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Intestinal Epithelial CD98: An Oligomeric and Multifunctional Protein

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Abstract

The intestinal epithelial cell-surface molecule, CD98 is a type II membrane glycoprotein. Molecular orientation studies have demonstrated that the C-terminal tail of human CD98 (hCD98), which contains a PDZ-binding domain, is extracellular. In intestinal epithelial cells, CD98 is covalently linked to an amino-acid transporter with which it forms a heterodimer. This heterodimer associates with β_1 -integrin and intercellular adhesion molecular 1 (ICAM-1) to form a macromolecular complex in the basolateral membranes of polarized intestinal epithelial cells. This review focuses on the many functional roles of CD98, including involvement in extracellular signaling, adhesion/polarity, and amino-acid transporter expression in intestinal epithelia. A role for CD98 in intestinal inflammation, such as Intestinal Bowel Disease (IBD), is also proposed.

Keywords

Intestinal Epithelial CD98; Extracellular PDZ binding domain; Adhesion; Cell polarity; Amino acid transporters; Intestinal inflammation

1) Introduction

Polarized intestinal epithelial cells contain distinct apical and basolateral membranes with unique protein and lipid compositions. This asymmetric distribution of plasma membrane components is a fundamental characteristic of epithelial cells (1–5). For example, the ability of epithelium to secrete and absorb fluid is closely linked to the asymmetric distribution of ion-

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transport processes in the membranes (6–11). Accumulating experimental and clinical evidence indicates that surface adhesion molecules, such as integrins, are required for normal epithelial development; mutation or absence of these molecular adhesins can disrupt growth control and/or alter epithelial cell function and cell polarity (12–13). Because epithelial cells are in direct contact with the extracellular matrix (ECM), it is reasonable to expect that specific interactions exist between basolateral "receptors", such as integrins, and their cognate extracellular "ligands" in the ECM. Indeed, on binding to ECM ligands, integrins deliver signals that control cell proliferation, gene expression, differentiation, and polarization (14–16).

The CD98 complex, a cell-surface amino-acid transporter formed by covalent linkage of the CD98 heavy chain (CD98hc) with several different light chains, has recently been shown to function as a β_1 -integrin regulator (17–25). Because most of the reported studies have been performed in non-polarized cells, the physiological relevance of this regulatory function is unclear. Here, we focus on CD98 in polarized intestinal epithelia, exploring its role as a potential regulator of multiple functions, including adhesion, epithelial cell polarity, and amino-acid transport.

