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The biological properties of HEK293T cell line transfected with mCD150 and nCD150 isoforms of CD150/SLAMF1 receptor

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Aim. To elucidate the role of CD150/SLAMF1 receptor isoforms mCD150 and nCD150 in regulation of HEK293T cells proliferation activity and clonogenicity. **Methods.** Cell culture, transfection, qPCR, flow cytometry, cells proliferation and cell cycle analysis, CFU assay and statistical analysis were used. **Results.** As a result of transfection of HEK293T cell line with mCD150 or nCD150 cDNA, two sublines were obtained with stable and exclusively cytoplasmic expression of CD150 isoforms – HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150. HEK293T cells transfected with mCD150 or nCD150 isoforms are characterized by higher proliferation supported by an elevated level of the IPO38 antigen expression compared to HEK293T and HEK293T-pBABE-puro. The analysis of cell cycle revealed a significantly higher percentage of HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150 cells in S phase and lower in G0/G1 in contrast to HEK293T and HEK293T-pBABE-puro. Compared to the control cell line, a significantly elevated percentage of HEK293T stably transfected with the nCD150, but not with the mCD150 isoform, were observed in G2/M phase of cell cycle. Both HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150 cells were able to form colonies at a low cell density, contrary to HEK293T and HEK293-pBABE-puro. **Conclusion.** Both mCD150 and nCD150 isoforms possess cell-growth promoting properties stimulating cell proliferation, cell cycle progression and clonogenic potential with more profound effect of the nCD150 isoform.

Keywords: CD150/SLAMF1 receptor, mCD150 isoform, nCD150 isoform, HEK293T cell line, cell proliferation, IPO-38, clonogenicity

Introduction

Studying the aberrant genes expression in malignant cells is not enough to understand the tumors pathogenesis. Recent studies have revealed that the differential isoforms expression of the key components of cell signaling pathways, or the enzymes with a high degree of homology also contribute to the tumors heterogeneity and clinical outcome for patients [1,

2]. Often, these isoforms demonstrate antagonistic functions and are associated with variable signaling pathways that regulate the biological properties of tumor cells. For example, analysis of the Akt1, Akt2, and Akt3 isoforms expression in tumors of different histogenesis, analysis of phosphoproteom and the usage of experimental model systems revealed the different signaling pathways mediated by these isoforms and their differential role in tumor cells migration, regulation of the cell cycle and RNA metabolism [3-6]. Various JNK isoforms can mediate both pro- and anti-apoptotic functions [7]. Recently, it has been shown that different kinases of the PKD family also have opposite effects on tumor cells survival and metastasis activity [8, 9]. Moreover, PKD1 is shown to act as a tumor suppressor whereas PKD2 is a promoter of the tumor growth.

Numerous experimental evidences of the heterogeneous isoforms expression of cell receptors, previously recognized as diagnostic markers and targets for tumor therapy, and their differential roles in the regulation of the tumor cells biological properties, have been reported. Among them there are estrogen and progesterone receptors [10], prolactin [11], androgen, epidermal growth factor [12] and 7 isoforms of Her (human epidermal growth factor receptor related) receptor [13]. It becomes clear that differential expression and functions of individual isoforms of target molecules should be taken into account for the development of molecular diagnostics and effective antitumor therapy.

