CD97 is a multifunctional leukocyte receptor with distinct roles in human cancers (Review)

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Abstract. G-protein coupled receptors (GPCRs) represent the most diverse and biologically ubiquitous protein receptors. The epidermal growth factor seven-span transmembrane (EGF-TM7) family consists of adhesion GPCRs with a diverse functional repertoire. CD97 is the most broadly expressed member with roles in cell adhesion, migration and regulation of intercellular junctions. CD97 is also expressed in a variety of human malignancies including those of the thyroid, stomach, colon and brain. CD97 confers an invasive phenotype and has been shown to correlate with tumor grade, lymph node invasion, metastatic spread and overall prognosis. More recently, CD97 was found to signal through Gα12/13, resulting in increased RHO-GTP levels. Proven roles in tumor invasion and signaling make CD97 an exciting novel therapeutic target. In this review, we will discuss the structure and function of this receptor, with a specific focus on its mechanistic significance in neoplastic diseases.

Contents

1. The EGF-TM7 family of G-protein coupled receptors

Adhesion G-protein coupled receptors (GPCRs) represent a diverse class of seven-span transmembrane (TM7) receptors that are ubiquitously expressed across species with diverse roles in cell adhesion and signaling (1). The epidermal growth factor-seven-span transmembrane (EGF-TM7) family is comprised of adhesion GPCRs expressed mainly on the surface of leukocytes, with six members identified to date: CD97, EGF-like module containing mucin-like receptor 2 (EMR2), EMR3, EMR4 and EGF-TM7-lactrophilin-related protein (ETL). Members of the EGF-TM7 family are known to play diverse roles in leukocyte development and activation (2-9).

CD97 is the most broadly expressed member of this family and is found on the surface of lymphocytes, monocytes, macrophages, dendritic cells, granulocytes and smooth muscle (10). It is upregulated during activation of lymphocytes and has been implicated in cell adhesion and migration through interactions with cell surface proteins and components of the extracellular matrix (ECM). CD97 has three known ligands: CD55, a negative regulator of the complement cascade (11), chondroitin sulfate, a component of the ECM (2,12,13) and the integrin α5β1 (14). The association with integrins is particularly noteworthy since they have been shown to mediate invasion, migration and angiogenesis in cancer (15,16). Additionally, proliferating endothelial cells are known to express chondroitin sulfate, suggesting a potential interaction between tumor cells and nascent tumor-associated vessels (17).

The unique structure of CD97 suggests dual roles in cell adhesion and signaling. Before reaching the cell surface, the receptor undergoes autocatalytic cleavage at the GPCR proteolysis site (GPS) motif, a unique cysteine-rich region just proximal to the first transmembrane domain, resulting in the formation of an extracellular α-subunit and transmembrane β-subunit (18). This process is critical, since failure of autocatalytic cleavage results in impaired receptor trafficking and function (19,20). Despite their physical separation the subunits are known to interact via non-covalent interactions to form heterodimers (18,20-22), with functional activity of the receptor dependent upon this interaction (1,23). Furthermore, studies in related proteins show that certain receptor isoforms exhibit a dominant negative phenotype mediated by their TM7 subunits, resulting in subsequent dimerization and degradation of receptor (21).

The functional contributions of these receptors towards immune cell adhesion are well established (1,2,11), however members of the EGF-TM7 family are also known to be

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expressed in a number of malignancies including those of the breast (24), thyroid (25), stomach (26,27), esophagus (26), colon (28), pancreas (26,29) and brain (30-32). Moreover, these receptors have been shown to confer an invasive cell phenotype (23,32-35) despite no known effects on tumor growth. More recently, CD97 became the first member of the EGF-TM7 family to be directly implicated in cell signaling. Ward *et al* demonstrated heterodimerization and functional synergy between CD97 and lysophosphatidic acid receptor 1 (LPAR1), with signaling mediated by Gα12/13, resulting in increased RHO-GTP levels in both thyroid and prostate cancers and correlating with increased tumor invasion (33,35). These proven roles in invasion and signaling make CD97 an attractive therapeutic target.