2) Expression of CD98 in intestinal polarized epithelia

CD98 is expressed in all cell types with the exception of platelets, and is expressed at highest levels in the tubules of the kidney and the gastrointestinal tract (26,27). In polarized epithelial cells, CD98 is targeted to the basolateral domain of the epithelium (26,27), and is found exclusively on the basolateral surface of intestinal epithelia in culture (24) and mouse small intestine (27). The intestinal epithelial CD98 cell-surface molecule is a 125-kDa type II membrane glycoprotein heterodimer composed of a 40-kDa non-glycosylated light chain (amino-acid transporter) and an 85-kDa glycosylated heavy chain (CD98) (24; and Figure 1). The CD98 transcript is 1863 base pairs (bp) long with an open reading frame of 1590 bp that encodes a glycoprotein of 529 amino acids with a predicted size of 80 kDa. The hydropathy plot of CD98 predicts the presence of a single membrane-spanning domain (24–31). Amino acids 1–81 form the cytoplasmic portion, amino acids 82–104 represent the single-pass transmembrane region, and amino acids 105–529 comprise the extracellular domain of the CD98 glycoprotein (30). Interestingly, the C-terminus of human CD98 (hCD98) contains a potential class II PDZ-binding domain (amino acids 520 to 529; GLLLRFPYAA), suggesting that hCD98 may associate with extracellular PDZ-domain-containing proteins (32). The primary sequence contains four potentially glycosylated asparagine residues at positions 264, 280, 323, and 405, all of which are located in the C-terminal half of the protein. Cys-109 has been shown to participate in disulfide-bond formation with amino-acid transporters. Five vertebrate "glycoprotein-associated" amino acid transporters, LAT1, LAT-2, y^+ LAT1, y^+ LAT-2, and xCT (33–39), have been shown to associate with CD98. However, intestinal CD98 is linked to the L-type amino-acid transporters LAT-2 and y^+ LAT1, which appear to be expressed only in epithelia (27,36). LAT-2, and presumably also y^+ LAT1, is exclusively expressed in the basolateral membrane domains of intestinal epithelial cells and is covalently linked to CD98 to form a functional heterodimer (39–42). Studies have demonstrated that basolaterally expressed CD98/LAT-2 in intestinal epithelial cells represents the minimal functional unit for an Na^+ -independent transporter of zwitterionic amino acids of any size (36,38). Hydrophobicity studies indicate that LAT2 is composed of 12 transmembrane domains with intracellular N- and C-terminal segments, a structure that is predicted to be shared by other proteins of this family (33,43). CD98 is responsible for recognition of LAT-2 and also ensures proper translocation of the transporter complex to the plasma membrane (44). However, CD98 has also been shown to be capable of guiding LAT-2 to the plasma membrane in the absence of a disulfide linkage between the two proteins, implying the existence of

important noncovalent, steric interactions (44). A model of the CD98/LAT-2 heterodimer is depicted in Figure 1.

3) Potential Role of the Extracellular PDZ Binding Domain of CD98

The C-terminal region of CD98 contains a potential PDZ class II-binding domain (amino acids 520 to 529; GLLLRFPYAA), indicating that the C-terminal tail of hCD98 might bind to PDZ class II proteins in the extracellular space (32). Such interactions might be expected to occur on the basolateral side of intestinal monolayers, given that proteins containing PDZ class II domains are often localized to specific subcellular sites near the plasma membranes of polarized cells, such as intestinal epithelial cells. One protein that may bind to the C-terminus of hCD98 in the extracellular space is human calcium/calmodulin-dependent serine kinase (hCASK), a member of the MAGUK protein family (45,46). This protein is the human homolog of the *Caenorhabditis elegans* LIN-2 and *Drosophila* Camguk proteins and is widely expressed in human tissues (47,48). The protein has a unique domain structure that includes an N-terminal region with homology to the calcium/calmodulin-dependent protein kinase, followed by the three characteristic MAGUK domains (PDZ class II consensus domain, SH3 domain, and GUK domain) (46). Although hCASK-associated protein complexes in epithelial cells have not yet been fully characterized, hCASK is reportedly basolaterally localized in intestinal epithelial cells (46). In agreement with these reports, we have recently shown that hCASK is basolaterally expressed in the Caco2-BBE intestinal epithelial cell line (32). We have further shown that hCASK may be inserted into the cell membranes of Caco2-BBE cells with an orientation that positions the C-terminus of hCASK and its PDZ domain on the extracellular side of the membrane (32). Although hCASK has previously been shown to access the intracellular side of the membrane (47), this is the first report that hCASK may also access extracellular ligands (32). In this orientation, hCASK would be capable of using extracellular nucleoside triphosphates as co-substrates (48,49). In addition, we have demonstrated that hCD98 and hCASK co-precipitate and co-localize both *in vitro* and *in vivo*, and that the PDZ-binding domain of hCD98 is directly involved in this interaction (32). The most critical residue for PDZ recognition was found to be a phosphorylatable amino acid (tyrosine), further suggesting that tyrosine phosphorylation is a common mechanism for regulating the hCASK/CD98 interaction (47), perhaps via the action of ectokinases (49).

