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ICAM-2, A Protein of Antitumor Immune Response in Mekong Giant Catfish (*Pangasianodon gigas*)

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***Abstract*—**ICAM-2 (intercellular adhesion molecule 2) or CD102 (Cluster of Differentiation 102) is type I transmembrane glycoproteins, composing 2-9 immunoglobulin-like C2-type domains. ICAM-2 plays the particular role in immune response and cell surveillance. It is concerned in innate and specific immunity, cell survival signal, apoptosis, and anticancer. EST clone of ICAM-2, from *P. gigas* blood cell EST libraries, showed high identity to human ICAM-2 (92%) with conserve region of ICAM N-terminal domain and part of Ig superfamily. Gene and protein of ICAM-2 has been founded in mammals. This is the first report of ICAM-2 in fish.

***Keywords*—**ICAM-2, CD102, *Pangasianodon gigas*, antitumor.

I. Introduction

INTERCELLULAR adhesion molecules (ICAMs), belonging to Ig superfamily, is cell surface proteins which involved in cell adhesion, mostly in white blood cell. The adhesion occurs in either same cells or endothelial cells. ICAMs involves in adhesion mechanisms and cell-cell communication in the immune system such as extracellular matrix or signal transduction for wound and inflammation. ICAMs are classified as 3 subgroups, i.e. immunoglobulin (Ig) superfamily, integrin family, and selectin family.

For subgroup of ICAMs Ig superfamily, it composes of ICAM-1, ICAM-2, ICAM-3, VCAM-1 and MadCAM-1. Functions of these molecules are binding to integrins on white blood cell and transcellular migration of leukoocytes through the endothelial basement membrane and pericyte sheath [1].

Subgroup of ICAMs integrin family, receptors on leukoocytes of ICAMs and VCAMs on vascular endothelium, composes of heterodimeric proteins; alpha and beta chain. Their function is mediated protein of leukocytes for adhesion to vascular endothelium or cell-cell interactions. While subgroup of selectin family composes of L-selectin, P-selectin and E-selectin which involving with lymphocyte binding to activated endothelium, rolling motion of endothelial surface and migration of lymphocyte to lymphoid tissues and inflaming site.

ICAM-2 or CD102 (Cluster of Differentiation 102) is type I transmembrane glycoproteins composing of 2-9 immuno-globulin-like C2-type domains and binding to leukocyte adhesion LFA-1 protein. ICAM-2 plays important role in lymphocyte recirculation by blocking LFA-1-dependent cell adhesion, mediator for adhesive interactions in specific immune mechanism.

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ICAM-2 is broadly expressed on leukocytes (exception of neutrophils), T-cells, B-cells, monocytes, platelets, early CD34+ hematopoietic progenitor cells and endothelial cells. The mechanism is involved in lymphocyte recirculation and tissue distribution [2]-[4]. There were reported that ICAM-2 associated with general immune response in human for critical physiology; i.e. decreasing lung inflammation from allergy by inhibiting eosinophil accumulation [5], [6], as a mediator for a survival signal sufficient to block apoptosis by activation of the PI3K/AKT pathway [7], enhancing activity of [gamma]-[delta]-T-cells to eliminating pancreatic cancer cells [8], involving in immune surveillance during pancreatic tumor development [9], showing antitumor effects by involving anti-angiogenesis and up-regulation in colon carcinoma [10] and being mediator in primary neuroblastoma cell with suppression of metastatic phenotype [11]. Moreover increased ratio of circulating ICAM-1/ICAM-2 discriminated HIV infected patients between progressor group and asymptomatic group [12]. For cancer treatment, ICAM-2 gene therapy was illustrated effective for peritoneal metastasis of gastric carcinoma [13] and neuroblastoma [14].

Gene and protein of ICAM-2 was reported only in mammals. This research was identification of putative ICAM-2 EST fragment from whole blood cells of Mekong giant catfish (*Pangasianodon gigas*), and the first report of this gene in non-mammal teleost.

II. Materials and Methods

A. Sampling and RNA Extraction

Whole blood samples were taken from caudal vessel of mature female Mekong giant catfish. Total RNA was extracted by TRIzol reagent (Invitrogen, Karlsruhe, Germany). The quantity and quality of total RNA was determined spectrophotometry. Poly(A) RNA was purified using a PolyATtract mRNA Isolation System III (Promega, USA).