2. Structure of CD97

Like all EGF-TM7 receptors, CD97 is composed of an extracellular α-subunit and transmembrane β-subunit. The extracellular portion of the receptor is notable for its large size, nearly 300 amino acids longer than other similar adhesion GPCRs (36) and mediates the interactions with receptor ligands. The extracellular subunit is composed primarily of 5 EGF-like domains (37), which share homology with other members of the EGF-TM7 family (1). The second and third EGF domains contain calcium binding sites, which results in certain ligand interactions that are calcium-dependent (36,38). Within the extracellular subunit there is also a single Arg-Gly-Asp (RGD) motif, which serves as a recognition site for integrin binding (39). CD97 and its isoforms are depicted in Fig. 1. Additionally, there are 8 potential N-glycosylation sites within the extracellular subunit, which can alter the molecular weight of the receptor (36). Although not directly involved in receptor-ligand interactions, the transmembrane β-subunit is important in mediating the dimerization of EGF-TM7 receptors, resulting in the formation of both homo- and heterodimers (21). This subunit also interacts with intracellular proteins such as Gα12/13, which is responsible for mediating CD97 signaling (33,35).

The CD97 gene is located on chromosome 19p13.12-13.2 and contains 18 exons separated by introns ranging in size from 100-2,000 bp, spanning a total distance of ~12 kb (36,40). The extracellular domain of the receptor, encoded by the first 11 exons, contains 433 amino acids and is significantly larger than other adhesion GPCRs. The EGF domains of CD97 likely arose by exon shuffling from a precursor gene of the secretin receptor superfamily (40,41). The seven-span transmembrane domain also exhibits significant homology with members of the secretin receptor family (36). CD97, like other members of the EGF-TM7 family, is characterized by multiple receptor isoforms generated through alternative gene splicing. In CD97, these isoforms vary in the number and arrangement of their extracellular EGF-like domains, resulting in receptors with distinct ligand binding properties (1,4,11,14,42). The isoform containing 5 EGF-like domains binds chondroitin sulfate (4), while the isoform containing 3 EGF-like domains binds CD55, also known as decay accelerating factor, a negative regulator of the complement cascade (11). In the latter case, the third and fourth EGF-like domains are spliced out, resulting in an isoform referred to as EGF(1,2,5), whereas the larger receptor capable of binding chondroitin sulfate is referred to as EGF(1-5). The interaction between CD97 and CD55 is characterized by a low affinity, off-rate (43) and is calcium-dependent. Calcium is believed to provide conformational stability to interdomain linkages, producing EGF domains that are highly resistant to proteolytic cleavage (44). Such properties are ideally suited for cell-cell or cell-ECM interactions, particularly those involved in adhesion, migration or invasion.

The EGF(1,2,5) and EGF(1-5) isoforms are both capable of binding the integrin α5β1 and to a lesser extent αvβ3, but there is a functional difference between these isoforms. In a study assessing the consequence of CD97 binding to human umbilical vein endothelial cells (HUVECs), EGF(1-5) stimulated both migration and invasion, while EGF(1,2,5) stimulated migration only (14). Since treatment with chondroitinase inhibited adhesion, migration and invasion of HUVECs to EGF(1-5), but not EGF(1,2,5), it is likely that coengagement of both an integrin and chondroitin sulfate are responsible for the observed invasive phenotype associated with CD97 (14). These findings are particularly important in tumor biology since these data suggest that the ratio of isoform, rather than total CD97 expression, determines the receptor phenotype.

3. Function and expression of CD97 in immune cells

CD97 has the broadest expression pattern among all EGF-TM7 family members. It is found on lymphocytes, monocytes, granulocytes and smooth muscle (10), as well as all types of macrophages and dendritic cells except microglia (45). Among lymphocytes, CD97 is expressed on T-cells, but rarely on B cells (45). However, during activation of lymphocytes and myeloid cells, expression of the EGF(1,2,5) isoform of CD97 is rapidly upregulated, likely facilitating adhesion and migration of these cells to sites of inflammation (10,36). By virtue of its expression on immune cells, CD97 is detectable in virtually all organs except the parathyroid glands, ovaries, pancreas and skeletal muscle (45).

