Biological Role, Clinical Significance and Potential Therapeutic Applications of CD157 in Ovarian Cancer

S. Morone[1], N. Lo Buono[1], A. Giacomino[1], R. Parrotta[1], R. Arisio[2], D. Katsaros[2], N. Biglia[3], G. Chiorino[4], I. Rapa[5], M. Volante[5], E. Ortolan[1,*] and A. Funaro[1,*]

[1]Laboratory of Immunogenetics, Department of Medical Sciences, University of Torino; [2]Città della Salute e della Scienza, Sant'Anna Hospital, Torino; [3]Department of Oncological Gynecology, Umberto I Mauriziano Hospital, Torino; [4]Cancer Genomics Laboratory, Fondazione Edo ed Elvo Tempia Valenta, Biella; [5]Department of Oncology, San Luigi Hospital, University of Torino, Torino, Italy

Abstract: Ovarian cancer is the leading cause of gynecologic cancer-related morbidity and mortality owing to the difficulty in detecting early-stage disease. Despite advances in surgical and chemotherapeutic strategies, only marginal improvement in patient outcome has been achieved. Hence, understanding the biological mechanisms underlying ovarian cancer development and progression is critical for its treatment. We reported that CD157 (also known as BST-1), a NAD-metabolizing ectoenzyme regulating leukocyte diapedesis in inflammatory conditions, is expressed in approximately 90% of epithelial ovarian cancers and high CD157 expression is associated with poor outcome in patients. Our experimental results showed that CD157 controls ovarian cancer progression by promoting mesenchymal differentiation. The increased aggressiveness associated with tumors with high CD157 can be reverted *in vitro* by CD157 gene silencing, or by monoclonal antibodies that block CD157. The overall picture inferred from our experimental and clinical findings suggests that CD157 could aid diagnosis by classifying ovarian cancers into molecular subtypes with different outcomes, CD157 could also represent a novel candidate as a target of antibody-based therapies. This review summarizes and assesses recent research into the emerging functions of CD157 in the control of ovarian cancer progression.

Keywords: Ovarian cancer, CD157, BST-1, tumor marker.

1. INTRODUCTION

Ovarian cancer is the second most common gynaecological cancer and the fifth leading cause of cancer-related deaths in women [1]. Although cancer may arise from all cell types composing the ovary, by far the most common arise from the single-cell layer coelomic epithelium surrounding the ovaries and from postovulatory inclusion cysts. These tumors are referred to as epithelial ovarian cancers (EOC) and are quite heterogeneous in terms of grade and histology. The anatomic location of the ovaries and the absence of early specific symptoms make the diagnosis of ovarian cancer very difficult. Moreover, because of the lack of reliable screening tools, most patients are diagnosed when the disease is at an advanced stage and metastases are diffuse throughout the peritoneal cavity [2].

Ovarian cancer typically spreads in a diffuse intra-abdominal fashion and, even after recurrence, remains mostly confined to the peritoneal cavity where it carries out immunosuppressive activities [3]. Peritoneal dissemination is frequently associated with formation of peritoneal effusion fluid (ascites) and, in most cases, represents an incurable disease [4,5].

*Address correspondence to this author at the Laboratory of Immunogenetics, Department of Medical Sciences, University of Torino, Italy; Tel: +390116705991; Fax: +390116966155;
E-mail: ada.funaro@unito.it, erika.ortolan@unito.it

Despite evidence of considerable heterogeneity, most cases of EOC are treated in a similar fashion: the current standard care consists of radical surgery combined with first-line chemotherapy with carboplatinum and paclitaxel. Usually, patients respond well to chemotherapy; however, the failure of chemotherapy to eliminate all tumor cells remains the principle obstacle to a curative treatment because it leads to the development of drug-resistant tumors and systematic disease recurrence that eventually leads to death. This scenario highlights the need for better therapeutic modalities supported by novel biomarkers to indicate tailored treatments for ovarian cancer patients. In the last decade, a synergistic effort by researchers and clinicians has led to the identification of a number of molecules that might provide insights into ovarian cancer progression and facilitate screening, diagnosis, prognosis and monitoring of anti-cancer therapies. The list of the newly identified markers includes several ectoenzymes, that is cell surface enzymes whose substrates are outside the cell and often capable of behaving both as enzymes and receptors [6]. Ectoenzymes might provide specific targets for antibody-based tumor therapy or for the design of specific inhibitory molecules. The recent clinical successes of the use of engineered antibodies in intraperitoneal immunotherapy have established this approach as a promising strategy of molecular targeting of therapy for EOC [7,8].

E-ISSN: 2309-4400/13

We focused our attention on CD157, a glycosyl-phosphatidylinisotol (GPI)-anchored ectoenzyme encoded by a member of a gene family of nicotinamide adenine dinucleotide (NAD)ase/Adenosine diphosphate(ADP)-ribosylcyclases, which includes CD38 [9,10]. Both CD38 and CD157 metabolize extracellular NAD generating cyclic ADP ribose (cADPR) and ADPR, two second messengers implicated in the regulation of intracellular calcium homeostasis [11]. Although its structure is unsuitable for the purpose, CD157 is implicated in intracellular signal transduction [12]. To accomplish this function, CD157 exploits its localization in selected membrane microdomains (lipid rafts) and its natural proclivity to establish functional and structural interactions with integrins [13,14].

