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Research paper

First characterization of fish CD22: An inhibitory role in the activation of peripheral blood leukocytes

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ABSTRACT

In mammals, CD22 is a member of the Ig superfamily that serves as an inhibitor during B cell responses to foreign antigens. In this study, we characterized for the first time a fish CD22 from tongue sole *Cynoglossus semilaevis* (CsCD22). CsCD22 possesses the conserved structural features of CD22 and shares 35%–54% sequence identities with other fish CD22. mRNA expression of CsCD22 was most abundant in head kidney and heart. CsCD22 protein was detected on the surface of peripheral blood leukocytes (PBL). In the presence of rCsCD22 antibody, the proliferation, phagocytosis, and antibacterial activity of PBL were significantly increased. These results indicate for the first time that fish CD22 plays an inhibitory role in PBL activation.

1. Introduction

The B-cell antigen receptor (BCR) on the surface of B lymphocytes plays a crucial role in immune response (Nitschke et al., 1997). Signal transduction from BCR depends on its co-receptors or accessory molecules (Cambier et al., 1994). Many accessory molecules modulating signaling through BCR have been identified, including those positively modulating BCR signaling (Doody et al., 1996) and inhibitors of BCR signaling (Muta et al., 1994). Accessory molecules can recruit additional signal-transducing proteins to the antigen receptor through their cytoplasmic tails. In this way, the strength of the signal or the threshold of antigen required for signaling through the antigen receptor can be modulated (Nitschke et al., 1997).

CD22 is a member of a family of sialic acid-binding, immunoglobulin (Ig)-like accessory molecules involved in regulation of cellular activation receptors and cell–cell adhesion (Crocker and Redelinghuys, 2008). Human and mouse CD22 have seven extracellular Ig-like domains (Tedder et al., 1997). The highest amount of conservation in CD22 occurs in the amino-terminal V-set Ig-like domain involved in ligand binding, as well as the ~140 amino acid cytoplasmic domain harboring the conserved tyrosine motifs including immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that recruit phosphotyrosine and phosphoinositide phosphatases SHP-1 and SHIP (Poe and Tedder, 2012).

In mammalian vertebrates, CD22 is a negative regulator of antigen receptor signaling, and its onset of expression in mature B cell may serve to raise the antigen concentration threshold required for B cell activation (O'Keefe et al., 1996). CD22 regulates BCR signaling chiefly via recruitment of the SHP-1 phosphatase and the absence of CD22 leads to decreased SHP-1 recruitment (Chappell et al., 2017). Splenic B cells from CD22-deficient mice consistently displayed decreased membrane IgM (mIgM) and increased expression of major histocompatibility complex (MHC) class II (Otipoby et al., 1996). Mutations in CD22 and its signaling machinery have been associated with dysregulated B cell development (Duong et al., 2010). Additionally, CD22 is also central to the regulation of peripheral B cell homeostasis and survival, promotion of BCR-induced cell cycle progression, and CD40 signaling (Tedder et al., 2005).

In contrast to mammalian CD22, no fish CD22 has been studied. Tongue sole (*Cynoglossus semilaevis*) is a species of flatfish mainly distributed in the Yellow Sea and the East China Sea. Due to its high economic value, tongue sole has become an important commercial fish species in China. In this study, with an aim to gain understanding of the function of teleost CD22, we investigated the expression and effect of tongue sole CD22 on the activity of peripheral blood leukocytes (PBL).