Given its ubiquitous expression, CD97 is an important mediator of host immune defense. In neutrophils, CD97 plays a critical role in the migration of these cells to active sites of inflammation. Using a mouse model of chemically-induced colitis and *Streptococcus pneumonia*, Lemans *et al* treated animals with a CD97 blocking antibody, resulting in impaired neutrophil migration to both sites and decreased survival in mice with pneumonia (8). Cytokine expression profiles of TNF-α, IL-1β, KC and MIP-2 were similar among treated versus control mice, suggesting that the observed decrease in neutrophil infiltration was not related to other humoral factors. Despite these compelling results, subsequent studies showed that CD97 is not absolutely required for granulocyte migration. Study by Veninga *et al* found that anti-CD97 antibody-mediated depletion of granulocytes only occurred under pro-inflammatory conditions and was mediated by an Fc receptor-dependent mechanism (46). Wang *et al* generated a CD97 null mouse model to explore the function of CD97 in the setting of Listeria-induced inflammation. The authors found that the granulocytes of CD97 null animals were produced in the bone marrow and migrated to sites of inflammation in a manner similar to their wild-type counterparts (47). Unexpectedly, CD97 null mice were significantly more resis-

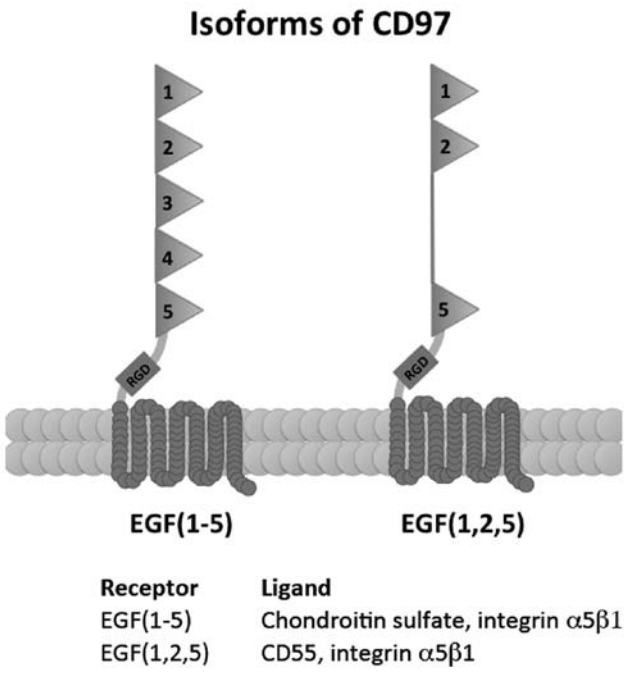

Figure 1. Structure of CD97 and its isoforms. CD97 is an EGF-TM7 adhesion G-protein coupled receptor with large extracellular EGF-like domains, an Arg-Gly-Asp (RGD) motif and seven-span transmembrane segment. Transcript splicing produces isoforms with three to five EGF domains with distinct ligand-binding properties.

tant to Listeriosis than wild-type controls, which the authors attributed to enhanced inflammation-induced granulocytosis. The impaired granulocyte migration in antibody-treated animals was likely due to technical artifacts, such as capture of antibody-bound cells by the reticuloendothelial system or destruction by the complement cascade. Although the authors could not explain the increased granulocytosis is CD97 null animals, the differences were not related to differences in development, migration, half-life or granulocyte-colony stimulating factor (G-CSF) levels. More likely, CD97 contributes to multiple processes involved in granulocyte homeostasis, such that its loss disrupts a delicate balance (47).