Initially characterized as a stromal [15] and myeloid surface antigen [16] playing a major role in the control of leukocyte adhesion, migration and diapedesis [17,18], CD157 was subsequently found to have a wider distribution than initially assumed [19,20]. In particular, we demonstrated that CD157 is expressed in EOC primary cell cultures and tissues, and it is involved in interactions among EOC cells, extracellular matrix (ECM) proteins, and mesothelial cells.

What follows is a portrait of CD157 focusing on its role in tumor progression and its potential clinical applications.

2. CD157 IN HUMAN EPITHELIAL OVARIAN CANCER TISSUES

We examined the expression of CD157 by flow cytometry, western blot and immunohistochemistry in ovarian cancer cell lines, fresh cells from primary tumors and surgical tissue samples from patients with ovarian cancer. Among the cell lines analyzed, SKOV-3, OV-90, OC314 and OC315 expressed CD157; by contrast, CD157 was undetectable in the OC316, A2780, IGROV-1, OVCAR-3 and OVCAR-5 cell lines. Western blot analysis of CD157 immunoprecipitated from selected positive cell lines confirmed that the protein migrates as a single polypeptide chain of approximately 45 kDa [21]. Fresh samples obtained from chemotherapy-naive patients with serous ovarian cancers expressed CD157, whereas the non-serous specimens were negative. However, this result was inferred from a small number of samples and requires further investigation.

The analysis of the expression and distribution of CD157 was extended to a cohort of 88 patients with EOC with several different histotypes, including serous papillary, endometrioid, undifferentiated, clear cells and mucinous tumors. CD157 was expressed by 93% of EOC analyzed by immunohistochemistry at varying levels and with discrete localization pattern (Figure **1**). In specimens containing residual areas of normal epithelium adjacent to the tumor, CD157 was mainly localized at the intercellular boundaries and at the basolateral surface of the epithelial cells. The extent of

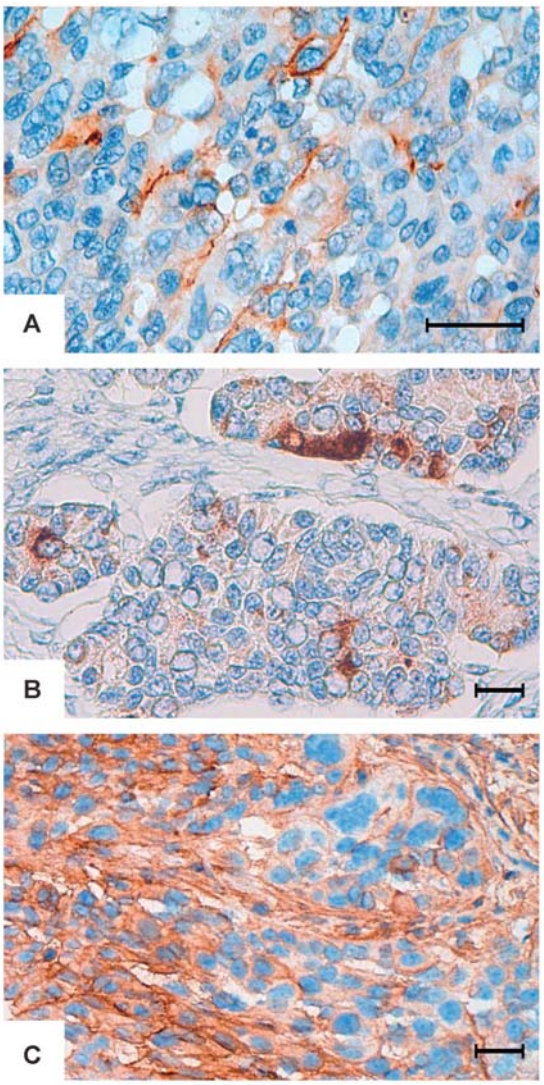


Figure 1: Immunohistochemical staining of CD157 in epithelial ovarian cancer tissue samples. Tissue sections from formalin-fixed paraffin-embedded epithelial ovarian cancers were stained with anti-CD157 mAb [RF3(MBL International Milan, Italy) diluted 1:100, brown] and a hematoxylin counterstain (blue). **A**. Serous papillary carcinoma showing dot-like cytoplasmic and membrane CD157 staining with apical localization (original magnification, ×400). **B.** Endometrioid carcinoma showing cytoplasmic localization of CD157 (original magnification, ×200). **C.** Serous papillary carcinoma showing intense membrane CD157 staining (original magnification, ×200). Scale bars = 50 μm.

CD157 immunohistochemical staining semiquantitatively measured by histological score (HS) was not associated with tumor histological type or grade or disease stage. However, high CD157 expression (e.g., above median HS) was associated with poor patient outcome on follow-up. Moreover, CD157 appeared to be a marker of poor prognosis in serous ovarian cancer, which is the most common and aggressive type. Multivariate survival analysis showed that high CD157 expression is an independent prognostic factor of tumor relapse shortly after surgical debulking of ovarian cancer [21].

These observations indicated that CD157 was associated with more aggressive ovarian cancers and prompted us to investigate the biological mechanisms through which CD157 could modulate tumor cell aggressiveness.