Although most protein phosphorylation studies have focused on intracellular protein kinases, some have reported evidence of ectokinase activities on the surfaces of a variety of cells (49–53). Importantly, a recent study identified ectokinase activity on the surface of human neutrophils (54), which can interact with the basolateral aspect of intestinal epithelial cells, and thus might regulate the hCD98/hCASK interaction. Recent reports demonstrate the importance of the 15 C-terminal residues of CD98 that contain the PDZ binding domain, showing that these residues are required for the transport function of the heterodimer. Mutation of the conserved C-terminal residue, leucine 523, to glutamine, reduced the $V_{\max}$ values for arginine and leucine uptake (66). Collectively, these observations suggest that the PDZ-binding domain of CD98 might be a functional entity capable of regulating important processes, such as amino acid transport activity via LAT-2, in intestinal epithelial cells.

4) Epithelial CD98/LAT2 as a regulator of intestinal epithelial adhesion and cell polarity

Integrins, including β_1 -integrins, are expressed in the basolateral domain and along cell–cell junctions (lateral domain), where they have a role in maintaining cell–cell adhesion and organization of the subcortical cytoskeleton (55). It has been suggested that integrin function is readily modulated by various proteins and protein complexes, including oncogenes (56). Several classes of cell-surface glycoproteins have been shown to play a role in integrin-

mediated events, including CD98, CD36, CD63, and CD9 (17,57,58,59). β_1 -integrins co-localize with CD98/LAT-2 to the intercellular contact sites in Caco2-BBE monolayers, which indicates that CD98 may also regulate β_1 -integrin function (24). Given the importance of integrin cytoplasmic tails in integrin activation, proteins such as CD98 that interact with integrin cytoplasmic domains are excellent candidates as modifiers of integrin activation. Integrins are dynamic molecules, and recent studies have reported that a number of surface transmembrane glycoproteins can associate with integrins and thereby modulate their function (17,60). Several classes of cell-surface glycoproteins have been shown to play a role in integrin-mediated events, including the integrin-associated protein (CD47) and members of the transmembrane 4 superfamily (TM4SF or tetraspanins). CD47 associates with $\alpha_v\beta_3$ -integrin and seems to affect integrin-mediated signal transduction, phagocytosis, and cell migration (58,59). Numerous TM4SF members such as CD36, CD63, and CD9 have been shown to be associated with β_1 -integrins (17,57). Experiments in non-polarized epithelial CHO cells have shown that CD98 can modify the function of β_1 -splice variants (19). In addition, a genetic complementation strategy identified an interaction between a transmembrane protein, CD98, and the integrin β_1 cytoplasmic domain (18,19,23). Together, such observations performed in non polarized cells indicate that CD98, if basolaterally expressed by polarized epithelial cells, could affect attachment, adherence, and integrin-mediated cytoskeletal functions.

