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Short communication

Identification of an intercellular cell adhesion molecule-1 homologue from grass carp: Evidence for its involvement in the immune cell adhesion in teleost

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ABSTRACT

Intercellular cell adhesion molecule-1 (ICAM-1) is a single-chain transmembrane glycoprotein which plays key roles in transendothelial migration of leukocytes and interaction between antigen presenting cells and T cells. In teleost, information of cell adhesion-related molecules is still lacking. In this study, we identified a gene from grass carp sharing similar exon and intron organization with human ICAM-1. Cloning and *in silico* analysis of its homologues in zebrafish and other two cyprinid fishes, respectively demonstrated the existence of the gene in these fishes. Moreover, the molecular features of these genes in fishes were conserved compared with human ICAM-1. In grass carp, the transcripts of this gene were detected with high levels in heart and liver and its mRNA expression in headkidney leukocytes was induced by Il-1β. Overexpression of this molecule in COS-7 cells could increase the adhesion of the cells with grass carp peripheral blood lymphocytes (PBLs), and the adhesion was further enhanced by lipopolysaccharide stimulation on PBLs. Further studies revealed that the mRNA levels of lymphocyte function-associated antigen-1, a ligand for ICAM-1, were much higher in the PBLs adhering to the COS-7 cells with overexpressing this molecule than in the PBLs alone. These results collectively showed that the newly cloned cDNA encodes grass carp intercellular cell adhesion molecule-1 (Icam-1) and it can mediate the adhesion of PBLs. This provides functional evidence for the existence of Icam-1 in teleost and will facilitate investigation on the transendothelial migration of leukocytes in fish species.

1. Introduction

Cell adhesion molecules are proteins at the surface of cell membrane that sustain cell-cell and cell-matrix interactions, and are indispensable for leukocyte trafficking and differentiation [1]. They also can directly activate signal pathways critical to cell functions and keep cellular contacts necessary for signaling through other receptors [2]. Given that the cellular communication based on physical contact of cells is critical for the coordination of immune responses, the roles of mammalian adhesion molecules in immune cells have been largely investigated, especially their roles in antigen recognition [3] and cellular adhesion [4].

Intercellular cell adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily, is a single-chain transmembrane glycoprotein [5] which contains five tandem immunoglobulin (Ig)-like domains in mammals [6]. ICAM-1 is expressed in both non-hematopoietic and hematopoietic cells [5], and its expression can be induced by some cytokines including IL-1, TNF-α and IFN-γ [7], lipopolysaccharide (LPS) [8] and phorbol ester [9]. ICAM-1 plays a crucial role in immune-related and inflammatory processes [10] and functions as a co-stimulatory molecule involving in trans-endothelial migration of leukocytes [11] and T cells activation [12]. Multiple ligands of ICAM-1 have been identified, including lymphocyte function-associated antigen-1 (LFA-1) [13], macrophage-1 antigen (Mac-1) [14], rhinoviruses [15], fibrinogen [16] and *Plasmodium falciparum*-infected erythrocytes [17]. It is well accepted that interaction of ICAM-1 with LFA-1 is traditionally associated with trans-endothelial migration of leukocytes from bloodstream into a tissue which is essential for the leukocytes to function at the sites of inflammation [11]. It is noteworthy that LFA-1 is tightly regulated since it actively contributes to cellular adhesion and migration in the immune system [18]. Interestingly, LFA-1 has three distinct conformations classified according to their binding affinity for ICAM-1:

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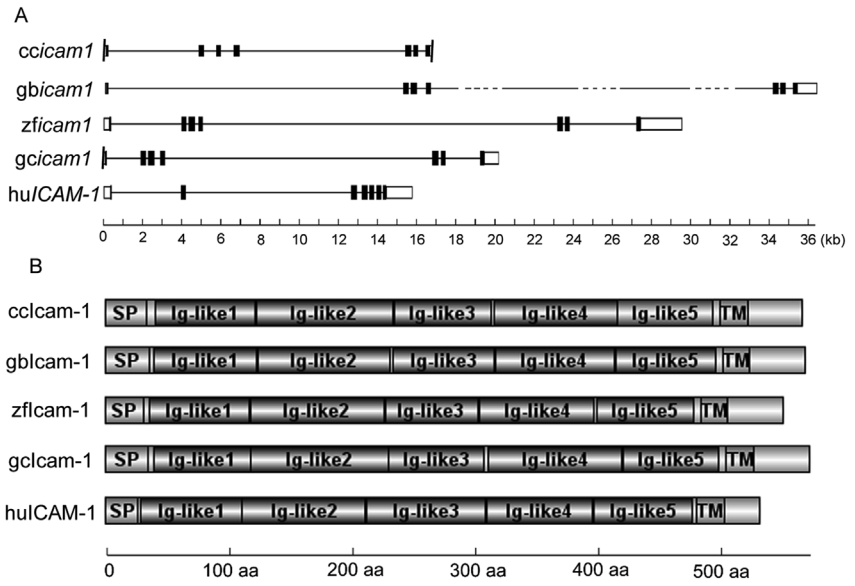

Fig. 1. (A) Exon and intron organization of common carp *icam1* (cc*icam1*), golden-line barbell *icam1* (gb*icam1*), zebrafish *icam1* (zf*icam1*), grass carp *icam1* (gc*icam1*) and human *ICAM-1* (hu*ICAM-1*). Open boxes, black boxes and solid lines represent untranslated regions, exons and introns, respectively. (B) Functional domains of ccIcam-1, gbIcam-1, zfIcam-1, gcIcam-1 and huICAM-1. SP: Signal peptide; Ig-like: Immunoglobulin (Ig)-like domain; TM: Transmembrane domain.

