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Complement regulatory protein genes in channel catfish and their involvement in disease defense response

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ABSTRACT

Complement system is one of the most important defense systems of innate immunity, which plays a crucial role in disease defense responses in channel catfish. However, inappropriate and excessive complement activation could lead to potential damage to the host cells. Therefore the complement system is controlled by a set of complement regulatory proteins to allow normal defensive functions, but prevent hazardous complement activation to host tissues. In this study, we identified nine complement regulatory protein genes from the channel catfish genome. Phylogenetic and syntenic analyses were conducted to determine their orthology relationships, supporting their correct annotation and potential functional inferences. The expression profiles of the complement regulatory protein genes were determined in channel catfish healthy tissues and after infection with the two main bacterial pathogens, *Edwardsiella ictaluri* and *Flavobacterium columnare*. The vast majority of complement regulatory protein genes were significantly regulated after bacterial infections, but interestingly were generally up-regulated after *E. ictaluri* infection while mostly down-regulated after *F. columnare* infection, suggesting a pathogen-specific pattern of regulation. Collectively, these findings suggested that complement regulatory protein genes may play complex roles in the host immune responses to bacterial pathogens in channel catfish.

1. Introduction

The complement system is a major antimicrobial defense system of innate immunity, providing vital host defense, especially early after infection before adaptive immunity is activated. It is crucial for phagocytes recruitment, clearance of invading pathogens, and elimination of altered cells. It serves as a bridge between innate immunity and adaptive immunity (Abbas et al., 2012; Carroll, 2004). The complement system can be activated by three major pathways: classical pathway, alternative pathway and lectin pathway. These three pathways merge to a common terminal pathway where membrane attack complex (MAC) is assembled, and MAC can lyse the invading pathogens directly. The complement system consists of more than 30 soluble and membrane-bound proteins, which interact with one another and form a highly regulated complement cascades (Abbas et al., 2012; Carroll and Sim, 2011; Mayilyan, 2012; Noris and Remuzzi, 2013). Although the complement system is under strict control, there are still potential risks of damaging the host cells. In order to maintain homeostasis of the organism, a set of complement regulatory proteins are responsible for avoiding excessive host responses after microbial infection.

Complement regulatory proteins, also known as complement regulators or complement inhibitors, can be categorized into two major classes: membrane-bound regulators and soluble regulators. Membrane-bound regulators mainly protect host cells from complement attacks in all three complement pathways and inactivate both C3 and C4 (Zipfel and Skerka, 2009). The membrane-bound regulators include complement component receptor 1 (CR1 or CD35), complement component receptor 2 (CR2 or CD21), membrane cofactor protein (MCP or CD46), dacay-accelerating factor (DAF

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or CD55) and protectin (CD59). Soluble regulators are more specific and control either the alternative, the classical or the lectin pathway (Zipfel and Skerka, 2009). They are needed to control the excessive activation in the fluid phase (Meri and Jarva, 2013). The soluble regulators include factor I (CFI), factor H (CFH) and factor H-related genes (CFHRs), carboxypeptidase N (CPN), complement component 1 inhibitor (C1INH), C4 binding protein α chain (C4BPα), clusterin (CLU, also named SP40-40 or apolipoprotein J), vitronectin (VTN, or S-protein), plasminogen (PLG) and the only known positive regulator properdin (CFP). Structurally some of the complement regulatory proteins belong to a family of proteins named regulator of complement activation (RCA), since they share a common tandem short consensus repeats (SCRs, also named CCP, complement control protein modules or sushi domain) which typically consist of 60–70 amino acids (HeineSuner et al., 1997; Norman et al., 1991). In humans, RCA genes are clustered on chromosome 1 (1q32), and can be categorized into two groups: RCA group 1 containing factor H and related genes, and RCA group 2 containing C4BPα, CR1, CR1L, CR2, MCP and DAF (Krushkal et al., 2000).

Complement regulatory proteins have been well studied in mammals (Zipfel and Skerka, 2009). Although not equally extensive, a number of studies of complement regulatory proteins were conducted with teleost fish. For instance, C1INH, CFP, CLU, VTN and CPN have been characterized in *Danio rerio* (Sun et al., 2013; Zhang et al., 2013b), *Oncorhynchus mykiss* (Chondrou et al., 2008; Londou et al., 2008; Marioli and Zarkadis, 2008; Papanastasiou et al., 2007; Wang and Secombes, 2003), *Oplegnathus fasciatus* (Godahewa et al., 2014), *Oreochromis niloticus* (He et al., 2013), *Pseudosciaena crocea* (Wei et al., 2010). Studies on RCA genes have also been conducted in teleosts. For example, the SBP1 gene was reported from barred sand bass (*Paralabrax nebulifer*) (Dahmen et al., 1994), and its related gene SBCRP-1 was also annotated (Zipfel et al., 1996). SBP1 gene was reported to have overlapped regulatory activities of mammalian factor H and C4BP (Kemper et al., 1998). Both RCA group 1 genes CFH and CFHL1-4 and RCA group 2 genes ZRC1 and ZRC2 have been reported from zebrafish (Sun et al., 2010; Wu et al., 2012). Furthermore, three membrane-bound complement regulatory protein isoforms gTecrem-1, gTecrem-2 and gTecrem-3 were identified in ginbuna crucian carp (*Carassius auratus langsdorfii*) (Nur et al., 2013). A CD46-like complement-regulatory membrane protein (cTecrem), an ortholog of a zebrafish RCA group 2 gene ZRC1, was cloned and characterized in common carp (Tsujikura et al., 2015). In channel catfish *(Ictalurus punctatus)*, the primary aquaculture species in the United States, complement factors Bf/C2 and Df (Zhou et al., 2012), and three complement regulatory protein genes, CD59, factor I, and C1INH have been reported (Abernathy et al., 2009; Yeh and Klesius, 2007), and C1INH was found to be significantly up-regulated at early stages after bacterial infection with *Edwardsiella ictaluri* (Li et al., 2014).