Epithelial cell polarity is determined by a combination of events mediated by cell-cell and cell-substratum adhesion. Cell adhesion to the ECM is mediated by the integrin superfamily of adhesion receptors and is thought to play a critical role in subsequent ordering of the cytoskeleton and formation of polarity. However, the interactions between integrins and the ECM, although triggering a crude initial polarity, are unlikely to be sufficient to organize the high level of polarity displayed by polarized columnar epithelia. For development of this level of polarity, it is likely that additional cell-cell interactions are required to restrict the localization of basolateral proteins. We have shown that expression of human heterodimeric (human CD98/endogenous canine light chain) or monomeric CD98 in a CD98-deficient cell line (MDCK) disrupts intercellular adhesion, leading to cytoskeletal disorder (24). This phenotypic conversion, which probably depends on the interaction of human CD98 with respective "ligands", is accompanied by reorganization of the actin cytoskeleton. However, interactions between CD98 and the amino-acid transporter are unlikely to be involved in this process, as overexpression of CD98 modified at a specific residue 109 (C109 was mutated to serine) preventing disulfide linkage between human CD98 and canine amino acid transporter does not inhibit the process. The later observation is in agreement with the finding that CD98/amino acid transporter association is not required for the interaction of CD98 with integrins (19). By contrast, it has recently been demonstrated that heterodimeric CD98, but not monomeric CD98, causes transformation of fibroblasts cells; however, in this case, expression of the amino-acid transporter was thought essential to achieve this phenotype (62). A potential ligand for CD98 is β_1 -integrin, which is expressed by MDCK cells. The phenotypic conversion observed with CD98 could be related to altered β_1 recognition of extracellular ligands (18), and CD98 has been shown to affect β_1 -integrin function (18,19,25,63). Consistent with this view, overexpression of a CD98 mutant protein lacking the cytoplasmic tail and part of the transmembrane domain did not induce this phenotypic change, whereas a CD98 protein with a partially truncated cytoplasmic tail did show this phenotypic change. These results indicate that the cytoplasmic juxtamembrane domain is crucial in this phenotype change and are in agreement with the recent study demonstrating that both the transmembrane domain and the proximal part of the cytoplasmic tail play an important role in modulating integrin-dependent cell adhesion and migration as well as branching morphogenesis of polarized renal epithelial cells. However, another study using non polarized cells has reported that CD98 interaction with the integrin β subunit cytoplasmic domain was necessary to mediate adhesive signaling (64). The reason for this discrepancy may be due to the different model system utilized. The co-localization of CD98/LAT-2 and β_1 -integrin suggests a possible

interaction between these three proteins. We speculate that a specific molecular ratio of CD98/LAT-2 heterodimer and β_1 -integrin may be required for polarization of epithelial cells. Expression of human CD98 in a human CD98-deficient cell line (MDCK) may change the canine CD98/amino-acid transporter and β_1 -integrin molecular ratio, which may have a consequent effect on cell adherence and polarity. The cytoplasmic juxtamembrane domain seems to be crucial in this process. Recently it has been proposed that CD98 expression participates in fibronectin matrix assembly by mediating integrin signaling (64). Under this context changes in CD98hc expression, or its association with integrins, may influence a wide range of developmental that include cell polarity processes through the regulation of matrix assembly (64).

5) Epithelial CD98 and ICAM-1 regulate the activity of amino acid transporter, LAT-2

The mechanisms by which CD98 and LAT-2 regulate amino-acid transport, and the possible interactions involved, remain largely unstudied. However, we have demonstrated that cross-linking of CD98 affects the intrinsic activity of the LAT-2 transporter by increasing the affinity and reducing the capacity of LAT-2-mediated leucine uptake in Caco2-BBE monolayers (65). In addition, we have reported that cross-linking of CD98 regulates LAT-2-dependent leucine efflux (65). Other studies have shown that a series of CD98 C-terminal truncates (ranging from 15 to 404 residues) caused a complete loss of light-chain function, although all heterodimers were expressed at the cell surface. This indicates that the 15 C-terminal residues of CD98 are required for the transport function of the heterodimer. Mutation of the conserved residue leucine 523 to glutamine in the C-terminus reduced the $V_{\max}$ of arginine and leucine uptake (66). Another study have demonstrated that the substitution of the extracellular domain of CD98hc with that of CD69 preserved effects on integrin function but abolished amino acid transport activity (19). Together, these observations suggest that extracellular domain of CD98 regulates the LAT-2 transport activity that contrast to the intracellular domain of CD98 which is crucial for regulating β_1 integrin activation.