The interaction between CD97 and CD55 plays an important role in regulating T-cell activation. CD55, a negative regulator of the complement cascade expressed by cells to prevent complement-mediated destruction, was the first known ligand of CD97 (11). CD55 is broadly expressed among hematopoietic and non-hematopoietic cells, exhibiting highest affinity to the EGF(1,2,5) isoform of CD97 (43). This interaction is calcium-dependent and mediated by the short consensus repeat (SCR) domains of CD55 and EGF-like domains of CD97. CD97 engagement of CD55, combined with CD3 stimulation, is capable of activating $CD4^+$ T-cells, resulting in increased proliferation and cytokine production (48). Additionally, monocyte-associated CD55 is capable of regulating the effector T-cell response through its interaction with CD97 on T-cells, since blockade of either CD97 or CD55 impairs both T-cell proliferation and secretion of IFN-γ (49). Expression of CD55 among epithelial and endothelial cells can be dramatically upregulated by surrounding inflammation (50), providing a unique mechanism by which CD55 aids in the recruitment and activation of T-cells to sites of active inflammation.

In addition to its role in T-cell activation, CD97 may play a role in the interaction of activated T-cells and B lymphocytes. Chondroitin sulfate, a glycosaminoglycan, is a ligand of CD97, specifically the EGF(1-5) isoform. While the first and second EGF-like domains are important in the CD97-CD55 interaction (8,11,12,43), the fourth EGF-like domain is critical for the CD97-chondroitin sulfate interaction (2). The EGF(1-5) isoform binds chondroitin sulfate-B, which is expressed specifically on B cells (4). Binding of CD97 to chondroitin sulfate does not induce B cell activation, immunoglobulin production, or class switching, but is likely involved in the adhesion of activated T-cells and differentiated myeloid cells to B cells. The recruitment of such cells to lymphoid organs or B cell follicles may pay a critical role in B cell homeostasis as well as their maturation, activation and proliferation (51-53). Both CD97 and EMR2 have been shown to bind chondroitin sulfate in the inflamed synovial tissue of patients with rheumatoid arthritis (54), suggesting that it serves to recruit and retain leukocytes in the synovium, thus perpetuating the pro-inflammatory state. Lastly, chondroitin sulfate is an important ligand since it is required for engagement of the integrin α5β1 with the EGF(1-5) isoform, but not EGF(1,2,5) isoform (14).

In addition to its role in host immunity, CD97 has also been linked to several autoimmune diseases. Among patients with rheumatoid arthritis, increased levels of soluble CD97 in synovial fluid, in conjunction with robust CD55 expression on fibroblast-like synoviocytes, suggest a potential mechanism for the interaction between macrophages and synovial tissue in this persistent inflammatory and destructive state (6). Kop *et al* used a collagen-induced model of arthritis is mice to demonstrate that CD97 blockade, via a neutralizing antibody, reduced arthritis-related joint damage and inflammation (55). Using a similar model of arthritis induction, Hoek *et al* showed that mice lacking CD97 and CD55 both displayed decreased arthritis compared to wild-type controls (56). CD97 has also been implicated in the demyelinating lesions of multiple sclerosis. The receptor is absent in normal white matter, although adjacent endothelium expresses low basal levels of CD55. In multiple sclerosis plaques, there is increased CD55 in the endothelium as well as robust CD97 expression on infiltrating macrophages, suggesting a mechanism for immune cell migration and activation across the blood-brain barrier (57). Interestingly, the same study found elevated levels of soluble CD97 in the serum, but not cerebrospinal fluid (CSF), of multiple sclerosis patients.

Outside of its role in immune cells, CD97 has been shown to play an important role in intercellular interactions. Within enterocytes, CD97 is an important mediator of E-cadherin-based adherens junctions, thus playing an important role in maintaining the integrity of the intestinal epithelium (58). Becker *et al* showed that CD97 participates in regulating the localization and stability of β-catenin, which is critical for the maintaining adherens junctions (58). The cellular distribution of β-catenin is important since it can translocate into the nucleus and induce expression of pro-oncogenic genes (59). In the normal state, β-catenin is targeted for destruction by glycogen synthase kinase-3β (GSK-3β), but in the setting of certain of neoplastic pathology GSK-3β is inhibited, thus increasing cytosolic β-catenin and increasing the transcription of pro-oncogenic genes (60). Becker *et al* showed that

Table I. Summary of CD97 in the non-pathologic state.

