

SLAMF1/CD150 EXPRESSION AND TOPOLOGY IN PROSTATE AND BREAST CANCER CELL LINES

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Background: SLAMF1/CD150 receptor is aberrantly expressed in malignant hematopoietic cells compared to ubiquitous expression in their normal analogues. However, the data about CD150 expression and function outside the hematopoietic system are limited. **The aim** of this pilot study was to examine the profile of mRNA expression of CD150 isoforms and the protein topology in highly and low malignant breast (BC) and prostate cancer (PC) cell lines. **Materials and Methods:** The study was performed on BC T47D, MDA-MB-231, BCC/P and BC/ML cell lines and PC LNCap, Du-145 and PC-3 cell lines. The quantitative polymerase chain reaction was applied for study of CD150 isoforms mRNA expression and flow cytometry was used for determination of protein localization. **Results:** Analyzed BC cell lines did not express CD150 on the cell surface membrane (csCD150⁻), but more than 45% of cells were positive for CD150 cytoplasmic reaction (cyCD150⁺). The cyCD150 expression level in T47D cells of luminal BC subtype was higher than in basal BC cell lines MDA-MB-231, BCC/P and BC/ML. The PC cell lines expressed CD150 both on the cell surface and in cytoplasm. The highest number of csCD150⁺ and cyCD150⁺ cells was revealed in less aggressive androgen responsive, non-metastatic LNCap cell line. All studied BC and PC cell lines expressed mRNA of canonical transmembrane mCD150 and novel nCD150 isoforms but with different pattern of prevalence. Soluble CD150 isoform was revealed at the low level only in BCC/P BC cell line and LNCap, PC-3 PC cell lines. **Conclusions:** We have shown that BC and PC cell lines differentially expressed multifunctional receptor CD150 at the mRNA and protein levels that may indicate its association with the degree of their malignancy.

Key Words: SLAMF1/CD150, prostate cancer, breast cancer, cell lines.

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Originally, signaling lymphocytic activation molecule family member 1 (SLAMF1/CD150) receptor was identified on the activated B- and T-lymphocytes [1, 2]. Plethora of studies concerning CD150 structure, signaling properties and function were conducted on the hematopoietic cells including normal and malignant B lymphocytes, T lymphocytes, dendritic cells, etc. [3]. CD150 is an active player in innate and adaptive immunity regulation, an essential component of malignant B cells signaling network, especially in Hodgkin lymphoma and chronic lymphocytic leukemia, bacterial sensor and receptor for measles virus. CD150 multifunctionality, existence of structurally different CD150 isoforms and their differential expression makes it an attractive candidate for development of new approaches to malignant cell fate modulation, and may open new prospects for the differential diagnosis and improvement of therapeutic strategy. However, our current knowledge about CD150 expression and functions outside hematopoietic system is limited by immunohistochemical studies in squamous cell carcinomas of uterine cervix, esophagus, rectum and oral cavity [4]. CD150 protein and mRNA expression was found in more than 70% tumors of central nervous system, including glioblastoma, astrocytoma, ependymoma and others [5]. It should be noted

that tumors of central nervous system in contrast to malignancies of B cell origin express exclusively novel CD150 (nCD150) isoform. High CD150 level was detected in methotrexate-resistant choriocarcinoma JEG3/MTX and JAR/MTX sublines [6]. Moreover, CD150 might be directly involved in forming methotrexate resistance via activating protective autophagy. A study that was performed on HEK293T cell line transfected with mCD150 and nCD150 isoforms may shed the light on the CD150 isoforms function outside hematopoietic system [7]. It was shown that both mCD150 and nCD150 isoforms in the *in vitro* model system show a growth-stimulating effect on cells, inducing proliferation, cell cycle progression and the ability to form colonies with a more pronounced effect of nCD150 isoform. Unfortunately, the data on CD150 functions and profile of its isoform's expression in malignant cells of non-hematopoietic origin are currently fragmentary and far from complete understanding.

In this study, we are focused on the detection of CD150 protein expression and cellular localization as well as evaluation of CD150 splice isoforms mRNA expression in breast cancer (BC) and prostate cancer (PC) cell lines. We believe this can help answering the question whether CD150 may be associated with phenotype of malignant cells outside the hematopoietic system.

