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CD157 at the intersection between leukocyte trafficking and epithelial ovarian cancer invasion

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1. ABSTRACT

CD157 is a member of the ADP-ribosyl cyclase gene family that is involved in the metabolism of NAD. CD157 behaves both as an ectoenzyme and as a receptor. Though CD157 is anchored to the membrane by a glycosylphosphatidylinositol moiety, which makes it unsuitable to transduce signals on its own, it exploits its localization in selected membrane microdomains and its proclivity to interact with integrins to accomplish receptor functions. Initially characterized as a stromal and myeloid antigen involved in the control of leukocyte adhesion, migration and diapedesis, CD157 was subsequently found to have a far wider distribution. In particular, CD157 was found to be expressed by epithelial ovarian cancer cells where it is involved in interactions among tumor cells, extracellular matrix proteins and mesothelium. The overall picture inferred from experimental and clinical observations is that CD157 is a critical player both in leukocyte trafficking and in ovarian cancer invasion and metastasis formation. In this review, we will discuss the biological mechanisms underpinning the role of CD157 in the control of leukocyte migration and ovarian cancer dissemination.

2. INTRODUCTION

Leukocytes are likely the best-adapted cells at migrating to different districts of our body through various microenvironments. Their effective migration is due to the ability to adapt their behavior according to the type of tissue they encounter. Increasing evidence indicates that cancer cells also use similar mechanisms to spread through the body albeit with significant differences (1). We have learned much about how both leukocytes and cancer cells move through the body and we realized that several key molecules that have a documented role in leukocyte migration through tissues also participate in cancer invasion. For example, integrins and proteases (mainly matrix metalloproteinases) are usually necessary for both leukocytes and cancer cells to traverse intact epithelial basement membranes. In addition to these “main actors”, many other molecules make a fundamental contribution to coordinate and fine-tune the whole process according to the particular circumstances.

Our group has spent the last several years assessing the role of CD157 in the human innate and adaptive immune response and has come to the conclusion that CD157 plays important roles in the control of migration and diapedesis of neutrophils and monocytes (2, 3). Exploiting this knowledge and other independent evidence indicating that CD157 has a wider distribution than originally thought, we demonstrated that CD157 is not only a myeloid marker, since it is also expressed by ovarian cancer epithelial cells where it is involved in the control of tumor cell migration and invasion.

3. CD157 AND THE ADPRC GENE FAMILY

In the early 1990’s, BST-1 was described as a surface antigen expressed by human bone marrow stromal cells (4), not knowing that BST-1 was identical to the antigen Mo5 identified a decade earlier in the myeloid lineage (5). In the VI Workshop on Differentiation Antigens, BST-1 and Mo5 were univocally designated as CD157 (5).

Human CD157 is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein encoded by a member of the NADase/adenosine diphosphate (ADP)-ribosyl cyclase (ADPRC) gene family, which also includes CD38 (6, 7). CD157 and CD38 behave both as ectoenzymes and receptors (8, 9). Their expression overlaps in a limited number of tissues and discrete lineage-specific differentiation stages, whereas it differs in most tissues, including the hematopoietic system. Comparative analysis of *CD38* and *CD157* genes showed a remarkable conservation between their intron-exon structures and those of the

Aplysia ADPRC gene, indicating that they originate from a common ancestral gene (10).

3.1. CD157 Gene

The human *BST-1/CD157* gene was assigned to chromosome 4p15, very close to that of its paralogue *CD38* (10). The murine *CD157* and *CD38* genes have a similar organization on chromosome 5 (in a region syntenic with human chromosome 4) (11, 12). The human *CD157* gene spans ~27 Kb and consists of nine exons and eight introns. Exon 1 encodes the 5'-untranslated region, the signal peptide and the first N-terminal 32 amino acids. Exon 9 encodes a short hydrophobic peptide which signals the attachment of a GPI moiety (pro-peptide), and the 3'-untranslated region. The genomic organization of exons 2-8 of the *CD157* gene encoding the rest of the extracellular region is identical to that of exons 2–8 of *CD38*: the introns have almost the same length and the exons code for the same amino acid number. Exons 5–8 of *CD38*, *CD157* and *Aplysia ADPRC* genes are quite similar. A difference was observed in exon 4 of *Aplysia ADPRC*, which in the *CD157* and *CD38* genes is split into two smaller exons, corresponding to exons 3 and 4 (6, 7). These observations support the view that the three genes have gone through the same evolutionary path, and that *CD38* and *CD157* originated through gene duplication before the divergence of mammals and amphibians (13).