Receptor feature	Leukocyte-associated expression or function	Refs.
Expression	Expressed on lymphocytes, monocytes, granulocytes and smooth muscle	(10)
	EGF(1,2,5) rapidly upregulated during lymphocyte and myeloid cell activation	(10,36)
	Increased CD97 expression on infiltrating macrophages in multiple sclerosis plaques	(57)
Ligands	Binds CD55, a negative regulator of the complement system, with highest affinity to the EGF(1,2,5) isoform	(11,43)
	EGF(1-5) isoform binds chondroitin sulfate in the inflamed synovial tissue of patients with rheumatoid arthritis	(4,54)
	Chondroitin sulfate required for engagement of integrin α5β1 with EGF(1-5), but not EGF(1,2,5)	(14)
Function	Engagement with CD55, along with CD3 stimulation, can activate $CD4^+$ T-cells	(48)
	CD97 null mice resistant to Listeriosis due to enhanced inflammation-induced granulocytosis	(47)
	Mice lacking CD97 and CD55 had decreased arthritis compared to controls	(56)
	Mediator of E-cadherin-based adherens junctions in enterocytes, maintaining integrity of intestinal epithelium	(58)

in addition to increasing the strength of enterocyte adherens junctions, overexpression of CD97 upregulated membrane-bound, but not cytoplasmic or nuclear β-catenin and decreased the amount of β-catenin targeted for degradation (58). This effect was associated with activation of Akt and subsequent inactivation of GSK-3β, suggesting that these proteins regulate the stability of β-catenin through CD97. These findings, when combined with evidence implicating Akt in the formation of E-cadherin-based adherens junctions (60), provide further evidence implicating dual roles for CD97 as a mediator of adhesion and cell signaling.

4. Function and expression of CD97 in cancer

Adhesion and migration are normal cellular processes mediated by a variety of receptors and cell surface ligands, including CD97. Migration through the ECM is an important component of normal cell function, however invasion represents a more aggressive process that involves both destruction of the ECM and dramatic changes in cell shape as it moves through the matrix. CD97 plays an important role in the adhesion and migration of normal immune cells, but is also a mediator of invasion in a variety of human cancers. The first report identifying CD97 in cancer came through an analysis of thyroid carcinoma. The authors found no expression of CD97 in normal thyroid, little to no expression in differentiated papillary or follicular thyroid carcinomas and high expression in undifferentiated anaplastic thyroid carcinomas and associated metastatic lesions (25). CD97 expression was lowest in tumors classified as T1 and highest in those classified as T4. These findings were corroborated in a study limited to medullary thyroid carcinoma (MTC), which found robust expression across all tumor samples compared to normal thyroid tissue, with increased CD97 expression in T3/4 tumors compared to T1/2 lesions (61). All MTC samples also expressed CD55, however there was no correlation between CD55 expression tumor stage (Table I).

Several groups have characterized a relationship between CD97 expression and the tumor's leading edge. Aust *et al* found CD97 expression in a high percentage of gastric, pancreatic and esophageal carcinomas (26). Interestingly, in over half of the gastric cancers analyzed, tumor cells at the invasive front exhibited stronger CD97 staining than those in solid formations. A similar expression pattern has been observed in colorectal carcinoma, with high CD97 expression at the invasive front, as well as an association with poor clinical stage and increased lymphatic invasion (28). In a study of rectal adenocarcinoma, patients with recurrent or metastatic disease had significantly higher CD97 expression at the invasion front compared to patients without recurrence or metastasis (62). Additionally, using a multivariate model, the authors also showed that lymph node involvement and strong CD97 expression at the invasive front were the only variables significantly associated with poor survival.