The cell surface CD150/SLAMF1 receptor that possesses signaling properties and has several structurally different isoforms could be considered as a potential diagnostic, prognostic marker or even a target for the measles virus based antitumor therapy. The experimentally confirmed CD150 isoforms include the following: full transmembrane (mCD150) with two ITSM signaling motifs in cytoplasmic tail, soluble (sCD150) – without 30 amino acids in the transmembrane part of the molecule and a novel isoform (nCD150), which has a unique cytoplasmic domain formed from the previously unrecognized Cyt-new exon [14]. Noteworthy, CD150 isoforms are differentially expressed in tumors of different tissue origin. Thus, mCD150, nCD150 and sCD150 isoforms are present in various hematological malignancies with the mCD150 expression predomination [15, 16]. However, central nervous system tumors are characterized exclusively by the nCD150 isoform expression [17]. The expression pattern of CD150 isoforms and their structural differences allow a hypothesis of the CD150 functional diversity. Up to date, all data on the CD150 signaling properties and function concern mostly the mCD150 isoform. Signaling and biological properties of other isoforms have not yet been investigated. The mCD150 and nCD150 isoforms are in focus of our present study since despite similar extracellular part they have structurally different cytoplasmic tails and potentially could possess various signaling properties underlaying the cell's biological properties. Construction of the experimental model systems with the expression of individual CD150 isoforms is one of the essential conditions to explore their function. Therefore, in order to understand the functions of mCD150 and nCD150 isoforms we have created HEK293T cells sublines with differential constitutive mCD150 and nCD150 expression.

Materials and Methods

Cell lines and transfection. The HEK293T cell line (human embryonic kidney cells) (Bank of cell lines of human and animal tissue, R.E. Kavetsky IEPOR of NASU) was used to obtain HEK293T sublines by transfection with pBABE-mCD150 (HEK293T-pBABE-mCD150) or pBABE-nCD150 (HEK293T-pBABE-nCD150) plasmids using GeneJuice® reagent according to manufacturer's protocol (Sigma-Aldrich, USA). The HEK293T cells transfected with pBABE-puro plasmid (HEK293T-pBABE-puro) were used as an additional control subline. The pBABE-puro plasmid and constructs of pBABE plasmids with mCD150 and nCD150 cDNA were kindly performed and provided by Prof. Alexander V. Taranin (Institute of Molecular and Cellular Biology of SB RAS, Novosibirsk, Russia). Selection of transfected cells was performed on the culture medium with selective antibiotic puromycin in the 1 μg/ml concentration during at least two weeks. All cell lines were maintained in DMEM culture medium containing 10 % FBS (Sigma, Germany), 1 % a mixture of essential amino acids (NEAA mix) (Sigma, Germany), 10 μg/ml penicillin, and 0.25 μg/ml streptomycin at 37°C with 5 % CO_2 in a humidified atmosphere.

Flow cytometry. Cell surface CD150 expression was detected using mouse anti-CD150 (IPO-3) monoclonal antibodies (mAbs) (IEPOR of NASU, Ukraine) as described earlier [15]. The standard indirect immunofluorescent method was used to study the intracellular CD150 expression and the detection of nuclear antigen of proliferation cells IPO-38, which was determined by mAbs IPO-38 (IEPOR of NASU, Ukraine). Cells were prefixed with 2 % paraformaldehyde for 15 min, followed by washing in PBS and then permeabilized with 0.3 % Triton X-100 for 15 min. Stained cells were examined with an EpicsXL fluorescence flow cytometer (Beckman Coulter, USA). The research results are presented in the GeoMFI index – GeoMean MFI ratio of antigen to isotype control.

qPCR analisys. Total RNA was isolated from $1x10^6$ 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 [15, 16]. The primers sequences to mCD150, nCD150 and control TBP (TATA-box binding protein) gene were published earlier [15]. The Ct values for target genes were determined and normalized to Ct value of TBP internal control gene using comparative Ct (ddCt) method.

Evaluation of cell proliferation. To assay the cell proliferation, each cell line was seeded in triplicates at concentration of $6x10^4$ cells per well in 24-well culture plates. The number of living and dead cells was calculated after 24, 48 and 72 h of cultivation using trypan blue exclusion test. The cell population doubling time (PDT) was calculated according to the following standard formula [18]: PDT = T / 3.32x(logXk – logX0), where Xk — number of obtained cells; X0 — number of plated cells; T — cell culture time.

