

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 leukocytes through the endothelial basement membrane and pericyte sheath [1].

Subgroup of ICAMs integrin family, receptors on leukocytes 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 immunoglobulin-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

Complementary 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™ enzyme mix through reaction of the Gateway BP recombination and transformed into competent Escherichia coli ElectroMax™ DH10B™ 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/nucleotide/>).

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 aa) of 330-aa human ICAM-2 (Fig. 1).

P. gigas ICAM-2 fragment was translated to deduced peptides of 170 amino acids (Fig. 2) and comprised 2 superfamily 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 GSPWLVPASPWRLEPMESSIGYRTLTLVALFTLICCPGSDSEKVFVHVRPKKLAVEPKGSLE 212
          GSPWLVPASPWRLEPMESSIGYRTLTLVALFTLICCPGSDSEKVFVHVRPKKLAVEPKGSLE 120
Sbjct 61 GSPWLVPASPWRLEPMESSIGYRTLTLVALFTLICCPGSDSEKVFVHVRPKKLAVEPKGSLE 120

Query 213 VNCGTTTCNQPEVGGLETSLDKILLEVOAQWQNLVSNISHDTVLQCHFTCSAKLESMNSN 392
          VNC TTCNQPEVGGLETSLDKILL+QAQWK LVSNI SHD TVLQCH FTCS K ESMNSN
Sbjct 121 VNCSTTCNQPEVGGLETSLDKILLDEQAQWKHYLVSNI SHD TVLQCH FTCSGRQESMNSN 180

Query 393 VSVYQPPRQVILTLQPTLVAVGKSFTIECRVPTVERLDSLRLMLFRGNDSL 545
          VSVYQPPRQVILTLQPTLVAVGKSFTIECRVPTVE LD SL L LFRGN+L
Sbjct 181 VSVYQPPRQVILTLQPTLVAVGKSFTIECRVPTVEPLDSLTLFLFRGNL 231
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Fig. 1 BLASTX alignment of EST clone O1005

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

GTTACAGGACCCTGACTGTGGCCCTCTTCACCCCTGATCTGCTGTCCAGGATCCGATGAGAAGGTATTCGAGGTACACGTGAGGCCAAAGA 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

AGCTGGCGGTTGAGCCCAAAGGTCCTCGAGGTCAACTGCGGCACCACCTGTAAACAGCCTGAAGTGGGTGGTCTGGAGACCTCTCTAG 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

ATAAGATTCTGCTGGAGGTACAGGCACAGTGGAAACAAAACCTGGTCTCAACATCTCCATGACACGGTCTCCTCAATGCCACTTCACT 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

GCTCCGCCAAGCTGGAGTCAATGAATCCAAACGTCACGCTGTACCAGCCTCCAAGGCAGGTCATCCTGACACTGCAACCCACTTTGGTGG 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