MATERIALS AND METHODS

Cell lines. BC cell lines T47D, MDA-MB-231, BCC/P, BC/ML and PC cell lines LNCap, Du-145, PC-3 were obtained from the Bank of Cell Lines from Human and Animal Tissues of the R.E. Kavetsky In-

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Abbreviations used: BC – breast cancer; GeoMFI – geometric mean fluorescence intensity; PC – prostate cancer; qPCR – quantitative polymerase chain reaction; SLAMF1 – signaling lymphocytic activation molecule family member 1.

stitute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine (Kyiv). The detailed characteristics of PC and BC cell lines are listed in the Table. All cell lines were maintained in IMDM (BioWest, France) medium supplemented with 20% FCS, 2 mM L-glutamine and antibiotics at 37 °C in a humidified atmosphere containing 5% CO₂.

Flow cytometry. Surface and cytoplasmic CD150 expression was detected by direct immunofluorescent method using anti-CD150 (IPO-3) FITC-conjugated mAbs (R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv). For cytoplasmic CD150 expression cells were pre-fixed before staining with 2% paraformaldehyde for 30 min, washed in PBS and then permeabilized with 0.2% Tween-20 for 30 min. Stained cells were examined using Beckman Coulter DxFLEX fluorescence flow cytometer (Beckman Coulter, USA) and CytExpert for DxFLEX software. CD150 expression analysis was performed three times for each cell line. The results are presented as the geometric mean fluorescence intensity (GeoMFI) index (GeoMFI ratio of antigen to isotype control) and as a percentage of CD150⁺ cells.

Quantitative polymerase chain reaction (qPCR). Total RNA was isolated from 1 × 10⁶ cells using NucleoZOL (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to manufacturer's protocol. A detailed description of cDNA synthesis and real-time polymerase chain reaction were reported elsewhere [8, 9]. The primers sequences for mCD150, nCD150 and control TBP (TATA-box binding protein) genes were published earlier [8]. qPCR reaction was performed on the QuantStudio 5 Dx Real-Time PCR System (Thermo Fisher Scientific, USA). The Ct values for target genes were determined and normalized to the Ct value of TBP internal control gene using comparative Ct (ddCt) method.

Statistical analysis. All statistical data processing was performed using Student's *t*-test with Prism software Version 4.0. The results were presented as mean of three independent experiments (± SEM). Differences between the comparison groups were considered as significant at *p* < 0.05.

RESULTS AND DISCUSSION

Rationality for studying CD150 expression in BC and PC cell lines was based on bioinformatic analysis of CD150 mRNA expression in different cancer types using a publically available data-

base Oncomine containing the published data of tumor DNA/RNA microarrays that have been collected, standardized, annotated and analyzed by Compendia (<http://www.oncomine.com>). Analysis of the large number of presented data sets on DNA microarrays of breast and prostate cancer revealed that majority of tissue samples expressed CD150 mRNA. Preliminary data showed high CD150 mRNA level in BC samples and its differential expression depending on BC histological subtype, whereas in PC tissue CD150 mRNA expression was low and depended on differentiation grade of PC. Thus, bioinformatic analysis in the Oncomine database justifies the need for further experimental study of CD150 expression and function in BC and PC with due regard to their molecular phenotype.

Analysis of primary BC or PC tissue samples for the CD150 mRNA and protein expression is complex since infiltration of CD150 positive immune cells can lead to false positive results. Thus for the first step of our research, we used highly and low malignant cell lines of BC (T47D, MDA-MB-231, BCC/P, BC/ML) and PC (LNCap, Du-145, PC-3), which have been previously characterized based on their molecular biological characteristics [10–13]. The abovementioned cell lines were analyzed for mRNA expression levels of CD150 isoforms as well as CD150 topology.

The CD150 cellular localization was studied by flow cytometry in the BC cell lines of different molecular subtypes and its expression was revealed only in the cytoplasm, but not on the cell surface. It should be noted that the intracellular CD150 level in the cells of the luminal BC subtype T49D (11.9 ± 0.3 r.u.) was significantly higher than that in the cells of the basal BC subtype MDA-MB-231, BC/ML and BCC/P (from 4.6 ± 0.1 to 6.8 ± 0.2 , *p* < 0.05) (Fig. 1). The percentage of CD150-positive cells was comparatively similar in all analyzed cell lines with the exception of MDA-MB-231 cell line that demonstrated the lowest percent of CD150-positive cells.