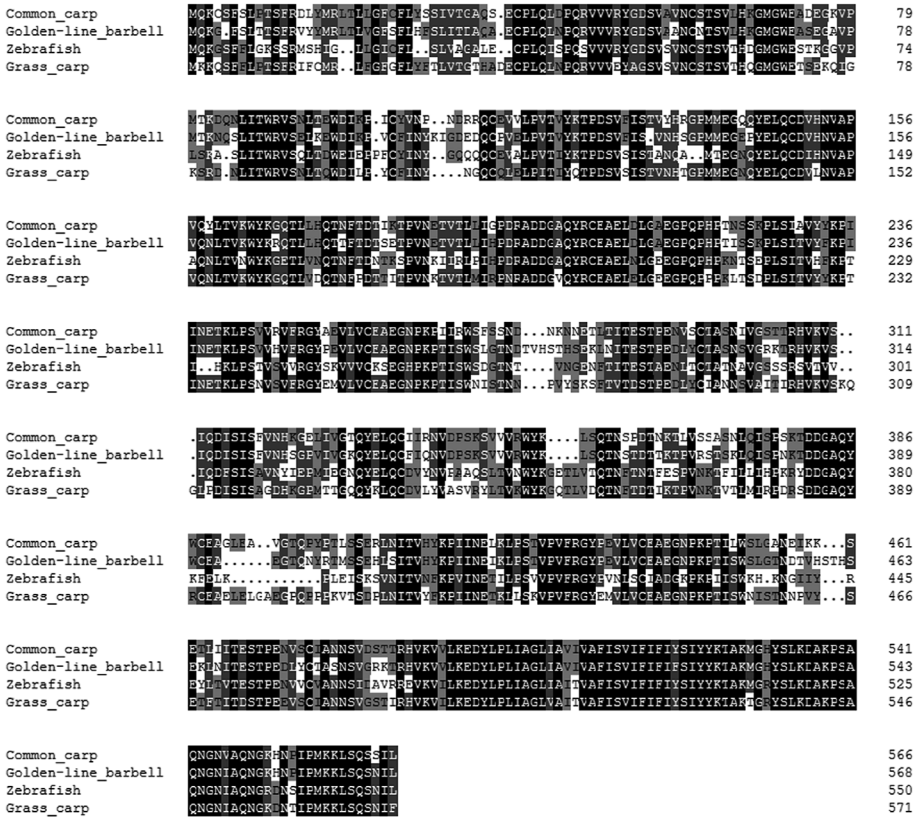

Fig. 2. Alignment of the deduced protein sequence of grass carp Icam-1 with its homologues in common carp, golden-line barbell and zebrafish. The conserved amino acid residues were shaded for homology. The amino acid sequences were deduced from the nucleotide sequences of Icam-1 homologues in these four fishes. (GenBank accession nos: Grass carp, KY963996.1; Golden-line barbell, XM_016275894.1; Zebrafish, XM_002661261; Common carp, NW_017541686.1).

the low-affinity, high-affinity and intermediate-affinity forms [19], and the conformation of high-affinity form can be induced by LPS [20], an anti-CD18 antibody, Mg^{2+}/EGTA, low pH [21], Mn^{2+} [22], or phorbol 12-myristate 13-acetate (PMA) [23].

In teleost, the study on cell adhesion in immune response is currently limited. Homotypic aggregation, a characteristic of activated immune cells, has been investigated in rainbow trout macrophage-like cells (RTS11) [24]. In this study, it has been proved that homotypic aggregation of RTS11 cells can be induced by poly I:C. Meanwhile, two commercial inhibitors (o-bromobenzoyl L-tryptophan and RGD) for lymphocyte function-associated antigen-1 (Lfa-1)/intercellular cell adhesion molecule-1 (Icam-1) interaction fail to block the cell aggregation in these cells [25], excluding the involvement of Lfa-1/Icam-1 interaction in the homotypic aggregation. In other two studies, the authors claimed that *icam1* can be detected in $CD8\alpha^+$ cells at the mucosal tip of olfactory lamella in rainbow trout [26] and in zebrafish challenged with virus infection [27]. However, compelling evidence for the existence of Icam-1 in fish species is currently lacking since Icam-1 has not been functionally characterized in these studies. Accordingly, the existence and molecular features of fish Icam-1, and its roles in immune cell adhesion are indispensable to be examined.

In the present study, grass carp (*Ctenopharyngodon idella*) Icam-1 (gcIcam-1) was found, and its molecular features and ability to mediate adhesion of grass carp peripheral blood lymphocytes (PBLs) were also