Cell cycle analysis. The cell lines were harvested by trypsin-EDTA and fixed in ice-cold 70 % ethanol. The fixed cells were washed with PBS and then suspended in 500 μL of FxCycle PI / RNAse Staining Solution (Invitrogen, USA) for 30 min at 4°C. Cell distribution in different cell cycle phases was

determined by flow cytometry (Beckman Coulter, USA) with following analysis by Modfit software.

Colony-forming unit (CFU) assay. To assess the ability of growing at a clonal density (at low cell seeding density resulting in formation of discrete colonies having a clonal origin, i.e. clonal colonies [18]), HEK293T, HEK293T-pBABE-puro, HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150 cells were seeded in triplicate in 6-well culture plates at concentration 1×10^3 cells per well and cultured in complete growth media during 10 days. After that, the cells were washed with PBS, fixed and stained with 0.1 % crystal violet in 20 % methanol. The number and size of colonies were calculated under the light microscope. To assess the effectiveness of colony formation (plating efficiency, PE) the following formula was used: PE (%) = (no. colonies counted/no. cells seeded) x100.

Statistical analysis. The data were analyzed using Student's T test. The results were presented as the mean ± SD. The differences were considered significant at $p < 0.05$. Each experiment was repeated at least 3 times.

Results and Discussion

To shed the light on the CD150 isoforms functions we have created experimental model systems based on the HEK293T cell line with differential mCD150 and nCD150 expression. To reveal how ectopic mCD150 and nCD150 expression may affect the biological properties of cells, the proliferation activity, cell cycle progression, and colony forming ability were examined in HEK293T cell line, HEK293T-pBABE-puro, HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150.

Before starting the experiments, the CD150 expression by qPCR and flow cytometry were detected to verify the transfection effectivity in HEK293T cell line. It was shown that HEK293T and HEK293T-pBABE-puro cells did not express mRNA of the mCD150 and nCD150 isoforms of mRNA. The mCD150 isoform mRNA expression was detected exclusively in HEK293T-pBABE-mCD150 cells (ΔCt=108.75 r.u.) whereas the nCD150 mRNA expression was specific for HEK293T-pBABE-nCD150 subline (ΔCt=49.9 r.u.). Noteworthy, PCR is the only method that allows differentiation of the CD150 isoforms expression because the known antibody recognized an extracellular part of CD150 which is identical in all CD150 isoforms.

The HEK293T cell line and HEK293T-pBABE-puro subline were negative on cell surface (data not shown) and cytoplasmic CD150 expression (Fig.1, A, B). Despite the fact that CD150 is the cell surface receptor its expression was not revealed on the surface membrane of HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150 cells (data not shown). At the same time, CD150 was detected in the cytoplasm of HEK293T-pBABE-mCD150 (Fig.1, C) and HEK293T-pBABE-nCD150 cells (Fig.1, D). We found that the CD150 expression level in cytoplasm of HEK293T-pBABE-mCD150 cells was slightly higher compared to the HEK293T-pBABE-nCD150 cells: the CD150 GeoMFI index were 5.33 and 5.07 respectively (Fig. 1).

Exclusively cytoplasmic CD150 localization in HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150 sublines seems not to be associated with the results of artificial transformation procedure, whereas the