In contrast to the BC cell lines, in the PC cell lines, CD150 was found both on the cell surface membrane and in the cytoplasm (Fig. 2). In general, cell surface CD150 expression level was significantly lower than that in cytoplasm in all studied PC cell lines (*p* < 0.05) (Fig. 2). The number of csCD150⁺ cells differed in PC cell lines (Fig. 2, a). The lowest number of csCD150⁺ cells ($3.0 \pm 0.2\%$) was detected in the most malignant PC cell line — PC-3, while the hormone-sensitive LNCap cell line had

Table. Characteristics of BC and PC cell lines

Cell line	Origin	Histological type	Molecular biological characteristics
BC cell lines			
T47D	Pleural effusion	Infiltrative ductal carcinoma	Luminal A subtype
MDA-MB-231	Pleural effusion	Adenocarcinoma	Basal subtype
BCC-P	Pleural effusion	Adenocarcinoma	Basal subtype
BC/ML	Pleural effusion	Adenocarcinoma	Basal subtype
PC cell lines			
LNCap	Metastasis in supraclavicular lymph node	Adenocarcinoma	Androgen responsive
Du-145	Metastasis in brain	Adenocarcinoma	Androgen non-responsive
PC-3	Metastasis in bone	Small cell neuroendocrine carcinoma	Androgen non-responsive

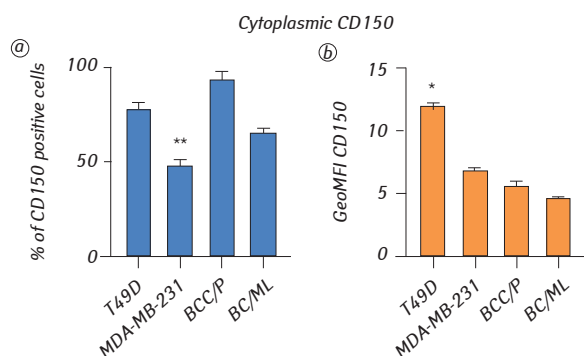


Fig 1. Cytoplasmic CD150 expression in BC cell lines. Results of flow cytometry analysis are presented as mean of three independent experiments ($\pm$ SEM): *a* — percent of CD150-positive cells; *b* — CD150 expression level, * $p < 0.05$ compared to highly malignant MDA-MB-231, BCC/P, BC/ML cell lines, ** $p < 0.05$ compared to T49D and BCC/P cell lines

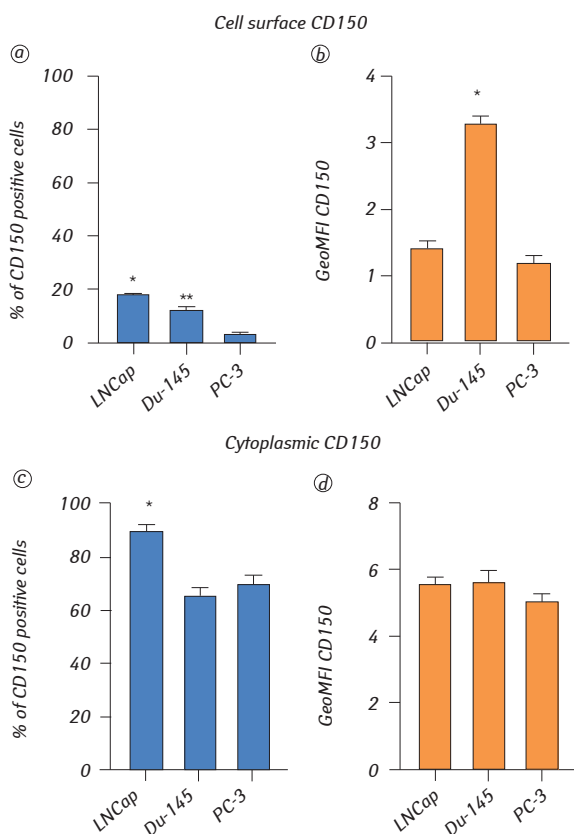


Fig. 2. CD150 protein expression and topology in PC cell lines. Results of flow cytometry analysis are presented as mean of three independent experiments ($\pm$ SEM): *a* — percent of cell surface CD150-positive cells; *b* — CD150 cell surface expression level; *c* — percent of cytoplasmic CD150-positive cells, *d* — CD150 expression level in the cytoplasm, * $p < 0.05$ compared to highly malignant cells, ** $p < 0.05$ compared to LNCap and PC-3 cell lines