In addition to localizing CD97 to the leading edge, a number of studies have characterized its expression with respect to CD55. In a study of >130 patients with carcinomas of the gallbladder, CD97 and its ligand CD55 were found in 70 and 65% of specimens, respectively, mainly at the invasive front (63). Furthermore, the authors found CD97 and CD55 to correlate with histologic grade, advanced pathological T stage, clinical stage and venous or lymphatic invasion. There was no association with patient age, gender or presence of nodal or distal metastasis. Using a multivariate model, only clinical stage and CD97/CD55 expression were associated with overall survival. In a sample of nearly 80 human oral squamous cell carcinomas, CD97 transcript and protein were increased in pathologic stage T3 and T4 tumors, as well as histologic grade 3 and 4, but weakly expressed in pathologic stage T1/T2 and histologic grade 1/2 tumors (64). CD55 expression was low in normal oral mucosa, but all oral squamous cell carcinoma samples, regardless of stage or grade, exhibited strong CD55 gene expression and immunostaining (Table II).

Table II. Summary of CD97 in cancer.

Tumor feature	Findings	Refs.
Expression	Expressed on 44/50 gastric, 14/18 pancreatic, 10/13 esophageal and 11/12 undifferentiated thyroid carcinomas	(25)
	Expressed in 18/18 colorectal carcinoma cell lines and 75/81 patient-derived colorectal carcinomas	(28)
	Among 78 oral squamous cell carcinomas (OSCC), CD97 expression weak in T1/T2 and G1/2 tumors but strong in T3/4 and G3/4 lesions All OSCCs express CD55	(64)
	Expressed in 3 glioblastoma cell lines and 5/5 low passage primary glioblastoma cell cultures	(71)
	CD97 expression in human thyroid cancer correlates with lysophosphatidic acid receptor, Ki67, and pAKT	(33)
Localization	In >50% of gastric tumors, CD97 expression is highest at the invasive front or leading edge	(26)
	In colorectal adenocarcinoma, CD97 staining strongest among cells at the invasive front	(28
	CD97 expression correlates with depth of invasion and TNM staging in gastric carcinoma	(27)
Isoform expression	EGF(1,2,5) associated with increased secretion of MMPs and IL-8 in fibrosarcoma cell lines	(23)
	EGF(1,2,5) promotes local growth and metastatic spread of gastric carcinoma	(34)
Invasion	CD97 associated with invasive phenotype in colorectal carcinoma cell lines	(28)
	Knockdown of CD97 decreases invasion and migration in glioblastoma cell lines	(71)
	Transgenic expression of CD97 increases vascular invasion and lung metastasis in an animal model of thyroid follicular cell carcinoma	(33)
Angiogenesis	CD97 promotes angiogenesis by serving as a chemoattractant for HUVECs and binding integrins α5β1 and αvβ3	(14)
Downstream signaling	CD97 heterodimerizes with lysophosphatidic acid receptor 1 (LPAR1) and signals through Gα12/13 to increase RHO-GTP levels in prostate carcinoma cell lines	(35)
	Expression of transgenic CD97 leads to upregulated ERK phosphorylation and increased Ki67-positive cells in developing thyroid tumors	(33)
Clinical correlations	High CD97 correlated with poor clinical staging and increased lymphatic invasion in colorectal carcinoma	(28)
	Increased expression of CD97 and CD55 at the invasive front of rectal adenocarcinoma correlates with tumor recurrence and metastasis	(62)
	In gallbladder carcinoma CD97 and CD55 correlate with histologic grade, advanced pathologic stage, clinical stage, venous or lymphatic invasion and overall survival	(63)
	Glioblastomas with upregulation of CD97 are associated with significantly worse overall survival compared to those with downregulation of CD97	(71)
Expression modulation	TGF-β decreases CD97 in colorectal carcinoma cell lines	(28)
	Sodium butyrate and retinoic acid decrease CD97 expression in OSCC cell lines	(64)
	Troglitazone induces redifferentiation and decreases CD97 expression in thyroid cancer cell lines	(78)