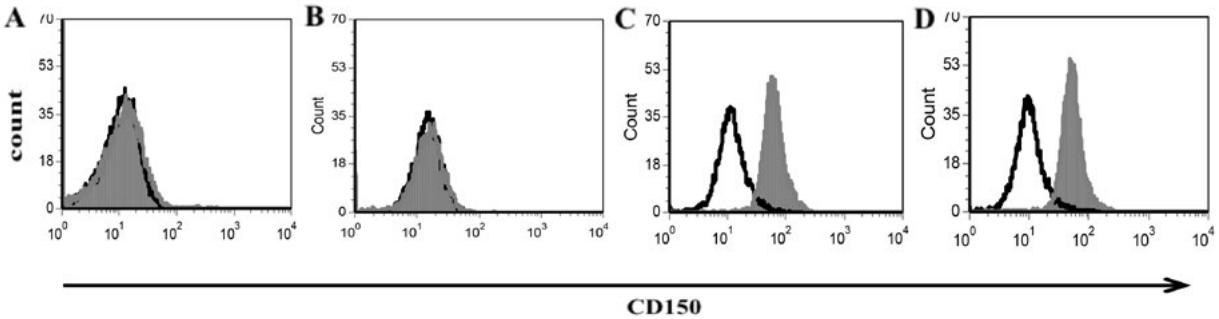

Fig.1. Intracellular expression of CD150 in HEK293T (***A***), HEK293T-pBABE-puro (***B***), HEK293T-pBABE-mCD150 (***C***) and HEK293T-BABE-nCD150 (***D***) cells. Gray histograms represent cells stained with anti-CD150 mAbs compared with black profile histogram of control isotype mAbs. Flow cytometry analysis.

intracellular CD150 expression was previously described in several types of normal and malignant cells. Thus, the cytoplasmic CD150 distribution was revealed in non-activated peripheral blood monocytes [19], glioma cell lines [17] and chronic lymphocytic leukemia B cells that do not express CD150 on the cell surface [15]. Up to now, the reasons of CD150 retaining in cytoplasmic compartment are not clear. Possibly, it could be associated with the disruption in an intracellular trafficking system.

Thus, we have created stable HEK293T sublines with differential expression of mCD150 and nCD150 isoforms that are localized exclusively in the cytoplasm, but not on the cell surface membrane.

Transfection of the HEK293T cell line with plasmids containing cDNA of mCD150 or nCD150 isoforms did not affect cell viability. It was shown that the number of dead cells in HEK293T, HEK293T-pBABE-puro, HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150 sublines after 72 h of cultivation was not significantly different and accounted in the range from 3.6 % to 5.1 %.

Proliferative activity is one of the key biological features of the cells, the rate of which is significantly different in the malignant cells in contrast to their normal counterparts. Several evidences indicate that CD150 could be involved in regulation of the cells proliferation activity. The first functional studies of CD150 showed that the CD150 ligation on naïve B lymphocytes enhances their proliferation induced by anti-CD40 mAbs and IL-4 [20]. Similarly, the CD150 engagement by the A12 mAbs leads to an increase of the anti-CD3-mediated proliferation of T cells [21]. Moreover, CD150 mediates Akt, MAPK, and NF-kB signaling pathways in normal and malignant B cells that may result in regulation of cell proliferation rate [15, 22–24].

In current study we showed for the first time that the HEK293T cells expressing mCD150 or nCD150 isoforms significantly differ in their proliferative activity compared to the control HEK293T and HEK293T-pBABE-puro cells (Fig. 2). After 24 hours of cultivation, the number of HEK293T-pBABE-mCD150 cells increased 2.5 times, HEK293T-pBABE-nCD150- 3.2 times, whereas the number of

HEK293T and HEK293T-pBABE-puro – only 1.8 times. A significantly higher proliferation rate of HEK293T cells that stably expressed the mCD150 or nCD150 isoforms compared to the control sublines was observed at all time points of cultivation, which is reflected by the population doubling time (PDT) values. The PDT was 30.02±1.05 h for HEK293T, 27.61±1.8 h for HEK293T-pBABE-puro vs 22.17±2.1 h for HEK293T-pBABE-mCD150 and 19.42±2.7 for HEK293T-pBABE-nCD150 cells ($p<0.03$). Important to note, the proliferation activity of HEK293T-pBABE-nCD150 cells was significantly higher than that of HEK-293T-pBABE-mCD150 cells during all time of cultivation ($p=0.001$) (Fig. 2). Similarly, the PDT in HEK293 transfected with nCD150 isoform was slightly shorter compared to mCD150 transfectants.