3. ROLE OF CD157 IN EPITHELIAL OVARIAN CANCER PROGRESSION

3.1. CD157 in Ovarian Cancer Migration and Invasion

To understand the possible role of CD157 in crucial aspects of ovarian cancer behavior, we analyzed 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 in OV-90 cells (expressing low levels of CD157) consistently increased cell motility. Conversely, shRNA-mediated CD157 down-modulation in OV-90 cells strongly reduced their migratory potential, compared to CD157-positive OV-90 cells transduced with a control shRNA.

Tumor cell motility is a prerequisite for cancer progression and for invasive migration of tumor cells to distant sites where metastatic growth occurs. This complex biological process involves a sequence of strictly connected events largely dependent on the ability of tumor cells to cross the mesothelial barriers. Using a three-dimensional culture specifically designed to mimic the metastatic process occurring *in vivo*, we demonstrated that the extent of transmesothelial migration achieved by EOC cell lines correlates with the level of expression of CD157, further implicating CD157 in the control of a crucial step of ovarian cancer metastasis [22]. These findings strongly support a role of CD157 in regulating EOC migration and peritoneal dissemination.

3.2. CD157 and Epithelial-Mesenchymal Transition

Movement of cancer cells from the site of origin to sites of metastasis is accompanied by transient, microenvironmentally controlled cell differentiation that converts adherent and strictly connected tumor epithelial cells into migratory fibroblast-like cells, capable of invading the ECM and establishing distant metastases. This process is known as epithelial to mesenchymal transition (EMT) because of its remarkable similarities with EMT occurring during development [23]. Using the above-mentioned engineered cell lines, we demonstrated that CD157 is involved in EMT of ovarian cancer cells. Indeed, exogenous expression of CD157 promotes morphological, phenotypic and functional changes that are considered the hallmarks of mesenchymal differentiation. These hallmarks include *i*) morphological change from a cobblestone-like epithelial to an elongated fibroblast-like cell with pronounced scattering; *ii*) loss of cell polarity and organized adhesive junctions with a consequent reduction of intercellular cohesion, *iii*) reduction of cortical F-actin localization and formation of actin stress fibers, *iv*) increased resistance to anoikis and *v)* enhanced secretion of matrix metalloproteinases (MMP) such as MMP2, MMP7 and MMP9. At the molecular level, CD157 expression promotes the cadherin switch, consisting in the repression of E-cadherin (an integral component of adherens junctions), counterbalanced by the induction of N-cadherin expression, and translocation of β-catenin into the nucleus. These profound phenotypic/morphologic changes act in concert to drive tumor cells toward a mesenchymal phenotype, characterized by a marked propensity to migrate. As expected, knockdown of CD157 reverted the mesenchymal phenotype and restored the original morphological, phenotypic and functional characteristics of epithelial cells [22].These data strongly support the idea that CD157 is involved in mesenchymal transdifferentiation of ovarian cancer cells, a dynamic and reversible process that confers on neoplastic epithelial cells the biological traits needed to accomplish most of the steps of the metastatic cascade [24].

3.3. CD157 and Cancer Stem Cells

EMT has been implicated in two of the most important processes responsible for cancer-related mortality: tumor progression towards metastatic disease and acquisition of drug resistance. Both of these processes may be linked, in turn, to a third: the

generation of cancer cells with stem cell-like characteristics [25,26]. Comparison of surface markers expressed by CD157-positive OVCAR-3 (transduced) *versus* CD157-negative OVCAR-3 (constitutive) cells highlighted that CD157 expression is paralleled by increased expression of EpCAM (Epithelial Cell Adhesion Molecule), CD24 and SSEA4 (Stage-specific embryonic antigen 4) (Figure **2**). These molecules are reported to identify a subset of cells with a stem-like phenotype. Moreover, EpCAM and CD24 are part of the signature of ovarian cancer-propagating cells and their expression correlates with tumour cell differentiation and invasiveness, and with patient survival [27,28]. The connection between EMT and cancer stem cell generation has a detrimental effect on cancer progression because it entails the acquisition of cellular traits associated with high-grade malignancy and with self-renewal ability, both of which foster metastatic dissemination. It is tempting to speculate that CD157 may be at the crossroad between mesenchymal and stem-like differentiation programs in ovarian cancer cells. However, currently available data refer to a limited number of *in vitro* models, which therefore cannot be considered conclusive. This intriguing aspect warrants further in-depth evaluation.

3.4. CD157 Expression and Gene Regulation

To dissect the transcriptional changes that may mediate the tumor aggressiveness associated with high expression of CD157, we performed microarray gene expression analysis of OVCAR-3 cells with and without CD157, and of OV-90 cells with low (constitutive) or high (induced by transfection of full-length CD157 gene) expression of CD157. We found 378 unique, significantly modulated transcripts (163 upregulated and 215 downregulated) that represent the signature of both OVCAR-3 and OV-90 cells overexpressing CD157 (Gene Expression Omnibus [GEO] database, ID:

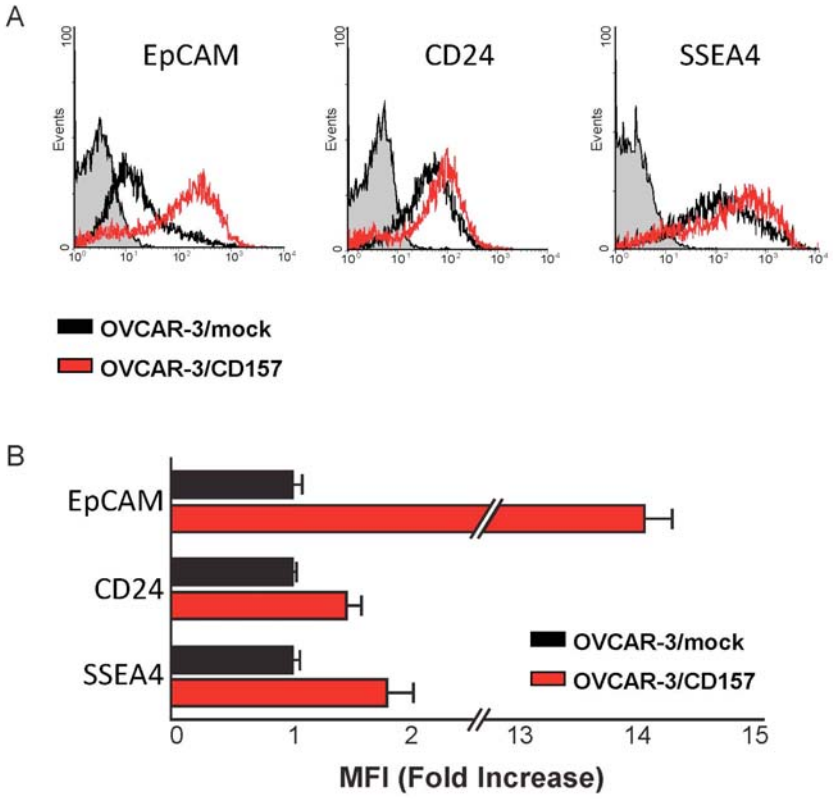


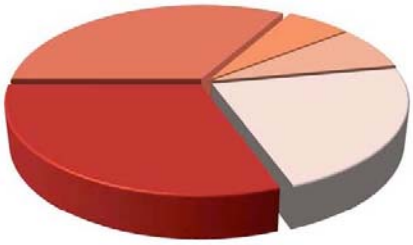
Figure 2: Flow cytometric analysis of the expression of cancer stem cell-like markers on OVCAR-3/mock and OVCAR-3/CD157 cells. **A.** Cells (10^5) were stained with 5 µl of FITC-labeled anti-EpCAM, anti-CD24 or anti-SSEA4 (BD Biosciences, Milano, Italy) for 30 minutes at 4°C. After washing, fluorescence was analyzed using a FACSCalibur flow cytometer and CellQuest software (BD Biosciences). Ten thousand cells were considered for each analysis. *x*-axis = fluorescence intensity, *y*-axis = number of cells (events). The results shown are representative of three experiments. The shaded histogram indicates the isotype-matched control IgG, the black and the red profiles represent the expression of the indicated surface molecules on OVCAR-3/mock and OVCAR-3/CD157 cells, respectively. **B.** Fold increase of the mean fluorescence intensity (MFI) in OVCAR-3/CD157 (red bars) compared to OVCAR-3/-mock (black bars) cells. The results are expressed as means of three separate experiments ± SEM.

GSE36364). This spectrum includes genes encoding proteins involved in crucial aspects of ovarian cancer dissemination (such as S100A4, BMP7, WNT10A, WNT6, FGF9, FZD4, FZD7, SFRP1, EPCAM) along with selected genes, such as EpCAM and CD24, which are considered ovarian cancer stem-like cell markers [29]. The latter products proved to be overexpressed in OVCAR-3 cells with high CD157 (see above). The comprehensive analysis of de-regulated genes indicated that forced expression of CD157 in ovarian cancer cells strengthens a number of biological processes favouring tumor progression (such as development, which also include EMT, and cell motility and migration) (Figure **3A**), and weakens several biological processes counteracting tumor progression (such as apoptosis, cell death and response to stress), corroborating the idea that CD157 is a marker of malignancy in EOC (See Figure **3B**).

3.5. CD157as a Therapeutic Target

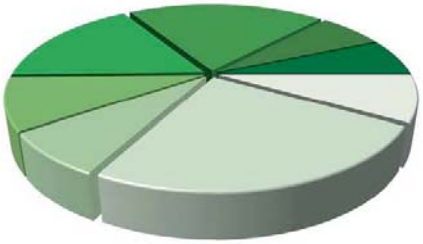
These findings led us to hypothesize that CD157 might be a good candidate as a therapeutic target for tumors characterized by high CD157 expression. To this end, we exploited the ability of blocking monoclonal antibodies (mAb) specific for CD157 [18] to dampen ovarian cancer invasiveness. Migration and ECM invasion of ovarian cancer cells were substantially inhibited by blocking anti-CD157 mAb (but not by irrelevant IgG used as control), on examination of both primary cell cultures obtained from fresh tumors or CD157-positive ovarian cancer cell lines. Conversely, anti-CD157 mAb treatment did not influence either motility or invasion of CD157-negative cells, confirming the specificity of the inhibitory effect [21]. Moreover, anti-CD157 blocking mAbs were able to reduce transmesothelial migration of single tumor cells or cell