$18.0 \pm 0.3\%$ csCD150⁺ cells. At the same time, the levels of CD150 expression did not significantly differ in these cell lines (1.4 ± 0.1 and 1.2 ± 0.1 , respectively) (Fig. 2, *b*). The highest level of csCD150 expression was found in Du-145 PC cells (3.3 ± 0.1) compared to LNCap and PC-3 cells ($p < 0.05$), while the number of csCD150⁺ Du-145 cells was $12.0 \pm 1.2\%$. Therefore, despite the fact that the percentage of csCD150⁺ Du-145 cells was significantly lower

than csCD150⁺ LNCap, the expression level of this marker on more aggressive PC cell line is significantly higher than on cells of less aggressive phenotype. This trend was not observed in PC-3 cells, which can be explained by the lower percentage of csCD150⁺ cells (at the limit of antigen detection). As for the cytoplasmic CD150 expression, the highest number of cyCD150⁺ cells was also recorded in the hormone-sensitive cell line LNCap ($90.0 \pm 2.2\%$), while in Du-145 and PC-3 cell lines, that are hormone-insensitive and more aggressive, percentage of cyCD150⁺ cells was significantly lower ($66.0 \pm 2.6\%$ and $70.0 \pm 3.2\%$, $p < 0.05$, respectively) (Fig. 2, *c*).

Thus, PC cell lines differed in topology and expression level of CD150. The highest number of CD150⁺ cells expressing CD150 both on the plasma membrane and in the cytoplasm was detected in less malignant hormone-sensitive non-metastatic LNCap cell line, but the highest expression level of this marker was found in the cells of hormone-insensitive Du-145 cell line.

CD150 receptor has several alternatively spliced structurally different isoforms that can possess different signaling properties and mediate multiple functions [3, 9]. What is the profile of CD150 isoforms expression in BC and PC cell lines? We analyzed mRNA expression level of canonical transmembrane mCD150 isoform with two ITSM signaling motifs in cytoplasmic tail, novel nCD150 isoform without known signaling motifs and soluble sCD150 isoform that lack transmembrane part and cannot be translocated to the cell surface.

All analyzed BC cell lines (T47D, MDA-MB-231, BCC/P and BC/ML) expressed conventional transmembrane mCD150 isoform and novel transmembrane isoform nCD150 (Fig. 3). Only BCC/P BC cell line expressed soluble or secreted sCD150 isoform. The highest mRNA expression level of mCD150, nCD150 and sCD150 isoform was observed in BCC/P cell line (Fig. 3). The mCD150 isoform was predominant in MDA-MB-231 and BCC/P cell lines, while in T47D and BC/ML mCD150 and nCD150 isoforms were expressed at the same level.

As for the PC cell lines, the mRNAs of three analyzed CD150 isoforms were found in LNCap and PC-3 cell lines with prevalence of nCD150 (Fig. 3). The Du-145 cells expressed only mCD150 and nCD150 isoforms. The highest mRNA expression level of CD150 isoforms was detected in LNCap cell line (Fig. 3).

We did not reveal any associations between CD150 protein expression, topology and CD150 isoforms mRNA expression. Exclusively cytoplasmic CD150 expression in BC cell lines shown in present study cannot currently be explained. However, CD150 cytoplasmic localization is not unique to studied cell lines. This phenomenon was observed in malignant csCD150⁺ CLL B cells, monocytes, inactivated dendritic cells, glioma cell lines [5, 8, 14]. Malignant cells may have disrupted vesicular trafficking that impairs CD150 transport from the intracellular compartments to the cell surface. For example, in dendritic

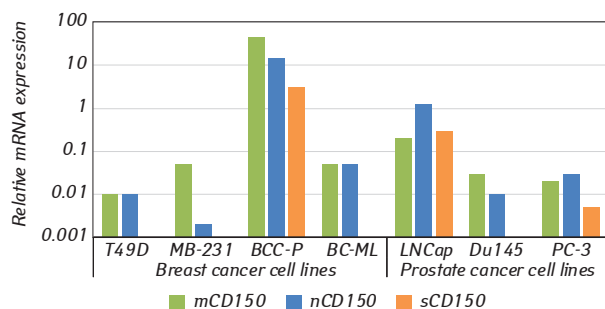


Fig. 3. Profile of CD150 isoforms mRNA expression in BC and PC cell lines. Quantitative real-time PCR. The presented results are from one of three independent experiments

cells, CD150 translocation to the cell surface depends on sphingomyelinase activity and ceramide generation that are essential for lipid raft formation [15]. Another possibility that could explain cytoplasmic CD150 localization is differential CD150 isoforms expression in BC and PC cell lines. However, our data demonstrate that predominant isoform in BC and PC cell lines is transmembrane mCD150 and nCD150 that potentially could be translocated to the cell surface. Most studied cell lines did not express sCD150 isoform. Thus, it is more likely that CD150 retention in cytoplasm is associated with the disruption of cell signaling that coordinate vesicular trafficking.