To clarify further the revealed differences in the proliferative activity of HEK293T cells expressing CD150 isoforms, we examined the expression level of nuclear antigen of proliferating cell IPO-38 [25], which is not identical to cyclo-dependent antigens such as proliferating cell nuclear antigen (PCNA) and Ki-67. The mAbs to PCNA and Ki-67 detect the cells in the late G1, S, G2 and M phase of the cell cycle, whereas the early G1 phase remains beyond analysis, that is a serious drawback of these mAbs [26, 27]. Anti-IPO-38 mAbs identify the expression of nuclear antigen that is inherent in all active phases of the cell cycle including the early G1 [28].

Flow cytometry analysis showed that HEK293T-pBABE-nCD150 and HEK293T-pBABE-mCD150 cells expressed IPO-38 antigen at a significantly higher level in comparison with control sublines ($p<0.02$). The IPO-38 GeoMFI index was 16.9±0.2 r.u. and 15.3±0.3 r.u. for HEK293T-pBABE-nCD150 and HEK293T-pBABE-mCD150 sublines respectively. No significant differences were found when comparing the IPO-38 GeoMFI index of HEK293T (13.4±0.4 r.u.) and HEK293T-pBABE-puro (13.6±0.3 r.u.) cells.

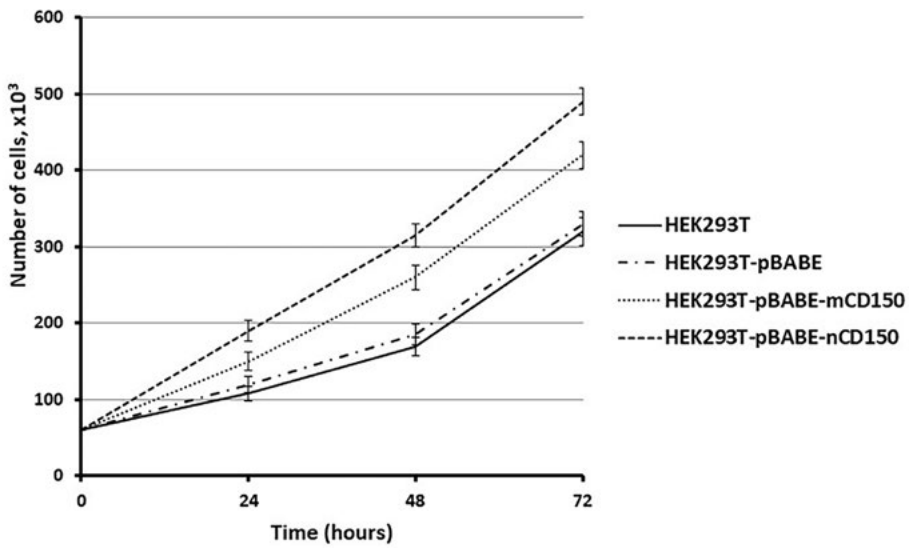

Fig. 2. The HEK293T, HEK293T-pBABE-puro, HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150 proliferation kinetic

Thus, the transfection of HEK293T cell line with the mCD150 or nCD150 isoforms sequences leads to increasing the cell's proliferation activity supported by the elevated IPO-38 expression level. The nCD150 isoform induces the cells proliferation significantly stronger than mCD150 isoform.