The *BST-1/CD157* gene has been associated with Parkinson's disease. Indeed, *BST-1* single nucleotide polimorphysms (SNPs) rs11931532, rs12645693, rs4698412 and rs4538475 were identified as risk factors in a sporadic late-onset Parkinson's disease genome-wide association (GWA) study in the Japanese population (14). The association between *BST-1/CD157* rs4698412 SNP and Parkinson's disease was confirmed in the European population (15), but not in the Northern Han Chinese population (16). A meta-analysis of GWA studies performed on a North American and a European population identified an association between the *BST-1* locus and Parkinson's disease (17). A plausible interpretation of these apparently discordant results is that ethnicity significantly influences the association between the *BST-1* locus and Parkinson's disease.

3.2. CD157 protein structure

The CD157, CD38 and *Aplysia* cyclase polypeptides share a central core with 25-30% amino acid sequence similarity (10, 18, 19). The determinants of their diverse protein topology reside instead in the N- and C-terminal modifications. Being a soluble protein, the *Aplysia* cyclase has an N-terminal signal peptide. A longer form of this hydrophobic region is found in CD38, accounting for its being type II membrane protein, whereas CD157 is GPI-anchored, by virtue of the presence of a ninth exon encoding the hydrophobic signal for GPI attachment. Thus, changes in *BST-1*/*CD157* exon 9 and *CD38* exon 1 are responsible for membrane attachment of the mammalian ADPRCs.

Human CD157 is a single chain cell surface glycoprotein of 318 amino acids including signal sequences (4) with a molecular mass of 42–45 kDa (4, 20), including N-linked glycosylation which accounts for ~12% of the total mass of the protein (21). The CD157 amino acid sequence encompasses a short C-terminal hydrophobic region, located closer to the membrane and a distal NH_2-terminal region, which includes the catalytic domain (22). CD157 is synthesized as a 41 kDa precursor, subsequently converted to the mature glycoprotein, when the signal sequence at its COOH-terminus is cleaved off in the endoplasmic reticulum and replaced by the GPI moiety (23). As most GPI-linked molecules, CD157 exists in a soluble form (sCD157), and high levels of sCD157 have been reported in chronic inflammatory diseases, such as rheumatoid arthritis (20).

The sequence alignment of CD38, CD157 and *Aplysia* cyclase proteins revealed the presence of 10 conserved cysteine residues located in the extracellular domain of CD38 and CD157, indicating that the three molecules have common features in their tertiary structures (11, 24). In addition to the cysteine motif, there is a highly conserved 18 amino acids sequence, which is presumed to be the binding site for cADPR. Cys160, Cys173, Cys119 and Cys201 are crucial residues for the synthesis and hydrolysis of cADPR by CD38 (25); among these, only Cys160 and Cys173 are conserved in CD157. The C-terminal part of CD38, including the Cys275 which contributes to the NAD^+ glycohydrolase activity (26), is conserved in CD157. Furthermore, the CD157 sequence includes a number of leucine residues, potentially forming leucine zipper motifs and allowing consequent homotypic and heterotypic associations. More details on CD157 protein structure are reported in (27).

3.3. CD157 enzymatic functions

Similarly to CD38, CD157 metabolizes NAD^+ to produce cADPR and subsequently ADPR, indicating that it is endowed with both ADP-ribosyl cyclase and cADPR hydrolase activities (21, 28). However, CD157 is a much less efficient cyclase than CD38 (29), its enzymatic activities are pH-dependent and require metal ions: the addition of Zn^{2+} and Mn^{2+} remarkably increases both the cyclase and hydrolase activities (21). In contrast, Cu^{2+} shows inhibitory effects on both catalytic activities of CD157 (21, 30).

The ability of CD38 and CD157 to synthesize cADPR strongly suggests their involvement in the control of cytosolic Ca^{2+} homeostasis (31). Indeed, cADPR is a potent Ca^{2+}-releasing agent acting through ryanodine-sensitive, inositol 1,4,5-trisphosphate (IP3)-independent intracellular stores involved in a number of biological functions both in human and mouse (31). CD157-mediated biological functions include lymphocyte proliferation (32, 33), cardiac and intestinal longitudinal muscle contraction (34) (35), activation of airway smooth muscle cells (36), inhibition of the cardiomyogenesis of mouse embryonic stem cells (37), glucoseinduced
insulin release in the endocrine pancreas (38, 39), and regulation of renal hemodynamics and excretory function in mice (40).

The products of NAD metabolism generated by CD157 can also act as extracellular immunomodifiers. For example, the concentrative uptake of cADPR generated by CD157-positive stromal cells stimulates proliferation of human hematopoietic progenitors (41). Moreover, emerging data indicate that cADPR and ADPR may operate outside the cell as paracrine factors. In a mouse model, calorie restriction was found to increase the expression of CD157 in Paneth cells. CD157 catalyses the extracellular production of cADPR, which can act in a paracrine manner to induce self-renewal of intestinal stem cells, suggesting that cADPR may have an application in improving intestinal regeneration and function in patients (42).