It has been reported in non polarized cells that CD98 can regulate different types of adhesion molecules such as CD147, LFA-1 through distinct mechanisms, reinforcing the notion that CD98 acts as a 'molecular facilitator' in the plasma membrane (19). In the context of intestinal inflammation, ICAM-1, a cell adhesion molecule is expressed in intestinal epithelial cells during intestinal inflammation (67,68). ICAM-1 plays an important role in cell-cell, cell-extracellular matrix interactions and cellular interactions such as the immune response (69). Furthermore, ICAM-1 is known to be the receptor to the heterodimer of CD11a, and CD18 (β_2 integrin) is expressed in leukocytes that interact with the intestinal epithelia during inflammation. ICAM-1 is constrictively expressed to both apical and basolateral domain membranes of the model intestinal epithelial cell line Caco2-BBE (65). Under this observation, it is conceivable that in the intestinal epithelia, ICAM-1 may be part of a multicomponent web that includes CD98/LAT2 and integrin β_1 . Recently, we have demonstrated that ICAM-1 associates with the heterodimer CD98/LAT2 (65). This result suggests that, in adhesion, ICAM-1 functions not only as an individual receptor but also as a component of supramolecular complexes at the plasma membrane in epithelial cells. In addition, association of the heterodimer CD98/LAT-2 and ICAM-1 indicate that this supramolecular complex may have a significant role in mediation of cellular regulation. We have shown that cross-linking of ICAM-1 reduces the affinity and increases the capacity of LAT-2 mediated leucine uptake (65).

Furthermore, cross-linking of ICAM-1 increases the rate of leucine efflux across the basolateral membranes of Caco2-BBE cells (65). However, it is not known if ICAM-1 interacts directly or indirectly with LAT2. Further investigations will have to be perform to elucidate the ICAM1

interaction(s) with CD98/LAT2 β_1 integrin complex. The supramolecular complex may signal via the amino-acid transporter LAT-2 to regulate multiple aspects of cell physiology. For example, LAT-2 mediated regulation of intracellular amino-acid availability may modulate the activity signaling pathway, leading to phosphorylation of an intracellular target protein. In addition, it has been shown that the intracellular amino-acid supply modulates several important regulatory translation factors through a variety of mechanisms (70). Furthermore, it has been shown that leucine availability regulates the activity of the signaling pathway, which leads to the activation of p70 S6 kinase (which is a 70-kDa protein kinase that acts on the ribosomal protein S6) (71,72). Indeed, we have demonstrated that cross-linking of CD98 or ICAM-1, which mimics natural ligands for these proteins, modifies LAT-2-mediated leucine transport activity. Interestingly, cross-linked CD98 and cross-linked ICAM-1 have different effects on LAT-2 transport activity. We suggest that the transport activity changes are the result of a direct or indirect phosphorylation of LAT-2. In conclusion, the amino-acid transporter LAT-2 is regulated by adhesion molecules such as ICAM-1 and CD98 in epithelial cells. CD98 and ICAM-1 may have roles in transduction of intracellular signals. Changes in amino-acid-transport activity resulting from CD98 and ICAM interaction may coordinate events such as cell adhesion.