The flow cytometry analysis of the cellular DNA content in both control cell lines, HEK293T-pBABE-nCD150 and HEK293T-pBABE-mCD150 was performed. A significantly higher percent of the HEK293T-pBABE-nCD150 and HEK293T-pBABE-mCD150 cells was detected in S phase of the cell cycle ($p<0.0003$) and, accordingly, lesser percent – in the G0/G1 phase ($p<0.03$) in comparison with the control HEK239T cells non-transfected and transfected with an empty vector (Fig. 3). The number of cells that undergo G2/M phase was increased in the HEK293T-pBABE-nCD150 sublines in contrast to HEK293T ($p=0.001$), HEK293T-pBABE-puro ($p=0.0005$) and HEK293T-pBABE-mCD150 ($p=0.02$). Notable, the percent of HEK293T-pBABE-mCD150 cells in the G2/M phase of cell cycle was similar to that in both control sublines (Fig. 3). The HEK293T-pBABE-nCD150 cell proliferation index (76.8±2.5 %) was notably higher compared to the HEK293T-pBABE-mCD150 one (66.5±1.7 %) ($p<0.03$). Thus, the results of our study indicate that the expression of mCD150 and nCD150 isoforms promotes progression of HEK293T cells through the cell cycle by reducing their number in the G0/G1 phase and accumulating the cell population in the S phase of cell cycle. Moreover, it seems that the nCD150 isoforms drive the cells to quick transition from S to G2/M phase of the cell cycle resulting in a higher proliferation rate. In contrast to the nCD150 effect, the mCD150 isoform transfection leads to retaining cells in the S phase. Therefore, using several methodological approaches, we have demonstrated that both mCD150 and nCD150 isoforms possess the properties promoting cell growth by stimulating the cell proliferation activity and cell cycle progression with more profound effect of the nCD150 isoform.

One of the biological characteristics of cells taken into account after different genetic interventions is the ability to reproduce viability

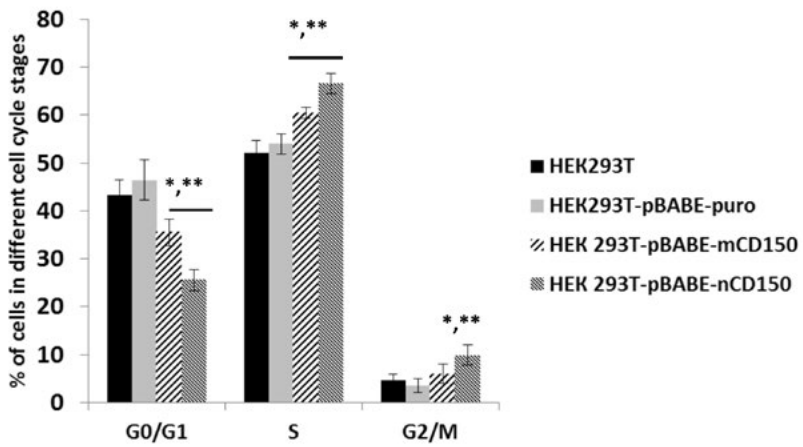

Fig.3. Distribution of HEK293T, HEK293T-pBABE-puro, HEK293T-pBABE-nCD150 and HEK293T-pBABE-mCD150 cells according to the cell cycle phases. The percent of cells in each phase of cell cycle was calculated using the cell ModFit software after flow cytometry analysis. * – $p<0.05$ compared to HEK293T; ** – $p<0.05$ compared to HEK293T-pBABE-puro.

or clonogenic potential. This property has been studied using a colony-forming unit (CFU) assay, the method that allows determination of the ability of one cell to form a colony. We used this approach to determine the transfection effect of the mCD150 and nCD150 isoforms on the HEK293T cells clonogenicity.

After ten days of cultivation none colony was observed in the control non-transfected HEK293T cell line and HEK293T cells transfected with empty vector. In contrast, both HEK293T-pBABE-mCD150 and HEK293T-pBABE-nCD150 cells formed colonies (Fig. 4). The clonogenicity of HEK293T-pBABE-mCD150 subline was slightly higher (PE=17.5±1.2 %) compared to HEK293T-pBABE-nCD150 cells (PE=15.6±1.0 %). It

