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Molecular cloning of two C1q-like cDNAs in mandarin fish *Siniperca chuatsi*

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Abstract

C1q, a subunit of the C1 complex, plays a key role in the recognition of immune complexes to initiate the classical complement pathway. In this study, we reported two C1q-like cDNAs from mandarin fish (*Siniperca chuatsi*), mC1q-like-1 (mC1qL1) and mC1q-like-2 (mC1qL2). The full-length cDNA of mC1qL1was 990 bp, containing a 71 bp 5′-untranslated region (UTR), an open reading frame (ORF) of 723 bp, and a 196 bp long 3′-UTR. mC1qL2 cDNA was 1193 bp, containing a 100 bp 5′-UTR, followed by an ORF of 756 bp and a 3′-UTR of 337 bp. mC1qL1 and mC1qL2 share 29% identity in amino acid sequence. Both mC1qL1 and mC1qL2 contained three parts: a short amino-terminal region, a collagen-like region and a carboxyl-terminal globular C1q domain. The phylogenetic analysis showed that mC1qL1 clustered with two *Danio rerio* hypothetical proteins and further grouped with C1q proteins, while mC1qL2 clustered with C1qA proteins from other species. In healthy mandarin fish, mC1qL1 and mC1qL2 were expressed in all tissues tested, including liver, spleen, head kidney, caudal kidney, intestine and gill. mC1qL1 was highly expressed in head kidney, while mC1qL2 was mainly expressed in spleen. The expression level of mC1qL1 and mC1qL2 in liver were not changed obviously and mC1qL2 was significantly changed ($p < 0.05$) in spleen after infectious spleen and kidney necrosis virus (ISKNV) infection. Mandarin fish C1q may play a role in response to ISKNV infection.

Keywords: mC1qL1; mC1qL2; Complement; *Siniperca chuats*; Tissue expression; ISKNV

1. Introduction

Mandarin fish, *Siniperca chuatsi* (Basilewsky), is a main economic cultured fish in China. With the rapid development of mandarin fish aquaculture, diseases, especially the viral disease, have become a major constraint and the most limiting factor in the mandarin fish culture industry. ISKNV (infectious spleen and

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kidney necrosis virus), a member of the Iridoviridae family, has caused high mortalities in mandarin fish and limited largely the mandarin fish cultures in China (He et al., 1998, 2000; Deng et al., 2000).

The complement system, a family of more than 35 soluble serum proteins, plays essential roles in innate and adaptive immunity in host defense (Nonaka and Smith, 2000; Boshra et al., 2006). Activation of the complement system also contributes significantly to the orchestration and development of acquired immune responses (Boshra et al., 2006). The complement system can be activated through three pathways: the antibody-dependent classical pathway, the antibody-independent alternative pathway, and the lectin pathway (Sunyer and Lambris, 1998). The classical complement pathway (CCP) is a major defense and clearance system in the blood circulation. It is activated at the level of C1q, a subcomponent of the C1 complex, by both immune complexes that react with the C1q globular region and nonimmune substances that react with the C1q collagen-like region (Kishore and Reid, 1999; Jiang et al., 1992). Fish possess complement pathways similar to those in mammals, and fish complement proteins identified thus far show highly homologies to their mammalian counterparts (Holland and Lambris, 2002).

The human C1q molecule is composed of 18 polypeptide chains (six each of A-, B- and C-chains). Each of these chains has a short amino-terminal region, followed by a collagen-like region (CLR, repeating Gly-X-Y triplets, where X is often proline and Y is often hydroxylysine or hydroxyproline) and a carboxy-terminal globular head region (Sellar et al., 1991). Every C1q chain also has four conserved cysteines, which can form inter- or intra-chain disulfide bridges that play an important role in formation of intact C1q molecules. C1q, the key component of the classical complement pathway in antimicrobial defense, can trigger rapid enhanced phagocytosis resulting in clearance of cellular debris, apoptotic cells and immune complexes, phagocytosis of bacteria, neutralization of retroviruses, and modulation of dendritic cells (DCs), B cells and fibroblasts (Bohlson et al., 2007; Kishore et al., 2004). Direct binding of C1q to a wide range of pathogens including viruses (Dierich et al., 1993; Kojouharova et al., 2003), parasites (Rimoldi et al., 1989), and both gram positive (Butko et al., 1999) and gram negative (Aubert et al., 1985; Kovacsovics et al., 1987) bacteria, has been reported. C1q is a versatile recognition protein and a major connecting link between classical pathway-driven innate immunity and acquired immunity (Kishore and Reid, 2000).

The complement system has been studied extensively in mammals, but considerably less known in lower vertebrates, particularly in teleost fish. The C1q just has been identified in zebrafish (*Danio rerio*) and *Tetraodon nigroviridis* because their full genomic DNA sequences are available. The full-length cDNA sequences of C1q in common carp (*Cyprinus carpio*) (BAD22535) and gibel carp (*Carassius auratus gibelio*) (AY662672) and an EST sequence (CV823415) similar to complement C1q subcomponent in killifish (*Fundulus heteroclitus*) can be found in the GenBank. Fish C1q has been reported in nurse shark (*Ginglymostoma cirratum*) (Smith, 1998) and channel catfish (*Ictalurus punctatus*) (Dodds and Petry, 1993), but the full-length cDNAs or genes are not available. In addition, full-length cDNA sequence containing the C1q globular domain but lacking the collagen domain has been cloned in color crucian carp (*Carassius auratus* color variety) (AY583317), and this C1q-like gene has unique functions in oogenesis, oocyte maturation and egg fertilization (Chen and Gui, 2004).

To our knowledge, there is no relevant paper on full-length fish C1q. Mandarin fish is an important economic fish in China. The breakouts of ISKNV do threaten the culture of mandarin fish and cause large loss every year. To understand the relationship between the complement system and ISKNV, here we report identification of two full-length C1q-like cDNAs in mandarin fish (*S. chuatsi*). Both mC1qL1 and mC1qL2 shared common features with mammalian C1q. The phylogenetic analysis showed that mC1qL2 were clustered with mammalian C1qAs and mC1qL1 clustered with zebrafish C1q-domain containing protein and further grouped with mammalian C1qs. mC1qL1 and mC1qL2 were expressed constitutively in all tissues tested. Both mC1qL1 and mC1qL2 were expressed at the lowest level 7 days after fish were challenged with ISKNV, while the ISKNV infected fish began to die at 7 days post-infection. The expression pattern of mC1qLs suggests that mandarin fish C1q may play a role in response to ISKNV infection.

2. Materials and methods

2.1. RNA extraction

Three healthy mandarin fish, about 500 g, were obtained from fish farm in Nan Hai (Guangdong Province, China) and acclimated for 2 weeks. The fish were assured free of ISKNV infection by PCR (Deng et al., 2000). Total RNA was extracted from fresh liver