3.4. CD157 tissue distribution

Originally identified as a myeloid cell differentiation antigen (5) and as a bone marrow stromal cell antigen (BST-1) (21), human CD157 has been found expressed by several other cell types and tissues (43) summarized in Table 1.

4. ROLE OF CD157 IN LEUKOCYTE TRAFFICKING

A fundamental requirement of any immune response is the migration of leukocytes from one site in the body to another to exert effector functions. Therefore, elucidating the molecular mechanisms underlying the migration of leukocytes from the blood to tissues and back is critical to our understanding of the immune response during inflammation and for the design of effective targeted therapies. The main steps of leucocyte trafficking involve leucocyte tethering and rolling on vessel walls of the vasculature, followed by firm adhesion to the endothelium and successive extravasation. This multistep process is choreographed by an increasing number of molecular interactions and by complex array of soluble factors in combination with the local intravascular and extracellular environment (44).

4.1. Role of CD157 in leukocyte adhesion, migration and diapedesis

The findings that CD157 i) is constitutively expressed by neutrophils, monocytes (5) and vascular endothelial cells (45), ii) is upregulated by soluble mediators of inflammation (46), and iii) undergoes redistribution and membrane compartmentalization in the presence of proinflammatory cytokines (47), fostered the hypothesis that it could be a crucial player in the control of leukocyte migration and transmigration during the inflammatory response.

4.1.1. Leukocyte adhesion

Adhesion of neutrophils and monocytes to ECM proteins - an essential step for their migration across blood vessels and subsequently into the stroma of inflamed tissues - is regulated by a wide variety of cell surface protein families, the most relevant of which belong to the β1 and β2 integrin subfamilies. The contribution of CD157 in regulating the adhesion of neutrophils and monocytes to fibronectin and fibrinogen (*i.e.* the main ligands of β1 and β2 integrin, respectively) was assessed by performing conventional *in vitro* experiments in the presence (or in the absence) of anti-CD157 monoclonal antibodies (mAbs). Ligation of CD157 by $F(ab')_2$ fragments of blocking mAb (able to bind two CD157 molecules) significantly reduced neutrophil and monocyte adhesion to both fibronectin and fibrinogen; moreover, combined blocking of CD157 and β1 or β2 integrin resulted in a more effective inhibition (47). Strikingly, CD157 clustering induced by ligation through blocking mAb, followed by cross-linking with a secondary antibody reversed the block and virtually completely restored leukocyte adhesion (3). Overall, these data suggested the existence of a functional interplay between CD157 and both β1 and β2 integrin governing critical steps of leukocyte trafficking and strengthened the hypothesis of a role of CD157 in signal transduction (2).

4.1.2. Leukocyte migration and transmigration

CD157 regulates neutrophil and monocyte chemotaxis stimulated by fMLP and MCP-1 chemoattractant factors, respectively. Indeed, ligation of CD157 by blocking mAb resulted in impaired directional migration of both neutrophils and monocytes in conventional *in vitro* assays (3, 47).

A central aspect of leukocyte trafficking is the continuous transition from the blood circulation into tissues and vice versa. This implies that leukocytes must repeatedly cross the vascular endothelium, which is located at the interface between the two compartments. The constitutive expression of CD157 both in leukocytes and vascular endothelial cells (mainly at interendothelial junctions) (2) was highly suggestive of its potential involvement in transendothelial migration. The potential role of CD157 in regulating leukocyte transmigration was explored *in vitro* using primary neutrophils or monocytes treated (or untreated) with an anti-CD157 blocking mAb. The results demonstrated that neutrophils treated with anti-CD157 mAb were able to adhere to the apical surface of the endothelium, but showed a prolonged and disoriented motility over the endothelial cell layer, which eventually resulted in a clearly impaired ability to cross the endothelium (46). Comparable results were obtained on monocytes, where CD157 ligation by a blocking mAb resulted in dose-dependent inhibition of transmigration across resting as well as activated endothelial cell monolayers. To mimic the extent of CD157 clustering likely occurring *in vivo* upon binding with a specific non-substrate ligand (currently unknown), monocytes were treated with anti-CD157 mAb, then, prior to their addition to the endothelium, crosslinking of CD157 was induced by means of an anti-mouse IgG antibody. Antibody-induced clustering of CD157 overcome the block and restored monocyte transmigration (3). These data suggest that *in vivo* clustering of CD157 might function as a switch that controls transmigration by eliciting intracellular signals.