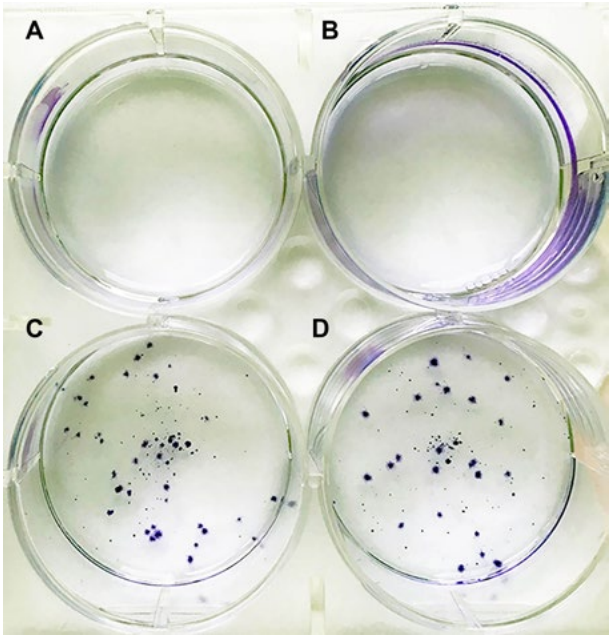

Fig.4. Colony formation assay of the HEK293T cells transfected with the CD150 isoforms encoding constructs. Representative cultured wells with HEK293T (A), HEK293T-pBABE (B), HEK293T-pBABE-mCD150 (C) and HEK293T-pBABE-nCD150 (D) cells.

should be noted the significant heterogeneity of HEK293T-pBABE-mCD150- and HEK293T-pBABE-nCD150-derived colonies in size. The major part of HEK293T-pBABE-mCD150 colonies was medium (up to 50 cells), whereas HEK293T-pBABE-nCD150 produced large (more than 100 cells) colonies. This suggests that HEK293T expressing nCD150 isoform acquired a higher viability and proliferation potential than HEK293T transfected with mCD150 cDNA.

Consequently, using the HEK293T cell line as a model system we showed that both mCD150 and nCD150 isoforms are involved in the regulation of cells proliferation activity and colony forming potential. Nevertheless, the mechanisms that could explain the revealed function of the CD150 isoforms are still elusive. One possibility is that the CD150 links to the regulation cyclin dependent kinases (CDK), the activity of which is essential to the cell cycle progression and proliferation. Thus, CD150 ligation in the primary chronic lymphocytic leukemia B cells causes increasing level of the CDK substrates phosphorylation [15]. However, this possibility is unlikely, given that the mCD150 and nCD150 isoforms are located exclusively in cytoplasm of HEK293T transfectant and could not be activated as a classic receptor via ligand interaction. Hypothetically, cytoplasmic CD150 could be incorporated into the membrane of some intracellular compartment and serve as a docking molecule regulating the signaling complex assembly, it transports *etc*. It was shown, that in human macrophages CD150 is localized in the endocytic recycling compartment and controls the traffic of Toll receptor-associated molecule (TRAM) modulating the TLR4 sig-

naling [19]. Thus, CD150 could act not only as a costimulatory molecule, but also as an adaptor protein and regulator of the signaling molecule transport.

Conclusion

Taken together, the transfection of HEK293T cells with mCD150 or nCD150 cDNA sequences leads to unidirectional changes associated with the increasing of cells proliferation activity and colony formation ability. At the same time, nCD150 isoform shows significantly stronger cell growth-promoting effect than mCD150. Though the mechanisms of the mCD150 and nCD150 isoforms functioning need to be studied, the obtained data about the mCD150 and nCD150 isoforms impact on the cell biological properties should be considered for understanding the CD150 role in tumors, especially outside the hematopoietic system.

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Біологічні властивості клітин лінії HEK293T, трансфікованих mCD150 та nCD150 ізоформами рецептора CD150/SLAMF1

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Мета. З'ясувати роль mCD150 та nCD150 ізоформ рецептора CD150/SLAMF1 у регуляції проліферативної активності та колонієутворювальної здатності клітин лінії HEK293T. **Методи.** В роботі були використані