The catfish industry continues to suffer from infectious diseases. In particular, the enteric septicemia of catfish (ESC) caused by *E. ictaluri* and columnaris disease caused by *Flavobacterium columnare* are the two major bacterial diseases that cause huge economic losses. Understanding of immune-relevant functional genes and their expression in the course of bacterial disease is important for designing strategies for disease management. With the advances in genomic sciences, rapid progress has been made in characterization and analysis of expression of innate immune genes such as pathogen recognition receptors (Baoprasertkul et al., 2007a, 2006, 2007b; Rajendran et al., 2012a; Rajendran et al., 2012b; Sun et al., 2014; Zhang et al., 2013a), chemokines (Bao et al., 2006a; Peatman et al., 2006), antimicrobial peptides (Bao et al., 2005, 2006b; Wang et al., 2006a; Wang et al., 2006b; Xu et al., 2005), lysozymes (Wang et al., 2013), lectins (Takano et al., 2008; Thongda et al., 2014; Zhang et al., 2012), NOS genes (Yao et al., 2014a) and protease inhibitors (Li et al., 2014; Yao et al., 2014b). However, systematic analysis of complement regulatory protein genes has not been conducted in channel catfish. Here in this study, we identified and characterized nine complement regulatory protein genes, and analyzed their expression profiles after bacterial infections with *E. ictaluri* and *F. columnare*.

2. Materials and methods

2.1. Identification and sequence analysis of complement regulatory protein genes

The complement regulatory protein genes in catfish were identified by searching the RNA-seq database (Liu et al., 2012) and the whole genome sequence database of catfish (Lu et al., 2011), using all available sequences of complement regulatory protein genes from fish, human, mouse, chicken and frogs retrieved from the GenBank (NCBI) and Ensembl as queries. TBLASTN similarity searches were conducted against RNA-seq database to identify all complement regulatory protein gene-related sequences using a cutoff E-value of e-5. The retrieved sequences were further analyzed using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) for the generation of coding sequences. To confirm the candidate complement regulatory protein gene sequences, BLASTN was conducted against the unpublished catfish whole genome sequence database with a cutoff E-value of e-10. The retrieved genome sequences were subjected to gene prediction by FGENESH program of MolQuest software version 2.4.3 using Fish model (Solovyev et al., 2006) and the resulted coding sequences were further confirmed by BLASTP against NCBI non-redundant (NR) protein sequence database.

2.2. Structural and phylogenetic analyses

Conserved domains of complement regulatory protein genes were identified using Simple Modular Architecture Research Tool (SMART) program (http://smart.embl-heidelberg.de/) (Letunic et al., 2012; Schultz et al., 1998), and confirmed by conserved domain prediction from BLASTP. In order to further identify channel catfish complement regulatory protein genes, phylogenetic analysis was conducted using all the amino acid sequences of complement regulatory protein genes from channel catfish and selected vertebrate species retrieved from GenBank and Ensembl, including those from human, cattle, mouse, chicken, lizard, frog, and several fish species such as zebrafish, tilapia, medaka, fugu, stickleback, platyfish, green spotted puffer and Atlantic cod. Separate phylogenetic trees for different complement regulatory protein genes were conducted, additional phylogenetic trees were also constructed to compare the sequences of each tandem SCR domain between zebrafish ZRCs and catfish CRCs. Alignment of multiple protein sequences were performed using Muscle v3.8 (multiple sequence comparison by log-expectation) (Edgar, 2004a, b) with default parameters. The phylogenetic trees of complement regulatory protein genes and homologies among SCR domains of RCA group 2 genes were constructed using maximum likelihood method in MEGA 5.2 (Tamura et al., 2011). Bootstrap test of 1000 replications was conducted to evaluate the phylogenetic trees.

2.3. Syntenic analysis

When required, such as for the RCA group 2 genes where phylogenetic analysis alone was not sufficient to provide annotation for gene duplicates, syntenic analysis was also conducted to determine orthologous relationship. Shared syntenies were assessed based on comparisons of the genes, their order on the