4.2. Functional, structural and molecular interactions between CD157 and integrins

CD157 lacks a cytoplasmic domain, therefore, it is unsuitable to transduce signals by its own. To overthrow this structural limitation, CD157 exploits its lateral mobility to establish functional interactions with other receptors. The existence of a functional interplay between CD157 and integrins ruling leukocyte adhesion to ECM suggested that integrins could be the partners of choice for CD157 to carry out its receptor functions. Immunolocalization and co-capping experiments in leukocytes showed that CD157 and integrins appear to closely associate spatially, as ligand-induced clustering of β2 integrin (CD18) or β1 integrin (CD29) caused colocalization
with CD157 and, vice versa, fibronectin-induced clustering of integrins caused co-localization of CD157 in living cells (3, 47). Moreover, co-immunoprecipitation experiments demonstrated that i) CD157 and CD11b/CD18 are spatially juxtaposed and are actually physically bound to one another in neutrophils (48), and ii) CD157 is structurally associated with both β1(CD29) and β2 (CD18) integrins in monocytes (3).

GPI-anchored proteins are usually concentrated in lipid rafts, which are microdomains within the plasma membrane enriched in glycosphingolipids, cholesterol and several signaling elements (49). Lipid rafts are thought to provide the optimal milieu to bring discrete receptors and downstream intermediates into close proximity, thereby favoring the formation of signalingcompetent membrane domains (50). The GPI-mediated anchorage to the membrane, suggested that CD157 could fulfill receptor functions in the context of membrane microdomains, as part of multimolecular complexes. As predicted, CD157 proved to be virtually entirely located within lipid rafts in neutrophil and monocyte cell membrane. CD157 clustering by means of specific antibodies caused β1/CD29 and β2/CD18 integrins to translocate into lipid rafts resulting in subsequent activation of downstream signaling pathways (3).The analysis of the phosphorylation status of selected core components of the integrin-mediated signaling cascade highlighted that CD157-mediated intracellular signaling relies on integrin/FAK/Src, leading to increased activity of downstream MAPK/ERK1/2 and PI3K/Akt pathways. This dynamic interplay between CD157 and integrins implies a close

functional cross-talk that is instrumental to the control of leukocyte adhesion to ECM proteins, migration and diapedesis. Indeed, concurrent engagement of CD157 and integrins promoted increased tyrosine kinase receptor phosphorylation and PI3K and MAPK signaling cascade activation, warranting optimal leukocyte transmigration (3). These findings confirm that CD157 is part of a multimolecular complex ruled by integrins orchestrating leukocyte transendothelial migration and adhesion to ECM proteins (Fig.1)

5. ROLE OF CD157 IN OVARIAN CANCER

Ovarian cancer is the most lethal of gynecological malignancies and has an extremely poor prognosis, primarily due to asymptomatic presentation of the disease, which makes early diagnosis extremely difficult. Although ovarian cancer may arise from all cell types composing the ovary, most ovarian carcinomas arise from the surface epithelium that covers the ovary and lines postovulatory inclusion cysts: they are defined epithelial ovarian cancers (EOC). The most common histological types of EOC are serous, endometrioid, clear-cell and mucinous. Whatever the site of origin, ovarian cancer progresses with a peculiar modality: cells shed from the tumor as single cells or multicellular aggregates are passively transported by the peritoneal fluid into the peritoneal cavity. Cells cable of escaping apoptosis attach to the mesothelium, giving origin to metastases (Figure 2A). This 'seeding' of the peritoneal cavity is frequently associated with the formation of ascites, and is the most widely recognized behavior of ovarian carcinoma, particularly serous carcinoma. Unlike most malignancies, ovarian cancers rarely metastasize through the hematogenous route until advanced stages (51). The high mortality associated with ovarian cancer underlines the urgent need to identify specific markers useful for the early diagnosis and for the control of neoplastic progression.
A huge number of molecules involved in ovarian cancer cell migration and invasion have been described, many of which were also found involved in crucial steps of leukocyte migration, highlighting the existence of remarkable similarities between metastatic dissemination and leukocyte trafficking. The list of these molecules includes a heterogeneous group of membrane proteins overall defined "ectoenzymes" as they are characterized by having the catalytic site facing the extracellular environment. One of these ectoenzymes is CD157 (52).