B. cDNA Library Construction

Complementaty DNA library was performed by protocol of CloneMiner cDNA Library Construction Kit (Invitrogen, Karlsruhe, Germany). Briefly, the double strand cDNA was synthesized and ligated with the attB adapter and size fractionated to remove excess primer, adapters, and small cDNAs by column chromatography (Sephacryl_ S-500 HR resin). The attB-flanked cDNA was ligated into pDONRTM-222 (attP containing vector, Invitrogen) with BP Clonase^{TM} enzyme mix through reaction of the Gateway BP recombination and transformed into competent Escherichia coli ElectroMax^{TM} DH10B^{TM} T1 Phage Resistant Cells

(Invitrogen).

C. Expressed Sequence Tags (EST) Analysis

Hundred microlitre of a 1:100 dilution of the *P. gigas* cDNA libraries was cultured on NA medium containing 50 ug/ml kamamycin and incubated overnight at 37°C. Single colonies were picked and grown in NB medium containing kamamycin. Plasmid DNA of each EST clone was extracted by alkaline lysis [15]. The EST clones were sequenced using ABI 3070 DNA Analyzer (Applied Biosystems; Foster City, CA). M13 forward primer: 5'-ACG ACG TTG TAA AAC GAC GGC CAG-3' and M13 reverse primer: 5'-TTC ACA CAG GAA ACA GCT ATG ACC-3'.

D. Sequence Analysis

Each nucleotide sequence was characterized by NCBI/ BLAST home (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared to same gene of other organisms from Genbank (http://www.ncbi.nlm.nih.gov/nuccore/).

III. Result and Discussion

We found that the EST clone O1005 with 545 base pairs, was putative ICAM-2. By BLASTX analysis, interestingly, it revealed that *P. gigas* ICAM2 showed high ortholog to human ICAM-2 isoform CRA_d with identities of 92% (158/171 amino acids) and similarities of 94% (161/171 amino acids) at middle peptide sequence (61-231 aâ) of 330-aâ human ICAM-2 (Fig. 1).

P. gigas ICAM-2 fragment was translated to deduced peptides of 170 amino acids (Fig. 2) and comprised 2 supperfamily domains; i.e. full ICAM N-terminal domain and partial Ig superfamily (Fig. 3).

In comparison, it was showed that *P. gigas* ICAM-2 domain was highly consensus peptide sequences to ICAMs of human and house mouse (Fig. 4).

There was reported that ICAM-1, ICAM-2 and ICAM-3 bind lymphocyte function-associated antigen-1 (LFA-1) via the N-terminal one or two Ig-fold domain(s). Melero et al. showed that ICAM-2 bound by EOL4G8 upregulates its adhesiveness to DC-SIGN, providing a mechanistic rationale to the enhancement of antitumor immunity [10]. Springer et al. reported that ICAM2 functional conservation of ICAM-2 across species. By in vivo, ICAM-2 is expressed on a variety of leukocyte cell lines, including T and B lymphoma, mastocytoma, and macrophage lines whereas in cell line, ICAM2 is well expressed on endothelioma cells [16], [17].

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> gb|EAW94220.1|intercellular adhesion molecule 2, isoform CRA_d[Homo sapiens]
Length=330

 GENE ID: 3384 ICAM2 | intercellular adhesion molecule 2 [Homo sapiens]
(Over 10 PubMed links)

 Score =  322 bits (826),  Expect = 7e-87
 Identities = 158/171 (92%), Positives = 161/171 (94%), Gaps = 0/171 (0%)
 Frame = +3

 Query  33   GSPWLVPASPWRLPEMSSIGYRTLTVALFTLICCPGSDEKVFEVHVRPKKLAVEPKGSLE  212
             GSPWLVPASPWRLPEMSS GYRTLTVALFTLICCPGSDEKVFEVHVRPKKLAVEPKGSLE
 Sbjct  61   GSPWLVPASPWRLPEMSSFGYRTLTVALFTLICCPGSDEKVFEVHVRPKKLAVEPKGSLE  120