A



Biological process	Genes
Developmental process	FRAS1, S100A4, ERMP1, MPZL2, TSHZ1, PLXNA4, FUT8, E2F5, FGF9, ONECUT2, PDE3B, FOXO1, TPM1, HOOK1, CTTNBP2, SHISA2, PVRL1, ITGAV, HOXA10, HS6ST1, TCTN1, NR2F2, WNT6, NR2F1, WNT10A, BST1, EFNB1, CSRP2, FZD4, GAS6, FZD7, TACC2, MYCN, HOXB3, SEMA6A, DLX1, SFRP1, HOXB8, HOXB5, ETS2, ZIC5, SIX1, MNX1, VCAN, HOXB9, LAMC2, IGFBP2, BMP7
Multicellular organismal process	S100A4, PLXNA4, E2F5, FGF9, FOXO1, PDE3B, HOOK1, CTTNBP2, KCNK5, CNTNAP3, WNT6, NR2F2, TFPI2, MAP2K6, NR2F1, WNT10A, BST1, EFNB1, MFGE8, TACC2, MYCN, SIX1, MNX1, VCAN, LAMC2, FRAS1, ERMP1, TSHZ1, FUT8, ONECUT2, TPM1, SHISA2, PVRL1, ITGAV, HOXA10, HS6ST1, TCTN1, SCNN1A, CSRP2, FZD4, GAS6, FZD7, HOXB3, SEMA6A, DLX1, SFRP1, HOXB8, HOXB5, ETS2, ZIC5, HOXB9, BMP7
Locomotion	CTTNBP2, VAV3, FUT8, PVRL1, EFNB1, PODXL, SIX1, VCAN, NR2F2, NR2F1
Biological adhesion	MPZL2, SORBS1, PVRL1, EFNB1, ITGAV, CNTNAP3, ITGB4, LAMC2, VCAN, MFGE8, PDZD2, ADAM15
Localization	PITPNC1, STEAP1, NR2F1, NR2F2, SEMA6A, FGF9, SORBS1, SLC37A1, ICA1, FRAS1, SCNN1A, TCN2, TPM1, KCNK5, ELMO3, TOM1L1, PODXL, VCAN, RAB3B, SIX1, FOLR1, RAB11FIP5, EFNB1, TMSB4X, NUDT4, HOOK1, ITGAV, ATP8A2, MFGE8, TACC2, FUT8, VAV3, CTTNBP2, CROT

B



Biological process	Genes
Multi-organism process	MAFF, IL6, HIST1H2BE, MPDZ, SOCS1, BNIP3, SIRT1, GCH1, FOS, MYD88, APOL1, ADM, HIST1H2BK, HIST2H2BE, HIST1H2BI, PI3, HIST1H2BJ, NLGN4X, GUCY1A3, MGST1
Response to stimulus	EDN1, BNIP3, FOS, MYD88, CASP9, HIST1H2BK, PLOD2, HIST1H2BI, HIST1H2BJ, GUCY1A3, CREB3L3, CASP1, NRG1, FAM129A, PTGER4, SOCS1, LIFR, GAL, LPIN1, DHRS2, ADM, HIST2H2BE, PPP1R15A, TPST1, HSD17B2, ERBB4, CXCL3, CXCL2, DUSP10, PPT1, C1S, TIMP3, GCH1, MSX2, PAPSS2, ND6, DNMT3B, PLAT, MAFF, KLF6, IL6, BMP2, HIST1H2BE, FADS1, AXL, TNFSF9, SLC10A3, SIRT1, PROM1, CD55, APOL1, DUSP1, NLGN4X, PBX3, KLF4, MGST1
Death	HYAL1, IL6, GULP1, APH1B, BCL2A1, BNIP3, PPT1, TNFSF9, SIRT1, CASP5, SYNE1, CASP4, APOL1, CASP9, GATA6, CASP1, PPP1R15A
Cellular component biogenesis	H1F0, HIST1H2AC, HIST2H2AA4, HIST1H2BE, HIST1H2AD, HIST1H2AE, HIST1H2BH, CD2AP, GCH1, HIST1H2BK, HIST1H2BL, HIST2H2BE, HIST1H2BI, ALOX5AP, HIST1H2BJ, TUBB6, HIST1H3D, PDGFC, QPRT, VCX, NRG1, HIST1H4H, TUBB4
Cellular component organization	HIST1H2AC, HIST2H2AA4, ERBB4, GULP1, HIST1H2AD, HIST1H2AE, BNIP3, PPT1, CD2AP, GCH1, HIST1H2BK, HIST1H2BL, HIST1H2BI, ALOX5AP, HIST1H2BJ, TUBB6, PDGFC, NRG1, EHD2, DNMT3B, HIST1H4H, TUBB4, H1F0, CPA4, IL6, BMP2, HIST1H2BE, HIST1H2BH, LIFR, SIRT1, LPIN1, RND3, SYNE1, ADM, HIST2H2BE, NLGN4X, HIST1H3D, QPRT, MAP9, VCX
Developmental process	ERBB4, TUFT1, MITF, EDN1, BNIP3, PPT1, C1S, TIMP3, MSX2, FOS, LBH, GATA6, POU5F1, CYP26B1, NKX3-1, PDGFC, NRG1, DNMT3B, PAPSS2, CRIM1, PLAT, ALDH6A1, KLF6, MAFF, BMP2, IL6, FADS1, SOCS1, FBN1, KRTDAP, PIM1, LIFR, AXL, TLE1, GAL, SIRT1, ZNF22, VCX3A, DHRS2, SYNE1, ADM, RGS2, ADAMTS1, PBX3, MGST1, KLF4
Reproductive process	MAFF, BMP2, ZP3, SIRT1, VCX3A, FOS, ADM, RGS2, DUSP13, CYP26B1, PI3, NKX3-1, ADAMTS1, VCX, MGST1
Reproduction	MAFF, BMP2, ZP3, SIRT1, VCX3A, FOS, ADM, RGS2, DUSP13, CYP26B1, PI3, NKX3-1, ADAMTS1, VCX, MGST1