5.1. CD157 expression and clinical significance

First evidence of the expression of CD157 in ovarian epithelial cells came from microarray analysis which indicated that *CD157* was included in the panel of genes upregulated in cells derived from primary ovarian cancers compared to cells derived from normal ovarian epithelium (53). This observation paved the way for a more detailed study that led us to demonstrate that CD157 is expressed by the majority of ovarian cancer tissues and primary cultures, and by selected ovarian cancer cell lines. The expression of CD157 is modulated during tumor progression: cells from primary tumors express high levels of CD157 (Figure 2B, left panels), whereas single cells or spheroids detached from the primary tumor express low levels of CD157, mainly confined to cells located at the periphery of the spheroid (Figure 2B, middle panels). However, CD157 expression is completely restored when spheroids are allowed to adhere to a substrate (Figure 2B, right panels) (54). This observation suggests that CD157 is turned-off when tumor cells move passively, and turned-on when they need to migrate through tissues. An alternative hypothesis is that the expression of CD157 is modulated on some cells by unknown microenvironmental signals, this could affect the interaction between CD157 and integrins on neighboring cells leading to the detachment of cells from the tumor mass. This intriguing hypothesis deserves future investigation.
Immunohistochemical staining of tissues from a cohort of 88 patients with known clinical history and follow-up demonstrated that CD157 is expressed by 93% of ovarian cancer tissues at variable expression levels and with different distribution pattern, including membrane and cytoplasmic localization with granular, dot-like, apical or perinuclear staining. In non-neoplastic epithelium adjacent to the tumor, CD157 localizes at the basolateral surface and at the intercellular boundaries (54). Semiquantitative evaluation of CD157 expression determined by histological score (HS), showed a significant correlation with patient outcome. Indeed, CD157 expression at or above the median H-score (HS = 60) was significantly associated with rapid tumor relapse: patients with tumors that expressed high CD157 had a poorer disease-free survival than those with low CD157. Association between high CD157 expression and reduced overall survival of patients showed a clear trend, even though it did not reach statistical significance in the analysed cohort. Furthermore, CD157 correlated with both disease-free and overall survival in patients with the serous ovarian cancer subtype, which accounts for more than 70% of ovarian cancer cases, and is the most aggressive histotype. Multivariable analysis with the Cox proportional hazard model confirmed that CD157 is an independent prognostic marker of reduced disease-free survival in patients with ovarian cancer, indicating that high CD157 expression is associated with higher risk of rapid tumor relapse after surgical resection of the tumor. Moreover, in patients with serous EOC, CD157 HS above the median value proved to be an independent predictive variable of both disease recurrence and reduced survival (54).

5.2. CD157 controls ovarian cancer cell migration and invasion

The ability to proliferate and migrate are physiological features of the epithelial cells of the ovary, which accompany ovulation (55). During ovarian cancer oncogenesis, epithelial cells exploit their inherent ability to migrate, lose any control mechanisms and acquire the ability to activate autocrine loops of growth signals that fuel tumor progression. Complex molecular networks relying on specific cell-cell and cell-matrix interactions drive ovarian cancer cell migration and dissemination.
As in neutrophils and monocytes, CD157 expressed by ovarian cancer cells controls adhesion to and migration through ECM proteins. This conclusion was inferred from the observations that ligation of CD157 by means of blocking mAb: i) reduced adhesion of CD157-positive primary ovarian cancer cells and cell lines to fibronectin, laminin and collagen type I, which represent the main components of the submesothelial basal lamina (56), and ii) inhibited tumor cell migration and invasion through ECM proteins (54).
To understand the direct contribution of CD157 in ovarian cancer migration and invasion, we analysed the effects of stable overexpression and knockdown of CD157 in selected cell lines. Using these tools in conventional *in vitro* assays, we demonstrated that exogenous expression of CD157 in CD157-negative OVCAR-3 cells and overexpression in OV-90 cells (showing low basal levels of CD157) consistently increased cell motility. Conversely, short hairpin (sh)RNA-mediated CD157 knock-down in OV-90 cells significantly reduced their migratory potential, compared to OV-90 cells expressing basal CD157 transduced with a control shRNA (57). The improved ability of CD157-positive ovarian cancer cells to migrate was further

confirmed in a different experimental setting in which a mesothelial layer was grown on the bottom of the well of a transwell chamber. Then, CD157-positive or CD157-negative OVCAR-3 cells were seeded on the membrane of the transwell chamber. CD157-positive cells migrated toward the mesothelial monolayer with an efficiency significantly higher than that of the CD157-negative control cells (S.M. unpublished results). Overall, these results indicate that CD157 confers increased motility to ovarian cancer.

5.3. CD157 controls ovarian cancer cell dissemination

Ovarian cancer progression is characterized by peritoneal dissemination of tumor cells shed from the primary tumor as single cells, small aggregates or spheroids (58, 59). The majority of ovarian cancer cells that detach from the primary tumor usually undergo anoikis, a form of apoptosis caused by loss of contact with neighboring cells or with ECM proteins (60). A small number of these tumor cells acquire the ability to escape death by anoikis and subsequently form invasive foci (61). The observed association between high CD157 expression and tumor relapse in patients suggested that CD157 might provide protection against anoikis. The results obtained *in vitro* have indeed confirmed that tumor cells expressing high CD157 are more resistant to anoikis than cells with low or absent CD157 (57). The increased resistance to anoikis accounts for the increased ability of CD157-positive cells to form colonies in soft agar, a conventional *in vitro* assay to measure the tumorigenic potential of cancer cells. The expression of CD157 not only affects the tumorigenicity of cells detached from the tumor, but also unravels the spherical architecture and enhances the invasive capacity of spheroids. Indeed, OV-90 cells with high CD157 form irregular clusters composed of loosely associated cells with high invasive potential (54, 57).