Taking together, we studied CD150 protein and mRNA expression in BC and PC cell lines that varied in CD150 cellular localization and CD150 isoforms expression profile. BC cell lines retained CD150 exclusively in the cytoplasm while PC cell lines showed differential levels of cell surface and cytoplasmic CD150 expression. High CD150 expression level was associated with less aggressive phenotype of BC and PC cell lines. Intracellular CD150 expression level was significantly higher in low malignant T49D BC cell line. Similarly, more CD150⁺ cells were detected in PC cell line LNCap with less aggressive phenotype.

Considering the functional significance of CD150 in hematopoietic cells, differential CD150 isoforms expression and aberration of CD150 expression in malignant hematopoietic cells, we assume the prospects of the comprehensive determination of CD150 topology and expression in primary BC and PC tissue samples with the focus on the association with malignancy degree, tumor stage, molecular profile, etc. These studies will be helpful for finding new CD150 functions outside hematopoietic system and developing the fundamental base for understanding biological features and improving prognosis of BC and PC.

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ЕКСПРЕСІЯ ТА ТОПОЛОГІЯ SLAMF1/CD150 В КЛІТИНАХ ЛІНІЙ РАКУ ПЕРЕДМІХУРОВОЇ ЗАЛОЗИ ТА РАКУ МОЛОЧНОЇ ЗАЛОЗИ

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Стан питання: Рецептор SLAMF1/CD150 абераційно експресується у злоякісних клітинах гемопоетичного ряду порівняно зі своїми нормальними клітинними аналогами. Однак дані про експресію і функцію CD150 поза межами гемопоетичної системи є досить обмеженими. **Метою** цього пілотного дослідження було вивчення профілю експресії мРНК ізоформ CD150 та топології білка у клітинах ліній раку молочної залози (РМЗ) та раку передміхурової залози (РПЗ) з високим і низьким ступенем злоякісності. **Матеріали та методи:** Дослідження проводили на клітинах ліній раку молочної залози (РМЗ) T47D, MDA-MB-231, BCC/P та BC/ML і клітинах ліній РПЗ LNCaP, Du-145 та PC-3. Для дослідження експресії мРНК ізоформ CD150 застосовували кількісну полімеразну ланцюгову реакцію, а для визначення локалізації білка — проточну цитометрію. **Результати:** Досліджені клітини лінії РМЗ не експресували CD150 на плазматичній мембрані (csCD150⁺), але біль-

ше як 45% клітин експресували CD150 у цитоплазмі клітин (cyCD150⁺). Рівень експресії cyCD150 у клітинах T47D люмінального молекулярного підтипу був вищим, ніж у клітинах ліній MDA-MB-231, BCC/P та BC/ML базального молекулярного підтипу. Клітини лінії РПЗ експресували CD150 як на плазматичній мембрані, так і в цитоплазмі. Найбільша кількість csCD150⁺ і cyCD150⁺ клітин була виявлена у менш агресивній, чутливій до андрогенів, неметастатичній лінії клітин LNCaP. Усі досліджені клітини лінії РМЗ і РПЗ експресували мРНК класичної трансмембранної mCD150 та нової nCD150 ізоформ, проте відрізнялися за їх домінуванням. Розчинна ізоформа CD150 була виявлена на низькому рівні лише у клітинах лінії BCC/P РМЗ та клітинах ліній LNCaP і PC-3 РПЗ. **Висновок:** Нами встановлено, що клітини лінії РМЗ і РПЗ експресують багатфункціональний рецептор CD150 на рівні мРНК і білка. Диференційна експресія і локалізація CD150 у клітинах ліній РМЗ і РПЗ може вказувати на участь CD150 у формуванні молекулярного фенотипу злоякісних клітин і асоціюється зі ступенем їх злоякісності. Це дослідження відкриває перспективи для подальшого дослідження експресії CD150 у зразках первинних пухлин з урахуванням їх індивідуальних особливостей.

Ключові слова: SLAMF1/CD150, рак передміхурової залози, рак молочної залози, клітинні лінії.