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IMMUNOHISTOCHEMICAL STUDIES OF CD150 EXPRESSION IN SOME HUMAN TUMORS

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ИММУНОГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ЭКСПРЕССИИ РЕЦЕПТОРА CD150 В НЕКОТОРЫХ ОПУХОЛЯХ ЧЕЛОВЕКА

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CD150 belongs to novel CD2/CD150 subfamily of cell surface receptors. Expression of this receptor was found on activated T and B lymphocytes, monocytes and dendritic cells. CD150 was shown to play a dual role in regulation of lymphocyte fate and a potential to transmit positive or negative signals. The objective of this paper was to study CD150 expression in normal human tissues and tumors of different histogenesis using immunohistochemical approach. Our pillot tissue screening studies revealed CD150 expression outside of hematopoietic system: in basalioma and squamous cell carcinomas of uterine cervix, rectum and oral cavity. These tumors have ectodermal origin and their normal counterparts did not show expression of CD150. Detailed studies of non-Hodgkin's lymphomas showed CD150 expression in T-cell lymphoma, mantle cell lymphoma (very low level) and in diffuse large B-cell lymphoma with activated B cell phenotype that is in accordance with CD150 expression in their normal counterparts. We also found CD150 expression in Hodgkin/Reed – Sternberg cells in Hodgkin's lymphoma. In these lymphomas, except mantle cell lymphoma, CD150 was coexpressed with the small adaptor protein SH2D1A. Since SH2D1A is involved in regulation of CD150 signaling and function, coexpression of SH2D1A and CD150 could contribute to pathogenesis of these types of lymphomas.
Key Words: **CD150, lymphocytes, non-Hodgkin's lymphomas, Hodgkin's lymphomas, immunohistochemistry.**

CD150 относится к новому суперсемейству клеточных поверхностных рецепторов CD2/CD150. Экспрессия данного рецептора была обнаружена на активированных Т- и В-лимфоцитах, моноцитах и дендритных клетках. Было показано, что CD150 играет двойную роль в регуляции клеточных программ лимфоцитов путем передачи положительных и отрицательных сигналов. Задачей данного исследования явилось изучение экспрессии CD150 в клетках некоторых нормальных тканей человека, а также в опухолях различного гистогенеза с использованием методов иммуногистохимии. В результате проведенного анализа установлено, что вне гемопоэтической системы рецептор экспрессирован на клетках базалиомы, плоскоклеточного рака шейки матки, пищевода, прямой кишки и ротовой полости. Данные опухоли имеют эктодермальное происхождение, однако на их нормальных аналогах экспрессия CD150 не наблюдалась. Детальное изучение неходжкинских лимфом и лимфогранулематоза показало, что CD150 экспрессируется на злокачественных клетках при Т-клеточной лимфоме, лимфомах из клеток мантийной зоны (слабая экспрессия) и при диффузных крупноклеточных В-клеточных лимфомах с фенотипом активированных В-клеток, что соответствует экспрессии CD150 на их нормальных аналогах. При лимфогранулематозе экспрессия наблюдалась в патогномоничных клетках Ходжкина и Березовского – Штернберга. При всех исследованных лимфомах, за исключением лимфомы из клеток мантийной зоны, CD150 был коэкспрессирован с адапторным белком SH2D1A. В связи с тем, что SH2D1A вовлечен в регуляцию передачи сигналов через CD150, коэкспрессия этих двух молекул может быть связана с патогенезом лимфом.
Ключевые слова: **CD150, лимфоциты, неходжкинские злокачественные лимфомы, лимфогранулематоз, иммуногистохимия.**

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Abbreviations used: ABC–DLBCL — diffuse large B–cell lymphoma with activated B cell phenotype; BCR — B cell receptor; BL — Burkitt's lymphoma; DLBCL — diffuse large B–cell lymphoma; GC — germinal center; GC–DLBCL — diffuse large B–cell lymphoma with germinal center phenotype; HL — Hodgkin's lymphoma; HRS — Hodgkin/Reed — Sternberg cells; LPL — lymphoplasmacytic lymphoma; MCL — mantle cell lymphoma; NHL — non–Hodgkin's lymphoma; SH2D1A — SH2 domain protein 1A; SLL — small lymphocytic lymphoma of B–cell origin; XLP — X–linked lymphoproliferative syndrome.

CD150 (IPO–3/SLAM) is a cell surface receptor that belongs to novel CD2/CD150 subfamily of Ig superfamily of cell surface receptors. Expression of this receptor was found on activated hematopoietic cells: T and B lymphocytes, monocytes and dendritic cells. High expression of CD150 was also revealed on thymocytes. CD150 was shown to play a dual role in regulation of lymphocyte fate, and as the B cell receptor (BCR), CD40 or CD95/Fas has a potential to transmit positive or negative signals [1, 2]. CD150 cytoplasmic tail possess specific signaling motif TxYxxI/V that serves a

docking site for a number of SH2–containing molecules — a key components of signal transduction pathways. These include: tyrosine phosphatase SHP–2, inositol phosphatase SHIP, Src–family kinases Lyn, Fgr, Lck and Fyn, and also small adaptor protein SH2D1A [3–7]. CD150 functions as a coreceptor molecule transmitting signals that initiate activation of ERK1/2 and Akt pathways [1, 4]. That is why CD150 is an attractive receptor for modulation of signal transduction pathways that regulate cell fate. However, a little is known about expression of this receptor outside of hematopoietic system and on malignant cells.