The initial step of tumor cell invasion is characterized by the breakdown of the basement membrane, a process dependent on ECM–degrading enzymes, mainly MMP2 and MMP-9 (62). We observed an intense proteolytic activity associated with CD157 overexpression in epithelial ovarian cancer cells documented by increased transcription and release of tumorspecific matrix metalloproteinases (MMPs), such as MMP2, MMP7 and MMP9, paralleled by reduced transcription of TIMP3 (an endogenous inhibitor of MMPs)(57). These findings strongly support the association between high CD157 expression and EOC aggressiveness that emerged from patient analysis.

5.4. CD157 promotes ovarian cancer cell transmesothelial migration

The adhesion of single epithelial ovarian cancer cells (or cell aggregates) to and migration throughout the mesothelium are key sequential steps during metastatic dissemination. We evaluated the contribution of CD157 in both steps and demonstrated that CD157 has no appreciable role in ovarian cancer cell adhesion to and dissemination over the mesothelial layer, whereas it plays a fundamental role in transmesothelial migration. Indeed, CD157-positive and CD157-negative OVCAR-3 cells showed comparable adhesion efficiency. Moreover, anti-CD157 blocking mAb did not interfere with tumor cell adhesion to mesothelium. These findings indicate that CD157 does not has a decisive role in tumor cell adhesion to mesenchymal tissues. Conversely, high expression of CD157 resulted in a significantly improved ability of both OVCAR-3 and OV-90 cells to cross the mesothelium, as compared to the respective control cells (Figure 2C, D), indicating that the extent of transmesothelial migration achieved by EOC cells correlates with the level of expression of CD157 (57).

The dissemination of ovarian cancer through the blood vessels is not a common route, but sometimes it occurs, especially in advanced stage disease. In this scenario it is likely to envision that tumor cells with high levels of CD157 can use it to transmigrate, as do leukocytes.

5.5. CD157 drives ovarian cancer cells toward epithelial-mesenchymal transition

Tumor progression from the place of origin to the site of metastasis involves profound morphological and functional alterations of the cells, which must transform from static into migrating cells. This transient and reversible differentiation program is known as epithelial-mesenchymal transition (EMT) because of its remarkable similarities with EMT occurring during embryonic development. Once the cancer cell has completed its journey and reached its final destination, it reverts its phenotype adapting to the new environment. Undergoing EMT, non-invasive cells composing the primary tumors acquire features essential for migration, invasion, metastatic dissemination and resistance to apoptosis (63). EMT is a physiological process during the postovulatory repair representing a homeostatic mechanism for maintaining an intact epithelial layer in normal ovary (64, 65). However, in tumors EMT may have detrimental consequences, promoting metastasis.

Using ovarian cancer cell lines engineered to overexpress or knockdown CD157, we demonstrated that forced expression of CD157 induces profound changes in tumor cell morphology, with loss of cell polarity and organized adhesive junctions resulting in reduction of intercellular cohesion. At the phenotypic level, CD157 overexpression promotes the so-called cadherin switch, which is considered the hallmark of EMT. The switch consists in repression of E-cadherin (an integral component of adherens junctions), counterbalanced by induction of N-cadherin, the prototype of mesechymal markers. The downregulation of E-cadherin is accompanied by relocation of β-catenin from the cell membrane to the nucleus. The acquisition of mesenchymal-like phenotype in CD157-positive cells is controlled by the induction of the E-cadherin transcriptional repressors Snail and Zeb1 (57). These observations clearly indicate that CD157 promotes mesenchymal differentiation which is considered a main driver of chemoresistance (66) and poor survival in ovarian carcinoma patients (67). Additional studies are needed to further validate the role of CD157 in the EMT process in animal models *in vivo*.

5.6. Transcriptome profiling analysis of genes modulated by overexpression of CD157

To dissect the transcriptional changes that may mediate tumor aggressiveness associated with high CD157 expression, we performed microarray gene expression analysis of i) CD157-negative and CD157-positive OVCAR-3 cells and ii) of OV-90 cells with increased or basal expression of CD157. We identified 378 significantly modulated genes (163 upregulated and 215 downregulated) representing the signature of CD157-overexpressing OVCAR-3 and OV-90 cells [Gene Expression Omnibus (GEO) database ID: GSE36364] (57). Noteworthy, functional grouping and assessment of the gene ontology designations of these 378 transcripts, indicated that many genes induced by CD157 expression are involved in biological processes such as developmental/differentiation processes (including EMT), cell-adhesion, motility, locomotion and adhesion, and cellular components assembly and organization. In contrast, many of the down-regulated genes take part in biological processes such as apoptosis, cell death and response to stress (Fig.3). The overall picture that has emerged from the analysis of the transcriptome is

consistent with the assumption that CD157 is a crucial player in tumor progression and aggressiveness, and motivate the observation that patients with tumors showing high CD157 have a worse prognosis than those with low or absent CD157.