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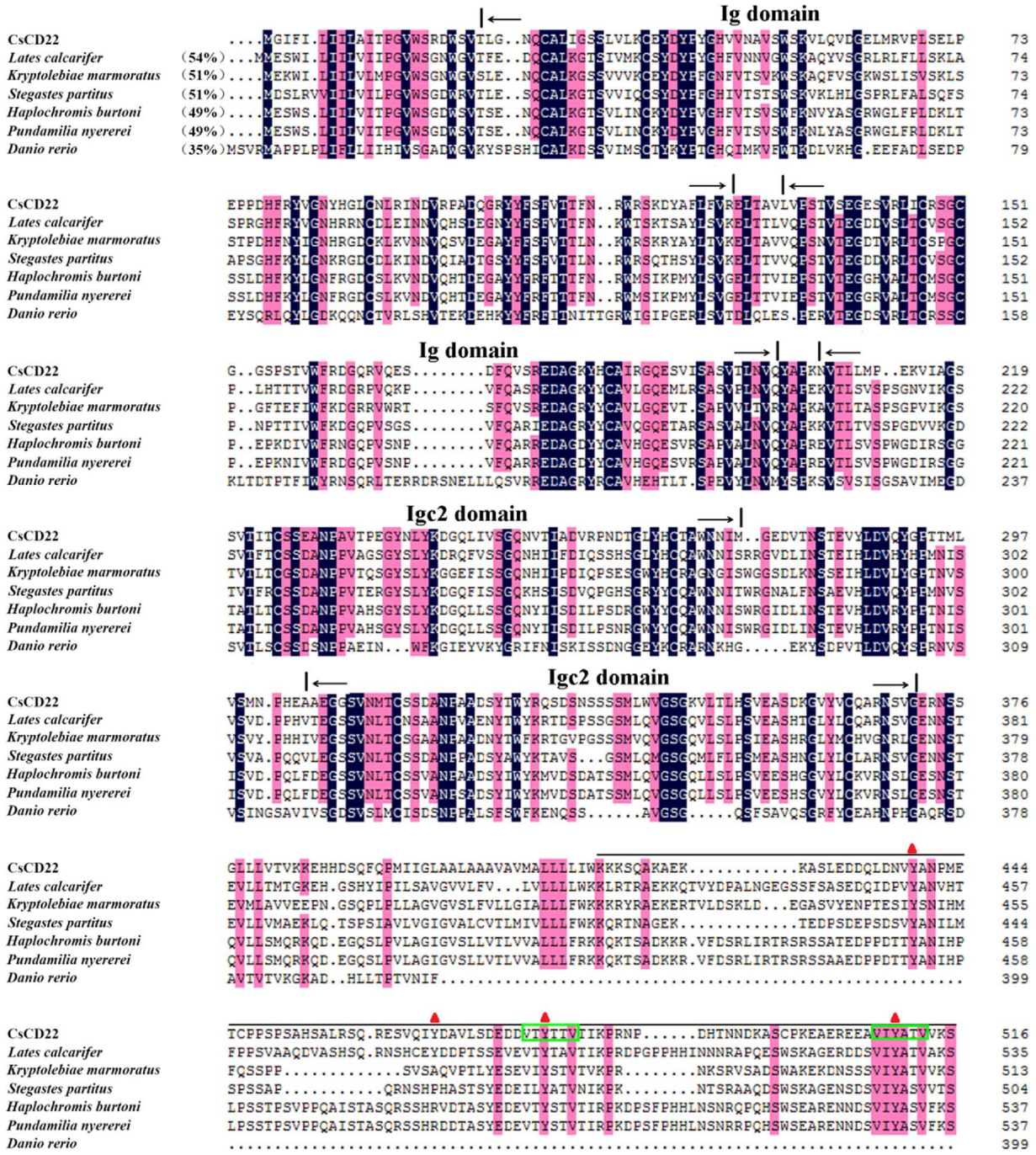

Fig. 1. Alignment of the deduced amino acid sequences of CD22 homologues. Numbers in brackets indicate overall sequence identities between CsCD22 and the compared sequences. The consensus residues are in black, the residues that are ≥75% identical among the aligned sequences are in pink; the Immunoglobulin domain (Ig domain) and Immunoglobulin C2 type domain (Igc2 domain) are indicated by arrows; the cytoplasmic tail is indicated by the black line; the immunorecepter tyrosine-based inhibitory motifs (ITIMs) and conserved tyrosine residues are indicated by green box and red triangle, respectively. The GenBank accession numbers of the aligned sequences are as follows: *Lates calcarifer*, XP_018539561.1; *Kryptolebias marmoratus*, XP_017265393.1; *Stegastes partitus*, XP_008285119.1; *Haplochromis burtoni*, XP_014196067.1; *Pundamilia nyererei*, XP_005743327.1; *Danio rerio*, XP_017211422.1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Fish

Fish were purchased, maintained, and manipulated as reported previously (Li and Sun, 2016). Briefly, healthy tongue sole (average 10 cm in length and 12.4 g in weight) were purchased from a commercial fish farm in Shandong Province, China and maintained at 20 °C in aerated seawater. Before experiment, fish were acclimatized in the laboratory for two weeks and confirmed to be absent of bacterial pathogens. For tissue collection, fish was euthanized with tricaine methanesulfonate (Sigma, St. Louis, USA) at the dose of 0.1 g/L.

2.2. Sequence analysis

The amino acid sequence (GenBank accession no. XP_008321463.1) of *CsCD22* was analyzed using the BLAST program at the National Center for Biotechnology Information (NCBI). Domain search was performed with the conserved domain search program of NCBI. The theoretical molecular mass and isoelectric point were predicted by using