Given the observed co-localization with CD97, CD55 appears to play an important role in the malignant state. CD55 is known to promote cancer growth through a variety of mechanisms including decreased complement-mediated lysis, inhibition of NK cells, phosphorylation of src-kinases, increased cell migration and tumor invasion via its interactions with CD97 (65). Given its diverse functional repertoire, some have even considered CD55 as a viable target for immunotherapy (66). Previous studies have shown that HUVECs and various cancer cell lines (cervical, osteosarcoma, colorectal) deposit CD55 into surrounding ECM at levels proportionate to their surface expression (52,67). The mechanism by which these cells deposit CD55 is not well understood, but may involve direct secretion, flipping of the cell membrane, or covalent linkage to ECM proteins before export from the cell (50,68). Additionally, CD55 expression on HUVECs can be upregu-

lated in the presence of either VEGF or tumor-conditioned media, thereby promoting angiogenesis (50). This may explain data suggesting that elevated CD55 expression is a predictor of more rapid tumor progression (69). Lastly, it is important to note that the CD97-CD55 interaction is dependent upon the tumor cells expressing the proper isoform of CD97, as well as the appropriate EGF domain glycosylation pattern, which is critical for epitope accessibility and ligand binding (70).

The role of CD97 isoforms in invasion is an active area of investigation. Studies have shown CD97 to confer an invasive cell phenotype in both glioblastoma (32,71) and colorectal carcinoma (28), although its expression does not appear to affect tumor cell proliferation in these tumors. Our group recently reported on the role of CD97 in glioblastoma (71). CD97 confers increased migration and invasion, but has no effect on cell proliferation. Using The Cancer Genome Atlas (TCGA) database, we found that patients whose tumors overexpress CD97 had significantly shorter survival than those with downregulated CD97. We are particularly interested in brain tumor initiating cells (BTICs), which like other cancer stem cells have the capacity for both self-renewal and differentiation. Our preliminary data suggest that CD97 is expressed in both BTICs and differentiated GBMs cultured from patients. Liu *et al* used gastric carcinoma as a model to study isoform-dependent migration. Earlier studies showed that CD97 correlated with both depth of invasion and TNM stage in human gastric carcinoma (27). Liu *et al* used the SGC-7901 gastric adenocarcinoma cell line, which expresses the EGF(1,2,5) isoform and found that they had increased migration and invasion compared to cells subjected to siRNA knockdown of CD97 (34). Furthermore, the cells with decreased EGF(1,2,5) exhibited a preference for aggregated growth rather than a pattern of detachment from the main tumor and disseminated growth. Using a mouse orthotopic gastric cancer model, the authors showed that tumors lacking the EGF(1,2,5) protein were smaller in size and had fewer lymph node metastases compared to controls. These findings, compared with data from HT-1080 cells, suggest that the EGF(1,2,5) isoform is an important mediator of tumor invasion. However, there is compelling immunohistochemical data suggesting that the EGF(1-5) isoform, by virtue of its interactions with CD55, is also an important mediator of this process. Future studies may show that the ratio of CD97 isoforms, rather than total amount, contributes to cell phenotype.

The function of CD97 in malignant cells has been well studied. Using the HT-1080 human fibrosarcoma cell line, Galle *et al* generated clones expressing the isoforms of CD97, EGF(1-5) and EGF(1,2,5). Using subcutaneous tumors in SCID mice, the authors found that overexpression of the EGF(1,2,5) isoform increased growth velocity compared to the EGF(1-5) isoform, but there was no significant difference in the doubling times (23). Interestingly, tumors expressing EGF(1-5) had increased microvessel density compared to EGF(1,2,5), leading authors to believe that the relatively faster growth velocity in the EGF(1,2,5) isoform could not be explained by increased vascularity (23). In the same study, tumor cells expressing the EGF(1,2,5) isoform were shown to express the highest levels of matrix metalloproteinases (MMPs), which serve as key mediators of tumor invasion; stimulation with TNF-α increased secreted MMP9 and pro-MP2. The EGF(1,2,5) isoform also resulted in increased basal levels of IL-8 secretion compared to the EGF(1-5) isoform. IL-8 contributes to cancer progression through its function as a mitogenic factor and has been shown to increase during tumor progression (72,73). Although the mechanism of increased IL-8 and MMPs is not entirely understood, cells expressing the EGF(1,2,5) isoform lacking a TM7 domain also secreted increased levels of IL-8 compared to the EGF(1-5) isoform, suggesting that the extracellular domain is mediating the observed upregulation of IL-8 and MMPs.

