). Similarities with other known C8α and C8β amino acid sequences were determined by comparison with amino acid sequences available in the NCBI and ENSEMBL databases. Characteristic domains and motifs of each sequence were identified using the PROSITE profile database (de Castro et al., 2006) and SMART proteomic database (Letunic et al., 2009). Prediction of signal peptides was accomplished using the SignalP World Wide Web server (Bendtsen et al., 2004). Promoter regions of each gene was examined using the prediction models such as TFSEARCH (v1.3) (Heinemeyer et al., 1998) AliBaba (v2.1) (http://www.gene-regulation.com/pub/programs/alibaba2/index.html), and Neural Network Promoter prediction in Berkeley Drosophila Genome Project (BDGP) (Hoskins et al., 2011) bioinformatics programs. Identity, similarity, and gap percentages were calculated using EMBOSS pair-wise alignment algorithms (http://www.ebi.ac.uk/Tools/psa/emboss_needle/). Multiple sequence alignments were performed with ClustalW (v2.0) (Thompson et al., 1994) using known orthologues. Aphylogenetic tree for both genes was reconstructed with the MEGA (v5.0) software package using neighbor-joining (NJ) method by the (Tamura et al., 2011) and bootstrapping values taken from 5000 replicates.

2.3. Experimental animals

Healthy rock breams (~50 g) were obtained from the Ocean and Fisheries Research Institute (Jeju, Republic of Korea) and acclimatized to laboratory conditions (salinity 34 ± 1%, pH 7.6 ± 0.5 at 24 ± 1 °C) in 400 L tanks for 1 week prior to experimentation. The animals were fed with a commercial feed and daily ration was