6) Regulation of CD98 Expression in intestinal Inflammation

In the context of intestinal inflammation, CD98 protein expression in human colonic epithelium was shown to be upregulated by pro-inflammatory cytokines such as interferon γ (73), and increased expression levels of lymphocyte-activation antigens for CD98 were found at the cell surface of intestinal B cells, T cells, CD4⁺ T cells, and CD8⁺ T cells isolated from patients with Crohn's disease and ulcerative colitis (74). These and other results indicate that markedly increased intestinal lymphocyte activation is an important immunological alteration in inflammatory bowel disease (IBD) (75). In addition, 5-aminosalicylic acid (which is used for the treatment of intestinal inflammation in IBD) dose-dependently inhibited expression of the CD98 cell-surface-activation antigen in mitogen-activated peripheral blood lymphocytes, further suggesting that CD98 plays an important inflammatory role. However, in contrast to the high expression of intestinal epithelial CD98 expression, it has been demonstrated that T-cells have low levels of CD98 transcripts and has been shown to be the result of a block to transcription elongation within the exon 1 intron 1 regions (76). These findings indicated that a removal of the block to mRNA elongation stimulates the induction of CD98 in activated T-cells (76). The latter observation suggests that regulation CD98 expression could be tissue dependent. Dextran sulfate sodium (DSS)-induced colitis is a useful model for examining the role of CD98 in the colonic mucosa (74,76), and we found that the effect of DSS on epithelial CD98 expression is mediated via interferon- γ (IFN- γ) (77). IFN- γ is present at high levels in tissues affected by IBD, where it helps enterocytes to function in host defense (75). Antibody-based inhibition of endogenous IFN- γ has been shown to ameliorate the chronic stage of colitis, indicating that IFN- γ is likely to be a key mediator of intestinal inflammation (78). Recently, we showed that CD98 transcription is activated in IFN- γ -treated intestinal epithelial cells, and investigated the underlying mechanisms in the colonic epithelial cells (77,79). We have isolated and functionally characterized the 5'-flanking region of the CD98 gene in the Caco2-BBE epithelial cell line. Sequence analysis revealed four GC/GT boxes potentially capable of binding Sp1 transcription factors, together with a nuclear factor- κ B (NF- κ B)-transcription-factor-binding site (79). This result is in agreement with results of sequence analysis of the cloned CD98 DNA from the HPB-MLT human T-cell tumor line, which revealed that the 5' flanking region of the CD98 gene contains a hypomethylated CpG island and four potential binding sites for the Sp1 transcription factors but does not contain TATA or CCAAT boxes (80). Both competition electrophoretic mobility shift assay (EMSA) and antibody supershift experiments revealed that Sp1 and NF- κ B interact with the promoter region. We found that different Sp1 binding sites have different DNA-protein interaction profiles, indicating that

each of these binding sites has distinct functional properties. Furthermore, chromatin immunoprecipitation studies have shown that DNA interacts with Sp1 and NF- κ B *in vivo* under basal and IFN- γ -stimulated conditions. The 5'-flanking region does not contain TATA or CCAAT boxes, and primer extension and rapid amplification of cDNA ends (RACE) assays revealed that a major transcriptional initiation site is located 129 bases upstream of the first ATG codon. This is in agreement with previous identification of single start sites for TATA-less promoters in other genes, including the genes encoding thymidine kinase (81), dihydrofolate reductase (82), and adenine deaminase (83). Transfection of Caco2-BBE cells with luciferase reporter constructs fused to the CD98 gene promoter region or its serially truncated mutants revealed that specific DNA regulatory elements are located within 0.33 kb of the major transcription start site. IFN- γ increases transcription of CD98 via the Sp1 and NF- κ B transcription factors in intestinal epithelial cells (79). These results indicate that, during intestinal inflammation, CD98 expression is upregulated in immune cells and in intestinal epithelial cells. The upregulation of CD98 in intestinal epithelial cells could affect functions such as β_1 -integrin mediated events (18,21) that have been implicated in the etiology of various pathologic conditions, including inflammatory disorders such as IBD.

7) Perspectives

Intestinal epithelial CD98 plays an important role in coordinating intestinal epithelia events such as adhesion/polarity, amino-acid transport, and the direct binding of cell-surface molecules. The unique molecular orientation of CD98, with a PDZ-binding domain in the extracellular C-terminal tail, suggests that extracellular signaling may play a role in the multiple functions of CD98. For example, one of the challenges would be to study the role of extracellular phosphorylation of CD98 and its effects in intestinal epithelial functions in the normal and disease states.

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Figure 1. Model of possible functional macro-complex including heterodimer of CD98 and LAT2, β 1 integrins and ICAM-1 in intestinal epithelia

The model shows that CD98 controls β 1 integrin activation that and amino acid transport activities via LAT2. In addition, ICAM-1 is also associated to CD98 and modulates amino acid transporter activities via LAT2. Extracellular C-terminal domain that contain PDZ binding domain controls LAT2 transport activity since intracellular/juxta-intramembrane N terminal domain of CD98 controls β 1 integrin activation in intestinal epithelia.