The regulation of CD97 expression in neoplastic cells is not entirely understood. Studies performed *in vitro* showed that cell density was an important regulator of CD97; cells grown in confluent layers expressed less CD97 compared to those grown in single cell cultures (23). Given the role of CD97 in regulating the localization and stability of β-catenin, some questioned whether CD97 was a target of β-catenin regulated by the Wnt pathway; however *in vitro* experiments using colorectal cancer cell lines showed that CD97 was regulated independently of β-catenin and is not a direct target of the Wnt pathway (74). The downstream targets of CD97, as well as other members of the EGF-TM7 family, remain largely uncharacterized. However, CD97 was recently shown to signal through Gα12/13 in prostate cancer cells, leading to increased levels of RHO-GTP (35). In the same study, the authors found that CD97 was able to heterodimerize with lysophosphatidic acid receptor 1 (LPAR1), a GPCR that has been previously implicated in prostate, ovarian and breast cancers, producing a synergistic effect and LPA-dependent RHO signaling (75-77). Perhaps most interestingly, by serving as as a chemoattractant for HUVECs, CD97 has been shown to promote angiogenesis though binding of the pro-angiogenic integrins α5β1 and to a lesser extent, αvβ3 (14). This function is dependent on the EGF(1-5) isoform, requiring coengagement of both an integrin and chondroitin sulfate to promote angiogenesis and further stressing the presence of isoform-dependent phenotypes (14).

Given its role in tumor invasion and angiogenesis, CD97 is becoming an attractive therapeutic target. Blocking antibodies have produced moderate success in neutralizing CD97 in inflammatory conditions, but they have not yet been tested in a tumor model. Several groups have identified pharmacologic agents capable of modulating CD97 expression. Transforming growth factor-β (TGF-β) decreased CD97 expression in certain TGF-β-sensitive colorectal carcinoma cell lines, but also decreased their proliferative rates (28). Sodium butyrate and retinoic acid were shown to decrease CD97 expression in oral squamous cell carcinomas, however, they simultaneously increased CD55 (64). Lastly, troglitazone, a peroxisome proliferator-activated receptor-γ (PPARγ) agonist, has been shown to downregulate CD97 and induce redifferentiation in thyroid cancer cell lines (78). Whether these agents are capable of decreasing CD97 expression in tumors *in vivo* remains to be seen, but represents an exciting area of future investigation.

5. Conclusions

The association of CD97 with human cancers poses many interesting questions. By what mechanisms are these receptors upregulated in diseased states? What is the relationship between their expression in differentiated tumor cells compared to

cancer stem cells? Given the physical separation of the α and β subunits, along with evidence suggesting a dual role for these receptors, one must ponder the importance of isoform expression, or whether isoform-ratios are important in determining phenotype. The potential for these receptors to participate in both adhesion and signaling also makes them attractive therapeutic targets. Will therapies targeting these markers provide a clinically relevant response? Given the presence of EGF-TM7 family members on the surface of normal leukocytes, one may assume that the host immune system will have difficulty mounting an effective response against these markers, making them ideal for cancer cells attempting to avoid immune detection. Advances in structural and molecular biology, genome wide analyses such as TCGA and an increased awareness of the EGF-TM7 family will shed new light on these markers and aid in the development of targeted therapeutic interventions with improved outcomes for patients.

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