 Query  213  VNCGTTCNQPEVGGLETSLDKILLEVQAQWKQNLVSNISHDTVLQCHFTCSAKLESMNSN  392
             VNC TTCNQPEVGGLETSLDKILL+ QAQWK  LVSNISHDTVLQCHFTCS K ESMNSN
 Sbjct  121  VNCSTTCNQPEVGGLETSLDKILLDEQAQWKHYLVSNISHDTVLQCHFTCSGKQESMNSN  180

 Query  393  VSVYQPPRQVILTLQPTLVAVGKSFTIECRVPTVERLDSLRLNLFRGNDSL  545
             VSVYQPPRQVILTLQPTLVAVGKSFTIECRVPTVE LDSL L LFRGN++L
 Sbjct  181  VSVYQPPRQVILTLQPTLVAVGKSFTIECRVPTVEPLDSLTLFLFRGNETL  231
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Fig. 1 BLASTX alignment of EST clone O1005

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AAGCTCAGACCTGCGGGCACCATCTCCCTCCAGGCAGCCCTTGGCTGGTCCCTGCGAGCCCGTGGAGACTGCCAGAGATGTCCTCTATCG   90
                              G  S  P  W  L  V  P  A  S  P  W  R  L  P  E  M  S  S  I  G   20

GTTACAGGACCCTGACTGTGGCCCTCTTCACCCTGATCTGCTGTCCAGGATCGGATGAGAAGGTATTCGAGGTACACGTGAGGCCAAAGA  180
  Y  R  T  L  T  V  A  L  F  T  L  I  C  C  P  G  S  D  E  K  V  F  E  V  H  V  R  P  K  K   50

AGCTGGCGGTTGAGCCCAAAGGGTCCCTCGAGGTCAACTGCGGCACCACCTGTAACCAGCCTGAAGTGGGTGGTCTGGAGACCTCTCTAG  270
  L  A  V  E  P  K  G  S  L  E  V  N  C  G  T  T  C  N  Q  P  E  V  G  G  L  E  T  S  L  D   80

ATAAGATTCTGCTGGAGGTACAGGCACAGTGGAAACAAAACTTGGTCTCAAACATCTCCCATGACACGGTCCTCCAATGCCACTTCACCT  360
  K  I  L  L  E  V  Q  A  Q  W  K  Q  N  L  V  S  N  I  S  H  D  T  V  L  Q  C  H  F  T  C  110

GCTCCGCCAAGCTGGAGTCAATGAATTCCAACGTCAGCGTGTACCAGCCTCCAAGGCAGGTCATCCTGACACTGCAACCCACTTTGGTGG  450
  S  A  K  L  E  S  M  N  S  V  S  V  Y  Q  P  P  R  Q  V  I  L  T  L  Q  P  T  L  V  A  V  140

CTGTGGGCAAGTCCTTCACCATTGAGTGCAGGGTGCCCACCGTGGAGCGGCTGGACAGCCTCAGGCTCAACCTGTTCCGTGGCAATGACA  540
  G  K  S  F  T  I  E  C  R  V  P  T  V  E  R  L  D  S  L  R  L  N  L  F  R  G  N  D  S  L  170

GTCTC                                                                                        545
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Fig. 2 Nucleotides (blue letters) and deduced peptides (red letters) of *P. gigas* ICAM2

