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Sevenspan transmembrane molecules: novel receptors involved in leukocyte adhesion¹

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Abstract

CD97 is a member of a new subgroup of seven-span transmembrane (7-TM) molecules which belong to the secretin receptor superfamily. Different from other members of the secretin receptor family, these recently characterized molecules have extended extracellular regions comprising several EGF domains near the NH₂ terminus. We recently demonstrated that the extracellular part of CD97 is involved in intercellular adhesion since it specifically binds to CD55 (decay accelerating factor), a regulatory protein of the complement cascade. To our knowledge this is the first demonstration of a cellular ligand for a 7-TM molecule. © 1996 Elsevier Science B.V. All rights reserved

Keywords: Sevenspan transmembrane molecules; Leukocyte adhesion; Secretin receptor family

1. Secretin receptor superfamily

Seven-span transmembrane molecules (7-TM) form one of the largest superfamilies of cell surface proteins with more than 100 members described by their primary structure so far [1]. A common feature of these receptors is their association with G proteins which engage signalling pathways leading to an increase of cAMP and/or the mobilization of intracellular Ca⁺⁺ [2]. Besides light-induced conformational changes in visual pigments, different types of molecules, e.g. cationic amines, arachidonic acid derivatives, peptides and glycoprotein hormones can ligate 7-TM receptors.

Molecular cloning of the secretin receptor by Ishihara et al. (1991) has revealed a sequence pattern of the membrane-spanning region different from that of classical 7-TM molecules [3]. Meanwhile, a number of homologous receptors have been described which bind

mammalian and insect peptide hormones, e.g. calcitonin, parathyroid hormone, vasoactive intestinal peptide, glucagon-like peptide 1, glucagon, pituitary adenylate cyclase-activating polypeptide, corticotropin-releasing factor and insect diuretic hormone [4].

2. 7-TM receptors with extended extracellular domains

The recent cDNA cloning of CD97 [5], EMR1 [6] and F4/80 [7] has shown that the secretin receptor group displays additional diversity. Their transmembrane region clearly places these molecules into the SecR group (20–25% amino acid (aa) identity). However, the extracellular parts of CD97, EMR1 and F4/80 exceed that from known members of the SecR superfamily (about 150 aa) and are characterized by several EGF domains near to the N-terminus.

The large similarity of CD97 and EMR1 (30.6% identity in 703 aa) as well as the assignment of the encoding genes to the same region on the short arm of human chromosome 19 [5,6] suggests a common pre-

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cursor gene that has probably evolved from the SecR supergene family. Analysis of the organization of the CD97 gene has confirmed this assumption and demonstrated that the precursor gene has assembled the N-terminal EGF domains by exon shuffling [8].

3. CD97 has a cellular ligand: CD55

The most relevant question to be answered is whether the acquisition of EGF domains has provided this new class of 7-TM receptors with novel functional properties. Indeed, by demonstrating that lymphocytes and erythrocytes specifically adhere to CD97-transfected COS cells we found that CD97, in parallel with its molecular evolution, has acquired the ability to bind cellular ligands. This interaction was found to be specific since it could be blocked by CD97 mAb (Table 1) [9].

To identify the cellular ligand for CD97 a mouse was immunized with human red cells, and spleen cells were fused with SP2/0. Hybridoma supernatants were screened for their ability to block the binding erythrocytes to CD97 transfectants and one mAb, designated CLB-CD97L, was selected in this way. Initial characterization of the mAb showed that it reacted with both leukocytes and red cells. Moreover, biochemical analysis showed that the antibody recognized a membrane glycoprotein with a molecular mass of 70 kD. Among antigens of this size characterized until now only a small number are expressed on both leukocytes and red cells. When we tested the capacity of mAbs against these molecules to inhibit the binding between CD97-transfectants and erythrocytes only antibodies directed against CD55 turned out to be inhibitory (Table 1). CD55 is a GPI-anchored molecule that by inhibiting C3/C5 convertases protects cells from complement-me-

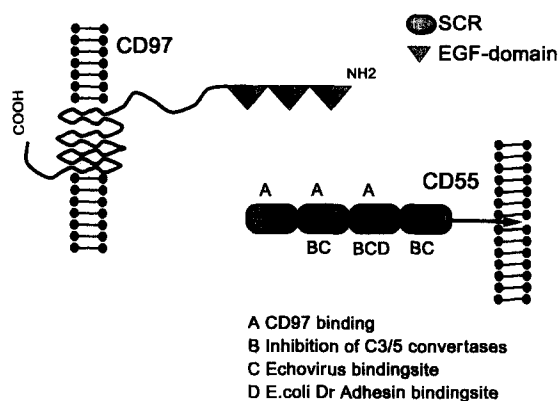


Fig. 1. Schematic representation of the interaction between CD97 and CD55.

diated damage [10]. CD55 is built from four so called 'short consensus repeat' (SCR) domains. The ability to dissociate and prevent assembly of C3/C5 convertases has been mapped to SCR-2, 3 and 4. In addition, Echovirus 7 also requires SCR-2, 3, and 4 for binding to CD55 [11] whereas *E. coli* Dr Adhesin recognizes SCR-3 [12]. Using mAb to distinct SCR we found that mAb to SCR-1, 2 and 3 can block the interaction between CD97 and CD55 (Fig. 1) implying for the first time a role for SCR-1 in ligand binding. Finally, the specificity of the interaction between CD97 and CD55 was confirmed by the finding that red cells that do not express CD55 do not adhere to CD97 transfectants [9].

4. Conclusions

CD97 is a 7-TM molecule that is rapidly up-regulated on leukocytes after activation [5,13]. Remarkably, among the hundreds of known 7-TM molecules CD97 is the first receptor for which a cellular ligand has been demonstrated [1]. The interaction between CD97 and CD55 implies the existence of a novel adhesion pathway primarily used by stimulated but not resting leukocytes. The signalling and functional consequences of this interaction are currently under investigation.

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Table 1

CD55 mAb inhibit the binding of ^{51}Cr -labeled erythrocytes to CD97-transfected COS cells

| Antibody | CD55 SCR-domain | Adhesion (%) ^a |
|----------------|-------------------|---------------------------|
| None | n.a. ^b | 100 |
| Control (IgG1) | n.a. | 103 |
| CLB-CD97/1 | n.a. | 13 |
| CLB-CD97L/1 | 1 | 8 |
| BRIC220 | 1 | 33 |
| BRIC230 | 1 | 77 |
| BRIC110 | 2 | 24 |
| BRIC216 | 3 | 16 |

Results are expressed as the percentage of erythrocyte binding relative to the amount bound in the absence of mAb. For experimental details see [9].

^aBinding of erythrocytes to CD97 transfectants: in the absence of antibodies was set at 100%.

^bNot applicable.

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