CTGTGGGCAAGTCTTCAACATTGAGTGCAGGGTGGCCACCGTGGAGCGGCTGGACAGCCTCAGGCTCAACCTGTTCCGTGGCAATGACA 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
```

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

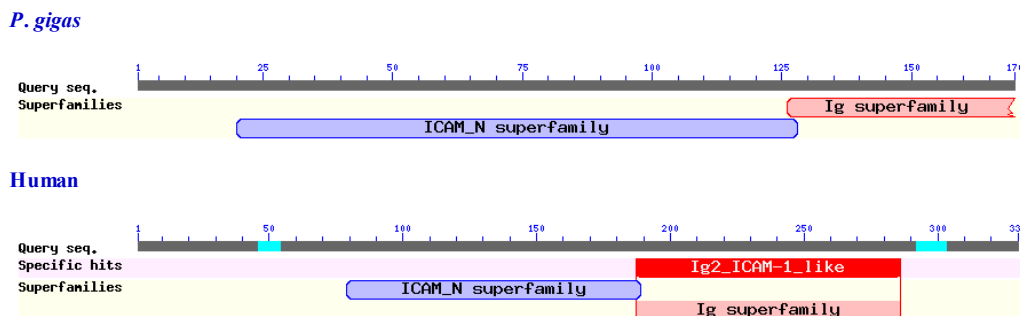


Fig. 3 Domain configuration of ICAM2

<i>P. gigas</i>	19	GYR ^T L ^T L ^F T ^L I ^C C ^P G ^S D ^E K ^F E ^V H ^V R ^P K ^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 ^K I ^L L ^E V	86
human ICAM-2	4	AC ^W S ^L S ^L L ^I L ^F Y ^S P ^G S ^G E ^K F ^E V ^I W ^S E ^K Q ^I V ^E A ^T E ^S W ^K I ^N C ^S T ^N C ^A A ^F D ^M G ^G I ^E T ^P T ^N K ^I M ^L E ^E	69
house mouse ICAM-2	4	GYR ^T L ^T L ^F T ^L I ^C C ^P G ^S D ^E K ^F E ^V H ^V R ^P K ^L A ^V E ^P K ^G S ^L E ^V N ^C S ^T T ^C N ^Q P ^E V ^G G ^L E ^T S ^L D ^K I ^L L ^E	71
human ICAM-1	5	PR ^P AL ^L EL ^L LV ^L LG ^L AL ^F PP ^G GA ^Q TS ^V SP ^S K ^V I ^L FR ^G GS ^V L ^V TC ^S T ^S CD ^Q PK ^L LG ^I E ^T PL ^E PK ^K EL ^L	71
house mouse ICAM-1	5	AK ^P T ^L EL ^L AL ^V T ^V IP ^G GA ^Q VS ^I HP ^R E ^A FL ^Q GG ^S V ^Q V ^N C ^S SS ^C K ^E DL ^S L ^G I ^E T ^Q WL ^K D ^E LES	71
human ICAM-3	12	AC ^W T ^L LV ^C LL ^T PG ^V Q ^Q E ^F L ^R LV ^E P ^Q N ^P V ^L S ^A GG ^S L ^F V ^N C ^S T ^D C ^P SS ^E K ^I A ^L E ^T S ^L S ^K E ^L V ^A S	76
<i>P. gigas</i>	87	A ^Q W ^K Q ^N L ^V S ^N I ^S H ^D T ^V L ^Q C ^H F ^T C ^S A ^K L ^E S ^M N ^S V ^S V ^Y Q ^P P ^R Q	128
human ICAM-2	70	Q ^G W ^K Q ^F L ^V S ^N V ^S K ^D T ^V F ^F C ^H F ^T C ^S G ^K Q ^H S ^E S ^L I ^R V ^Y Q ^P P ^A Q	114
house mouse ICAM-2	72	Q ^A W ^K H ^L V ^L S ^N I ^S H ^D T ^V L ^Q C ^H F ^T C ^S G ^K Q ^E S ^M N ^S V ^S V ^Y Q ^P P ^R Q	114
human ICAM-1	72	N ^N R ^K V ^Y E ^L S ^N V ^Q E ^D S ^Q P ^M C ^Y S ^N C ^P D ^G Q ^S T ^A K ^L T ^V Y ^W T ^P E ^R	115
house mouse ICAM-1	72	F ^N W ^K L ^F E ^L S ^E I ^G E ^D S ^S E ^L C ^F E ^N C ^G T ^V Q ^S S ^A S ^A I ^T V ^Y S ^F P ^E S	114
human ICAM-3	77	M ^G W ^A A ^F N ^L S ^N V ^T G ^N S ^R I ^L C ^S V ^Y C ^N G ^S Q ^I T ^G S ^S I ^T V ^Y R ^L P ^E R	119

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 domains; 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.

ACKNOWLEDGMENT

This research was supported by The Office of Agricultural Research and Extension, Maejo University, Thailand.

The Mekong giant catfish sample was kindly provided by Dr. Jaral Chaiongkarn from Jaral Farm, Chiangrai, Thailand.

REFERENCES

- [1] K. Ley, C. Laudanna, M.I. Cybulsky and S. Nourshargh. "Getting to the site of inflammation: the leukocyte adhesion cascade updated." *Nature Reviews Immunology*, vol. 7, pp. 678-689, September 2007.
- [2] D. E. Staunton, M. L. Dustin and T.A. Springer. "Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1." *Nature*, pp. 339: 61-64. 1989.
- [3] A. R.de Fougerolles, S. A. Stackler, R. Schwarting and T. A. Springer "Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1." *Journal of Experiment Medicine*, vol. 174, pp. 253-267, 1991.
- [4] T. G. Diacovo, A. R.de Fougerolles, D. F. Bainton and T. A. Springer. "A functional integrin ligand on the surface of platelets: inter-cellular adhesion molecule-2." *Journal of Clinical Investigation*, vol. 94, pp.1243-1251, 1994.
- [5] N. Gerwin, J. A. Gonzalo, C. Lloyd, A. J. Coyle, Y. Reiss, N. Banu, B. Wang, H. Xu, H. Avraham, B. Engelhardt, T. A. Springer, J. C. Gutierrez-Ramos. "Prolonged eosinophil accumulation in allergic lung interstitium of ICAM-2 deficient mice results in extended hyper-responsiveness." *Immunity*, vol.10, pp. 9-19, 1999.