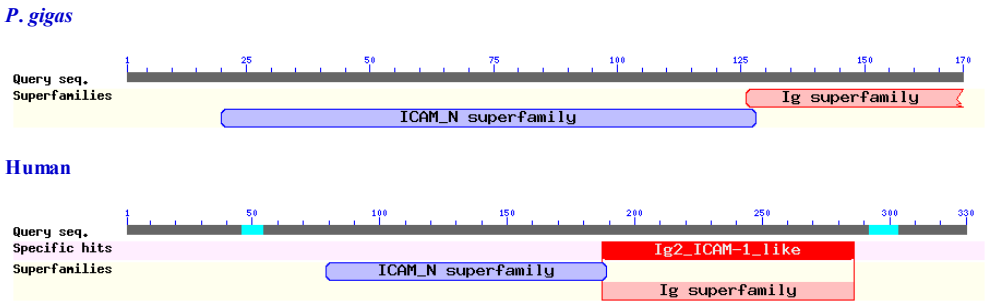

Fig. 3 Domain configuration of ICAM2

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P. gigas          19 GYRTLTLFTLICCPGSDEKFEVHVRPKKLAVEPKGSLEVNCGTTCNQPEVGGLETSLDKILLEV 86
human ICAM-2       4 ACWSLSLLILFYSPGSGEKFEVYIWSEKQIVEATESWKINCSTNCAAPDMGGLETPTNKIMLEE 69
house mouse ICAM-2 4 GYRTLTLFTLICCPGSDEKFEVHVRPKKLAVEPKGSLEVNCSTTCNQPEVGGLETSLDKILLDE 71
human ICAM-1       5 PRPALPLLVLLGALFPGPGAQTSVSPSKVILPRGGSVLVTCSTSCDQPKLLGIETPLPKKELLL 71
house mouse ICAM-1 5 AKPTLPLLALVTVVIPGPGAQVSIHPREAFLPQGGSVQVNCSSSCKEDLSLGLETQWLKDELES 71
human ICAM-3      12 ACWTLLVCCLLTPGVQGQEFLLRVEPQNPVLSAGGSLFVNCSTDCPSSEKIALETSLSKELVAS 76

P. gigas          87 AQWKQNLVSNISHDTVLQCHFTCSAKLESMNSVSVYQPPRQ 128
human ICAM-2      70 QGWKQFLVSNVSKDTVFFCHFTCSGKQHSESLIRVYQPPAQ 114
house mouse ICAM-2 72 QAWKHYLVSNISHDTVLQCHFTCSGKQESMNSVSVYQPPRQ 114
human ICAM-1      72 NNRKVYELSNVQEDSQPMCYSNCPDGQSTAKTLTVYWTPER 115
house mouse ICAM-1 72 PNWKLFELSEIGEDSSPLCFENCGTVQSSASAITVYSFPES 114
human ICAM-3      77 MGWAAFNLSNVTGNSRILCSVYCNGSQITGSSITVYRLPER 119
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Fig. 4 Multi-alignment of ICAM domain

Accession numbers were human ICAM-1 (*Homo sapiens*) (gi 68067956), human ICAM-2 (gb: EAW94220.1), human ICAM-3 (gi 206729872), house mouse ICAM-1(*Mus musculus*) (gi 124099) and house mouse ICAM-2 (gi 462381)

Later, they showed that ICAM-1, ICAM-2 and ICAM-3 are recognized by an I domain-containing integrin, lymphocyte-function-associated antigen 1 (LFA-1, or CD11a/CD18). Additionally, the glutamic acid residue at position 37 of ICAM-2 is critical for LFA-1 binding and is proposed to coordinate the Mg2+ ion in the I domain of integrin. This Glu 37 is surrounded by a relatively flat recognition surface and lies in a beta-strand. And this finding suggests that there are differences in the architecture of recognition sites between integrins that contain or lack I domains. A bend between domains 1 and 2 of ICAM-2 and a tripod-like arrangement of N-linked glycans in the membrane-proximal region of domain 2 may be important for presenting the recognition surface to LFA-1. This model based on the ICAM-2 structure provides a framework for understanding its recognition by pathogens. [18].

Godwin et al. revealed molecular interaction of interspecies between pig ICAM-2 to human LFA-1. This often leads to confusion because equations do not balance dimensionally. If you must use mixed units, clearly state the units for each quantity in an equation [19].

With the high similarities of *P. gigas* ICAM-2 to human ICAM2, this protein may play important function for immune response in Mekong giant catfish as well.

IV. Conclusion

EST clone O1005 was suggested to be messenger RNA gene of *P. gigas* ICAM-2 with partial N-terminus of 545 base pairs (deduces peptides of 170 amino acids) that consisted of highly conserved region of 2 domians; ICAM_N superfamily and Ig superfamily.

The complete sequence of *P. gigas* ICAM-2 cDNA and its mRNA expression should be further investigated for utilities not only of understanding immune mechanism to abnormal cells and risk stage in *P. gigas* but also of medical database for mammals.

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