6. SUMMARY AND PERSPECTIVES

In the last ten years we have learned much about leukocytes and cancer cells migration. Our knowledge has been significantly fueled by the identification of many key molecules governing fundamental steps of cell migration through tissues. Studying leukocyte trafficking and cancer cell dissemination, we realized that leukocytes and cancer cells use similar strategies and share many of the molecules they exploit to spread through the body, adapting each time to the surrounding environment. CD157 was found to have a starring role in the control of myeloid cell migration and diapedesis during inflammation, a function which is fully consistent with its distribution pattern on leukocytes and endothelial cells. At the molecular level, CD157 clustering by antibody-induced cross-linking (mimicking the effects of an unknown physiological ligand) recruits β1 and β2 integrins into signaling-competent membrane microdomains, thus influencing their three-dimensional organization and promoting the transduction of intracellular signals which drive efficient cytoskeletal rearrangements, cell adhesion, and transmigration.

The anecdotal report of the expression of CD157 in human mesothelial cells (68) on the one hand, and the demonstration that *BST-1/CD157* is among the genes differentially expressed in epithelial ovarian cancer cells *versus* normal ovarian surface epithelial cells (53) on the other, prompted us to hypothesize that CD157 could be expressed by epithelial ovarian cancer cells. Our studies confirmed this hypothesis and highlighted that CD157 is an independent prognostic factor of poor prognosis in patients, suggesting that CD157 expression characterizes more aggressive tumors. The association of CD157 with epithelial ovarian cancer aggressiveness has been further substantiated by the observations that CD157 is directly implicated in the control of tumor progression. The functional contribution of CD157 to ovarian cancer progression relies on its ability to activate a differentiation program that drives cells toward a mesenchymal phenotype, a prerequisite for cancer cell invasion and metastatic dissemination. We envision that CD157 cooperates with other transmembrane receptors to fulfill its functions in ovarian cancer. Lateral partners of the CD157 interactome and molecular mechanisms regulating CD157-driven ovarian cancer progression currently under investigation in our lab will shed light on this issue.

Overall, clinical and experimental data lead us to believe that CD157 may be helpful in clinical practice. In chronic inflammatory conditions, such as arthritis and atherosclerosis, CD157 represents a potential target for the design of novel therapeutic strategies. Indeed, due to its structural and functional partnership with integrins, CD157 offers the opportunity for fine-tuning integrin functions without interfering directly with them, an approach that has generated serious side effects in patients (69). In ovarian cancer patients, it is tempting to predict that CD157 may serve as a novel therapeutic target for intraperitoneal antibody-based therapies aimed at controlling invasion and dissemination of the peritoneal cavity by residual ovarian cancer cells, after surgical intervention. Furthermore, CD157 is anchored to the plasma membrane by a GPI moiety and can be easily shed into the serum. Therefore, soluble CD157 could be measured in serum (or ascites) of ovarian cancer patients to determine if there is a correlation between the levels of soluble CD157 and the progression of the disease. Given the ability of membrane-bound CD157 to increase the tumorigenic potential of ovarian cancers, it is tempting to speculate that high soluble CD157 could indicate a highly aggressive tumor requiring a particular line of therapy.

Over the last decade, a large body of information has emerged from different perspectives contributing to the overall picture of the human ADPRC family. The dual receptor/enzyme nature of both members of this family has been clearly established. What remains an unsolved riddle is the relationship between enzymatic activities and receptor functions of both CD157 and CD38 in specific physiological and pathological contexts.

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Abbreviations: GPI: glycosylphosphatidylinositol; ADPRC: adenosine diphosphate ribosyl cyclase; cADPR: cyclic ADPR; SNP: single nucleotide polimorphysms; GWA: genome-wide association; sCD157: soluble CD157; ECM: extracellular matrix proteins; mAb: monoclonal antibody; EOC: epithelial ovarian cancer; HS: histological score; shRNA: short hairpin RNA; MMP: matrix metalloproteinase; EMT: epithelial-mesenchymal transition

Key Words: Leukocyte Trafficking, CD157, BST-1, Signal Transduction, Ovarian Cancer, Tumor Dissemination, Epithelial-Mesenchymal Transition, Review