- [6] J. T. Pai and E. Ruoslahti. "Identification of endothelial genes up-regulated in vivo." *Gene*, vol.347, pp. 21-33, 2005.
- [7] O. D. Perez, S. Kinoshita, Y. Hitoshi, D. G. Payan, T. Kitamura, G. P. Nolan and J. B. Lorens. "Activation of the PKB/AKT pathway by ICAM-2." *Immunity*, vol. 16 issue 1, pp. 51-65, 2002.

- [8] L. Zhiyong, G. Ben and D.L. Richard. "Expression of intercellular adhesion molecule (ICAM)-1 or ICAM-2 is critical in determining sensitivity of pancreatic cancer cells to cytotoxicity by human $\gamma\delta$ -T cells: Implications in the design of $\gamma\delta$ -T-cell-based immunotherapies for pancreatic cancer." *Journal of Gastroenterology & Hepatology*, vol. 24 issue 5, pp. 900-911, 2009.
- [9] N. Hiraoka, R. Yamazaki-Itoh, Y. Ino, Y. Mizuguchi, T. Yamada, S. Hirohashi and Y. Kanai. "CXCL17 and ICAM2 are associated with a potential anti-tumor immune response in early intraepithelial stages of human pancreatic carcinogenesis." *Gastroenterology*, vol. 140, pp. 310-321, 2011.
- [10] I. Melero, I. Gabari, A. L. Corbi, M. Relloso, G. Mazzolini, V. Schmitz, M. Rodriguez-Calvillo, I. Tirapu, E. Camafeita, J. P. Albar, and J. Prieto. "An Anti-ICAM-2 (CD102) Monoclonal Antibody Induces Immune-mediated Regressions of Transplanted ICAM-2-negative Colon Carcinomas." *Cancer Research*, vol. 62, pp. 3167-3174, June 1, 2002.
- [11] P. Galea, C. Vermot-Desroches, C. Le Contel, J. Wijdenes and J. C. Chermann. "Circulating cell adhesion molecules in HIV1-infected patients as indicator markers for AIDS progression." *Research Immunology*, vol. 148, pp. 109-117, 1997.
- [12] K. J. Yoon, D. A. Phelps, R. A. Bush, J. S. Remack, C. A. Billups and J. D. Khoury. "ICAM-2 Expression Mediates a Membrane-Actin Link, Confers a Nonmetastatic Phenotype and Reflects Favorable Tumor Stage or Histology in Neuroblastoma." *PLoS ONE*, vol. 3 issue 11, e3629 November 2008.
- [13] H. Tanaka, M. Yashiro, T. Sunami, Y. Sakate, K. Kosaka, and K. Hirakawa. "ICAM-2 Gene Therapy for Peritoneal Dissemination of Scirrhous Gastric Carcinoma." *Clinical Cancer Research*, vol. 10, pp. 4885-4892, July 15, 2004.
- [14] K. J. Yoon and M. K. Danks. "Review : Cell adhesion molecules as targets for therapy of neuroblastoma." *Cancer Biology & Therapy*, vol. 8 issue 4, pp. 306-311, 15 February 2009.
- [15] J. Sambrook, E.F. Fritsch, and, T. Maniatis, "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor: Cold Spring Harbor Laboratory Press.,1989.
- [16] A. R. de Fougerolles, S. A. Stacker, R. Schwarting and T. A. Springer. "Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1." *Journal of Experimental Medicine*, vol. 174, pp. 253-267, July 1991.
- [17] H. Xu, J. K. Bickford, E. Luther, C. Carpenito, F. Takei, T.A. Springer. "Characterization of murine intercellular adhesion molecule-2." *Journal of Immunology*, vol. 156 issue 12, pp. 4909-4914. June 15, 1996.
- [18] J. M. Casasnovas, T. A. Springer, J. H. Liu, S. C. Harrison and J. H. Wang. "Crystal structure of ICAM-2 reveals a distinctive integrin recognition surface." *Nature*, vol. 387 issue 6630, pp. 312-315, May 15, 1997.
- [19] J. W. Godwina, A. J. F. d'Apicea and P. J. Cowana. "Characterization of Pig Intercellular Adhesion Molecule-2 and its Interaction with Human LFA-1." *American Journal of Transplantation*, vol. 4, pp. 515-525, 2004.