Figure 3: Gene ontology analysis (GO) of genes modulated by CD157 overexpression in OVCAR-3 and OV-90 cells. **A**. Main biological processes to which upregulated genes shared by OVCAR-3 and OV-90 cells overexpressing CD157 contribute. **B**. Main biological processes to which downregulated genes shared by OVCAR-3 and OV-90 cells overexpressing CD157 contribute.

aggregates, suggesting that CD157 is a key mediator of tumor-mesothelial cell interactions [21]. Collectively, these results show that anti-CD157 blocking mAb actually counteract tumor progression *in vitro.*

3.6. CD157 and Tumorigenicity of Ovarian Cancer Cells

It is widely accepted that *in vitro* colony-forming ability reflects the tumorigenic potential of cancer cells, and that the greater the propensity for metastasis, the greater the ability to form colonies in soft agar. According to this dogma, forced expression of CD157 increased (while CD157 knockdown reduced) the colony-forming ability in OVCAR-3 and OV-90 cells, compared to the corresponding control cells, supporting the existence of a direct correlation between the levels of CD157 and the tumorigenic potential of ovarian cancer cells *in vitro* [22]. Since *in vitro* studies may not thoroughly recapitulate the situation occurring *in vivo*, we determined if high expression of CD157 could enhance tumorigenesis *in vivo*. Female NOD/SCID/IL2rg-/- mice were subcutaneously inoculated with either CD157-negative OVCAR-3 cells (mock) or with OVCAR-3/CD157-positive cells and tumor growth was monitored over time. By day 18 post-inoculation, tumor masses were detectable in mice injected with OVCAR-3/CD157 cells. In contrast, tumors were not detected in mice inoculated with OVCAR-3-mock cells until day 22 (Figure **4A**). The size of OVCAR-3/CD157-positive tumors was significantly larger than CD157-negative tumors until a very late stage of growth (day 46), when mice were compassionately euthanized (Figure **4B**). These data indicate that CD157-positive cells show accelerated growth and a higher proliferation rate than the negative counterpart, resulting in a significantly greater tumorigenic potential *in vivo*.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

The involvement of CD157 in the control of leukocyte trafficking - [30] on the one hand, and its expression outside the myeloid compartment - primarily in mesothelial cells [31] on the other, led us to hypothesize that CD157 could be expressed by epithelial ovarian cancer cells and could be involved in the control of tumor dissemination. Collectively, our experimental and clinical data lead to the conclusion that CD157 plays a leading role in the progression and aggressiveness of ovarian cancers both *in vitro* and *in vivo*.