методи культури клітин, трансфекції, кількісної ПЛР, проточної цитометрії, аналізу проліферації клітин, клітинного циклу, формування колоній та статистичні методи. **Результати.** В результаті трансфекції клітин лінії HEK293T послідовностями кДНК mCD150 або nCD150 ізоформ CD150 було отримано дві сублінії зі стабільною диференційною та виключно цитоплазматичною експресією цих ізоформ – HEK293T-pBABE-mCD150 та HEK293T-pBABE-nCD150. Клітини HEK293T, трансфіковані mCD150 або nCD150 ізоформами, характеризуються більш високою проліферативною активністю та рівнем експресії ядерного антигена проліферуючих клітин IPO-38 порівняно з клітинами HEK293T та HEK293T-pBABE-puro. Аналіз розподілу клітин за фазами клітинного циклу виявив, що достовірно вищий відсоток клітин HEK293T-pBABE-mCD150 та HEK293T-pBABE-nCD150 перебуває у S і менший у G0/G1 фазі проти такого в клітинах HEK293T і HEK293T-pBABE-puro. Крім того, достовірно вищий відсоток клітин HEK293T, трансфікованих кДНК nCD150, але не mCD150 ізоформою, встановлено і у G2/M фазі клітинного циклу порівняно із клітинами контрольних сублінії. З'ясовано, що за низької щільності посіву як клітини HEK293T-pBABE-mCD150, так і HEK293T-pBABE-nCD150 здатні формувати колонії, чого не спостерігається у клітинах HEK293T та HEK293-pBABE-puro. **Висновок.** mCD150 та nCD150 ізоформи мають властивості, що сприяють посиленню проліферативного та клоногенного потенціалу клітин з більш вираженим ефектом ізоформи nCD150.

Ключові слова: рецептор CD150/SLAMF1, mCD150 ізоформа, nCD150 ізоформа, лінія клітин HEK293T, проліферація, IPO-38, колонієутворення.

Биологические свойства клеток линии HEK293T, трансфицированных mCD150 и nCD150 изоформами рецептора CD150/SLAMF1

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Цель. Выяснить роль mCD150 и nCD150 изоформ рецептора CD150/SLAMF1 в регуляции пролиферативной и клоногенной активности клеток линии HEK293T. **Методы.** В работе были использованы методы культуры клеток, трансфекции, количественной ПЦР, проточной цитометрии, анализа пролиферации клеток, клеточного цикла, формирования колоний и статистические методы. **Результаты.** В результате трансфекции клеток линии HEK293T последовательностями кДНК mCD150 или nCD150 изоформ CD150 было получено две сублинии со стабильной дифференциальной и исключительно цитоплазматической экспрессией этих изоформ – HEK293T-pBABE-mCD150 и HEK293T-pBABE-nCD150. Клетки HEK293T, трансфицированные mCD150 или nCD150 изоформами, характеризуются более высокой пролиферативной активностью и уровнем экспрессии ядерного антигена пролиферирующих клеток IPO-38 по сравнению с клетками HEK293T и HEK293T-pBABE-puro. Анализ распределения клеток по фазам клеточного цикла обнаружил, что достоверно больший процент клеток HEK293T-pBABE-mCD150 и HEK293T-pBABE-nCD150 находится в S и меньший в G0/G1 фазе против такого в клетках HEK293T и HEK293T-pBABE-puro. Кроме того, достоверно более высокий процент клеток HEK293T, трансфицированных кДНК nCD150, но не mCD150 изоформы, установлено и в G2/M фазе клеточного цикла по сравнению с клетками контрольных сублиний. Установлено, что при низкой плотности посева как клетки HEK293T-pBABE-mCD150 так и HEK293T-pBABE-nCD150 имеют колониеобразующую способность, которая не наблюдается в клетках HEK293T и HEK293-pBABE-puro. **Вывод.** mCD150 и nCD150 изоформы обладают свойствами, способствующими усилению пролиферативного и клоногенного потенциала клеток с более выраженным эффектом изоформы nCD150.

Ключевые слова: рецептор CD150/SLAMF1, mCD150 изоформа, nCD150 изоформа, линия клеток HEK293T, пролиферация, IPO-38, колониеобразование.

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