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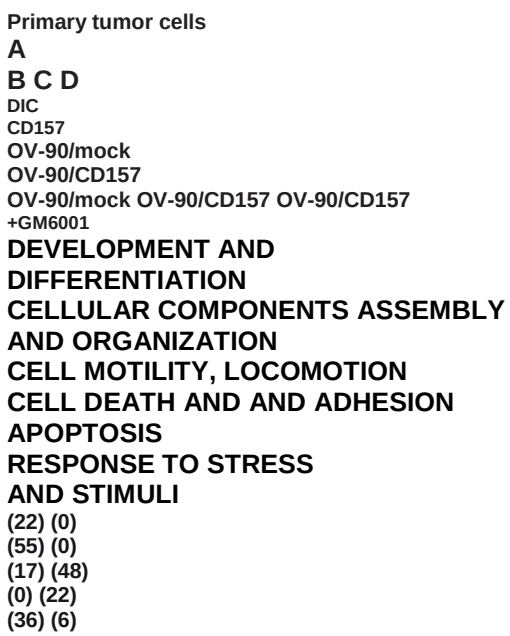
Figure 1. CD157 and integrin partnership. (A) CD157 engagement by its non-substrate ligand recruits integrins into signallingcompetent
microdomains (lipid rafts), thus influencing their three-dimensional organization and promoting the assembly of a network of interconnected signal transduction pathways (B). The experimental data indicated that CD157 and β1 and β2 integrins converge on activation of Src family kinases. This leads to increased activity of downstream MAPK-ERK1/2 and PI3K-Akt pathways, known to regulate cell adhesion and migration.

Figure 2. Key steps of ovarian cancer cell dissemination analysed by confocal microscopy. A) Tumor cells (green) shed into the peritoneal cavity as spheroids or single cells adhere to mesothelium (red), then compromise the integrity of the mesothelial layer and migrate through it. Met-5A non-malignant pleural mesothelial cells were labeled with CellBriteTM Red and grown to confluence on fibronectin-coated coverslips. CellTracker green-stained OV-90/mock (left panels) or OV-90/CD157 (middle and right panels) cells were plated onto the monolayer. Samples were analysed by sequential scanning of the XY planes recorded along the Z-axis (step size: 1.5 μm) at different time points. Series of confocal optical XY images were processed using a 3-dimensional reconstruction program (bioView3D software, Bio-Image Informatics, University of California, Santa Barbara, CA) and visualized as orthogonal views. B) Expression of CD157 in confluent primary tumor cells (left panel), in spheroid from the same patient (middle panel) and in the spheroid-derived monolayer (right panel). Sample were analyzed with an Olympus FV300 laser scanning confocal microscope (top panels) or by Nomarski differential interference contrast (DIC) optics (bottom panels). C) Phase contrast microscopy images of spheroids disseminating through mesothelium. Digital photographs of spheroids generated from OV-90/mock cells (top panel) or OV-90/CD157 cells (bottom panel) plated on human peritoneal mesothelial cell layers for 7 days. Arrows delineate the perimeter of invading spheroids. Images were acquired using an IX70 inverted microscope equipped with an F-View II camera (Olympus Biosystems). D) Ovarian cancer cell migration through a mesothelial monolayer. OV-90/mock (left panels) or OV-90/CD157 (middle and right panels) cells were plated onto the monolayer. In the right panel, OV-90/CD157 were treated for 1 h with GM6001 broad spectrum inhibitor of matrix metalloproteinases (25 μg/ml) before seeding onto Met-5A mesothelial cell layer. After migration for 2.5 h at 37°C, sample were fixed and analyzed using an Olympus FV300 laser scanning microscope (as described in A). Top and bottom views are shown.

Figure 3. Gene ontology analysis of transcripts modulated by CD157 overexpression. Differentially expressed genes in OVCAR-3 and OV-90 cells overexpressing CD157 are grouped in five major biological processes. The number of transcripts up-regulated (red arrows) and down-regulated (green arrows) in a specific biological process is indicated in brackets. Grey segment indicates the remaining biological processes.

Table 1. Schematic distribution of CD157 in tissues


AKT
c-Src
FAK
Actin
PI3K
MEK
pTEN
c-Src
PI3K
FAK
P
P
P
ERK1
P ERK2
P
P
P
P
Casp130 P
P
P P
P
MEK AKT
Vinculin
Pax Talin
Actinin
Actin
Fibers
PIP3
P
IN
β-integrin
α-integrin
CD157
CD157
OUT
OUT
IN
α-integrin
β-integrin
PIP2
751DC
CD157
A
B


Primary tumor cells
A
B C D
DIC
CD157
OV-90/mock
OV-90/CD157
OV-90/mock OV-90/CD157 OV-90/CD157
+GM6001
DEVELOPMENT AND
DIFFERENTIATION
CELLULAR COMPONENTS ASSEMBLY
AND ORGANIZATION
CELL MOTILITY, LOCOMOTION
CELL DEATH AND AND ADHESION
APOPTOSIS
RESPONSE TO STRESS
AND STIMULI
(22) (0)
(55) (0)
(17) (48)
(0) (22)
(36) (6)