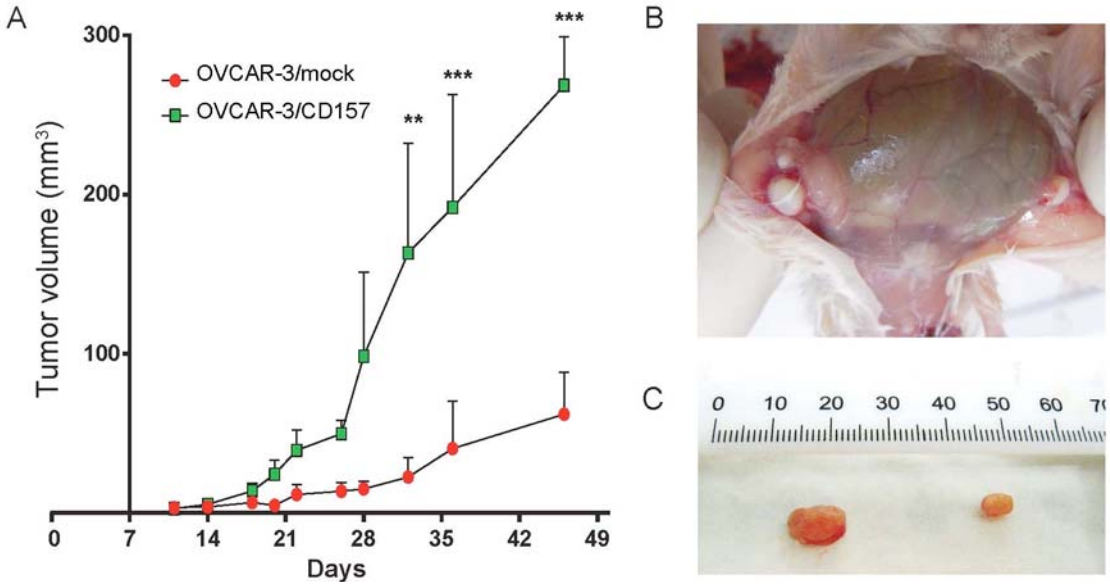


Figure 4: Effects of CD157 expression on tumor growth in NOD/SCID/IL2rg-/- mice. Five female 5- to 7-week-old immunodeficient NOD/SCID/IL2rg-/- mice were subcutaneously inoculated with 10^5 OVCAR-3/CD157 (right flank) or OVCAR-3/mock (left flank) cells suspended in 200 µl of ECM gel (Sigma Aldrich) and tumor growth was measured at the indicated times. **A.** Increase of tumor size over time. Tumor growth was monitored biweekly from day 7 to day 46. Tumor masses were measured with a manual caliber, and tumor volumes were calculated approximating the tumor mass to a sphere, according to the following equation: volume = π/6 [major diameter × (minor diameter)^2]. Data are averages of multiple determinations (n=5) of OVCAR-3/CD157 and OVCAR-3/mock tumors. *P* values were derived from analysis of variance with Bonferroni correction by comparing OVCAR-3/CD157 (green) with mock cells (red). ***P* <0.01, ****P* <0.001. **B**. (left side) Representative subcutaneous tumors derived from CD157-positive (right flank); (right side) CD157-negative (left flank) OVCAR-3 cells. **C**. Tumor masses excised at day 46 post-inoculum.

We envision that CD157 may be helpful in clinical practice. Indeed, used in conjunction with conventional diagnostic markers, it could help formulate dependable prognostic criteria and address the classification of ovarian cancers into molecular subtypes with different outcomes. 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. Given the ability of CD157 to increase the tumorigenic potential of ovarian cancers, high soluble CD157 could indicate a highly aggressive tumor requiring a particular line of therapy. Finally, the ability of CD157 blocking mAb to prevent EOC invasion and dissemination *in vitro* suggests that CD157 could be a promising therapeutic target for intraperitoneal antibody-based therapy as an additional modality complementing surgery and chemotherapy. Despite favorable preclinical results, mAb used as mono therapies in ovarian cancer have often yielded disappointing results in improving clinical outcome. However, their administration in combination with cytotoxic chemotherapy may be a promising option [32]. Based on this assumption, we plan to generate CD157-based nanoparticle formulations (such as liposomal doxorubicin or protein-bound paclitaxel) for active tumor targeting as a strategy to increase specificity and anticancer potency of conventional chemotherapy drugs in the treatment of EOC.

ACKNOWLEDGEMENTS

N.LB is supported by a fellowship from the Fondazione Umberto Veronesi, S.M. and A.G. are members of the Ph.D. program in ''Complex systems applied to post-genomic biology''. This work was supported by grants from the Italian Association for Cancer Research (AIRC, MFAG6312 and IG 11602 to EO), from the Italian Ministry for University and Scientific Research (60% Projects to AF) and from the International Foundation for Research in Experimental Medicine, Torino, Italy.

REFERENCES

[1] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60(5): 277-300. http://dx.doi.org/10.3322/caac.20073

[2] Coleman RL, Monk BJ, Sood AK, Herzog TJ. Latest research and treatment of advanced-stage epithelial ovarian cancer. Nat Rev Clin Oncol 2013; 10(4): 211-24. http://dx.doi.org/10.1038/nrclinonc.2013.5

[3] Yigit R, Massuger LF, Figdor CG, Torensma R. Ovarian cancer creates a suppressive microenvironment to escape immune elimination. Gynecol Oncol 2010; 117(2): 366-72. http://dx.doi.org/10.1016/j.ygyno.2010.01.019

[4] Feki A, Berardi P, Bellingan G, Major A, Krause KH, Petignat P, *et al*. Dissemination of intraperitoneal ovarian cancer: Discussion of mechanisms and demonstration of lymphatic spreading in ovarian cancer model. Crit Rev OncolHematol 2009; 72(1): 1-9. http://dx.doi.org/10.1016/j.critrevonc.2008.12.003

[5] Malayev Y, Levene R, Gonzalez F. Palliative chemotherapy for malignant ascites secondary to ovarian cancer. Am J Hosp Palliat Care 2012; 29(7): 515-21. http://dx.doi.org/10.1177/1049909111434044

[6] Lo Buono N, Morone S, Parrotta R, Giacomino A, Ortolan E, Funaro A. Ectoenzymes in epithelial ovarian carcinoma: potential diagnostic markers and therapeutic targets. In: Farghaly SA Ed. Ovarian Cancer – Basic science perspective. Rijeka, Croatia: InTech - Open Access Publisher 2011; pp. 245-70.

[7] Seimetz D, Lindhofer H, Bokemeyer C. Development and approval of the trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3) as a targeted cancer immunotherapy. Cancer Treat Rev 2010; 36(6): 458-67. http://dx.doi.org/10.1016/j.ctrv.2010.03.001

[8] Leone RMU, Bellati F, Ruscito I, Gasparri ML, Alessandri F, Venturini PL, *et al*. Monoclonal antibodies therapies for ovarian cancer. Expert Opin Biol Ther 2013; 13(5): 739-64. http://dx.doi.org/10.1517/14712598.2013.767328

[9] Ferrero E, Malavasi F. Human CD38, a leukocyte receptor and ectoenzyme, is a member of a novel eukaryotic gene family of nicotinamide adenine dinucleotide+-converting enzymes: extensive structural homology with the genes for murine bone marrow stromal cell antigen 1 and aplysian ADP-ribosylcyclase. J Immunol 1997; 159(8): 3858-65.

[10] Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, *et al*. Evolution and function of the ADP ribosylcyclase/CD38 gene family in physiology and pathology. Physiol Rev 2008; 88(3): 841-86. http://dx.doi.org/10.1152/physrev.00035.2007

[11] Galione A, Chuang KT. Pyridine nucleotide metabolites and calcium release from intracellular stores. Adv Exp Med Biol 2012; 740: 305-23. http://dx.doi.org/10.1007/978-94-007-2888-2_13

[12] Ishihara K, Hirano T. BST-1/CD157 regulates the humoral immune responses *in vivo*. Chem Immunol 2000; 75: 235-55. http://dx.doi.org/10.1159/000058772

[13] Lavagno L, Ferrero E, Ortolan E, Malavasi F, Funaro A. CD157 is part of a supramolecular complex with CD11b/CD18 on the human neutrophil cell surface. J Biol Regul Homeost Agents 2007; 21(1-2): 5-11.

[14] Lo Buono N, Parrotta R, Morone S, Bovino P, Nacci G, Ortolan E, *et al*. The CD157-integrin partnership controls transendothelial migration and adhesion of human monocytes. J Biol Chem 2011; 286(21): 18681-91. http://dx.doi.org/10.1074/jbc.M111.227876

[15] Kaisho T, Ishikawa J, Oritani K, Inazawa J, Tomizawa H, Muraoka O, *et al*. BST-1, a surface molecule of bone marrow stromal cell lines that facilitates pre-B-cell growth. Proc Natl Acad Sci USA 1994; 91(12): 5325-29. http://dx.doi.org/10.1073/pnas.91.12.5325

[16] Goldstein SC, Todd RF, 3rd. Structural and biosynthetic features of the Mo5 human myeloid differentiation antigen. Tissue Antigens 1993; 41(4): 214-8. http://dx.doi.org/10.1111/j.1399-0039.1993.tb02007.x

[17] Funaro A, Ortolan E, Ferranti B, Gargiulo L, Notaro R, Luzzatto L, *et al*. CD157 is an important mediator of neutrophil adhesion and migration. Blood 2004; 104(13): 4269-78. http://dx.doi.org/10.1182/blood-2004-06-2129

[18] Ortolan E, Tibaldi EV, Ferranti B, Lavagno L, Garbarino G, Notaro R. *et al*. CD157 plays a pivotal role in neutrophil transendothelial migration. Blood 2006; 108(13): 4214-22. http://dx.doi.org/10.1182/blood-2006-04-017160

[19] Ortolan E, Vacca P, Capobianco A, Armando E, Crivellin F, Horenstein A, *et al*. CD157, the Janus of CD38 but with a unique personality. Cell Biochem Funct 2002; 20(4): 309-22. http://dx.doi.org/10.1002/cbf.978

[20] Quarona V, Zaccarello G, Chillemi A, Brunetti E, Singh VK, Ferrero E, *et al*. CD38 and CD157: A long journey from activation markers to multifunctional molecules. Cytometry B 2013; in press.

[21] Ortolan E, Arisio R, Morone S, Bovino P, Lo-Buono N, Nacci G, *et al*. Functional Role and Prognostic Significance of CD157 in Ovarian Carcinoma. J Natl Cancer Inst 2010; 105(16): 1160-77. http://dx.doi.org/10.1093/jnci/djq256

[22] Morone S, Lo Buono N, Parrotta R, Giacomino A, Nacci G, Brusco A, *et al*. Overexpression of CD157 contributes to epithelial ovarian cancer progression by promoting mesenchymal differentiation. PLoS ONE 2012; 7(8): e43649. http://dx.doi.org/10.1371/journal.pone.0043649

[23] Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science 2011; 331(6024): 1559-64. http://dx.doi.org/10.1126/science.1203543

[24] De Craene B, Berx G. Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer 2013; 13(2): 97-110. http://dx.doi.org/10.1038/nrc3447

[25] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, *et al*. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133(4): 704-15. http://dx.doi.org/10.1016/j.cell.2008.03.027

[26] Scheel C, Weinberg RA. Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. Semin Cancer Biol 2012; 22(5-6): 396-403. http://dx.doi.org/10.1016/j.semcancer.2012.04.001

[27] Munz M, Baeuerle PA, Gires O. The emerging role of EpCAM in cancer and stem cell signaling. Cancer Res 2009; 69(14): 5627-29. http://dx.doi.org/10.1158/0008-5472.CAN-09-0654

[28] Gao MQ, Choi YP, Kang S, Youn JH, Cho NH. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. Oncogene 2010; 29(10): 2672-80. http://dx.doi.org/10.1038/onc.2010.35

[29] Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008; 8: 755-68. http://dx.doi.org/10.1038/nrc2499

[30] Funaro A, Ortolan E, Bovino P, Lo Buono N, Nacci G, Parrotta R, *et al*. Ectoenzymes and innate immunity: The role of human CD157 in leukocyte trafficking. Frontiers in Bioscience 2009; 14: 929-43. http://dx.doi.org/10.2741/3287

[31] Ross JA, Ansell I, Hjelle JT, Anderson JD, Miller-Hjelle MA, Dobbie JW. Phenotypic mapping of human mesothelial cells. Adv Perit Dial 1998; 14: 25-30.

[32] Sessa C, Del Conte G. Targeted therapies: tailored treatment for ovarian cancer: are we there yet? Nat Rev Clin Oncol 2010; 7(2): 80-2. http://dx.doi.org/10.1038/nrclinonc.2009.233

Received on 15-05-2013 Accepted on 03-06-2013 Published on 30-06-2013

DOI: http://dx.doi.org/10.14205/2309-4400.2013.01.01.5