The aim of this study was to carry out wide and detailed analysis of CD150 in normal human tissues and tumors of different histogenesis. Since CD150 is upregulated on lymphoid cells after activation, special emphasis of this paper was on human lymphoma studies. We chose immunohistochemical methods as the most adequate approach for studies of CD150 in normal and malignant tissues. This allowed us to analyze CD150 expression in distinct tissues zones, and what is the most important — directly in tumor cells.

MATERIAL AND METHODS

CD150 expression was investigated in normal tissues and also in some benign and malignant tumors of different histogenesis. Biopsies and after–surgery tissues were obtained from liver, intestine, rectum, kidney, lung, thyroid gland, salivary glands, breast, skin, lymph nodes, and tonsils. At least 3 samples of normal tissue were analyzed. Each disease entity was defined on the basis of combination of morphologic and clinical features. Verification of diagnosis for non–Hodgkin's lymphoma and Hodgkin's disease was performed on the basis of combination of morphologic, immunophenotypic, and clinical characteristics. Tumor samples included: non–Hodgkin's lymphoma (26), Hodgkin's lymphoma (14), basalioma (2), fibromioma of the uterus (2), colorectal adenocarcinoma (6), adenocarcinoma of the breast (5), salivary glands (2), lung (4), stomach (2), kidney (2), squamous cells carcinomas of oral cavity (12), esophagus (2) and lower rectum (5).

Two step immunohistochemical method with monoclonal antibodies IPO–3 followed by dextran polymeric conjugate (EnVision) technique was used for CD150 antigen detection on cryostat tissue sections. Expression of SH2D1A was studied only on paraffin sections using either PAP complex or EnVision. Anti–SH2D1A rabbit antiserum was a kind gift from Dr. K. Nichols (USA).

The pieces of normal and tumor tissues were frozen in liquid nitrogen and placed into cryostat. 4–6 μm–thick sections were mounted further on poly–L–lysine coated slides, dried in air and fixed in acetone for 5 min at room temperature. Antigen expression was assayed at the same day, otherwise the specimens were wrapped into aluminum foil and stored at –20 °C. Immediately before immunohistochemical assay the frozen specimens were brought up to the room temperature for 20 min. In order to reduce nonspecific background, section were treated with normal rabbit serum and 0.1% solution of BSA. Then incubation with optimal dilutions of monoclonal antibody (mAB) anti–CD150 was performed. EnVision system was used in 30 min second step incubation. After washes in phosphate–buffered saline peroxidase activity was assayed using DAB. Then sections were counter–stained with haematoxylin for 1–2 min, embedded in balm and studied by light microscopy.

For investigation of SH2D1A expression on paraffin sections, pieces of tissues were fixed in 4% paraformaldehyde and embedded in paraffin. The 4–6 μm–thick paraffin sections were left for a day at 37 °C. Prior to immunohistochemical assay the specimens were deparaffined, placed in citrate buffer (pH 6.0) for antigen retrieval in microwave. Immunohistochemical investigation was carried out as described above.

Lymphomas were classified according to WHO classification [8]. Standard set of mAbs antibodies was used for immunophenotyping of lymphomas, which included: CD3 (RIV9), CD4 (RIV7), CD5 (CRIS–1), CD7 (124–1D1), CD8 (RIV11), CD10 (CB–CAAL), CD15 (BRA4F1), CD20 (93–1B3), CD22 (MYG13), CD37 (IPO–24), CD38 (AT1), CD45 (BRA–55), CD54 (1H4), CD95 (IPO–4), CD150 (IPO–3), kappa (L1C1), lambda (ICO–107), HLA–DR (IPO–10), IPO–38 (all mAbs produced and purified at the Institute of Experimental Pathology, Oncology and Radiobiology NAS, Kyiv, Ukraine), CD19 (HD37) and anti–IgM (4B8) mAbs were kindly provided by Dr. E.A. Clark, CD30 (Ber–H2) was from DAKO Corporation (USA). Final concentration of mAbs in immunohistochemical procedures was 20 μg/ml.

RESULTS AND DISCUSSION

Outside the hematopoietic system a limited number of cells in normal tissues expressed CD150 antigen. In skin CD150 was detected only on lymphocytes, which were localized under the epidermis. However, high density of CD150 receptor was detected in skin basalioma (data not shown). Cells in normal liver, kidney, lung, thyroid gland, salivary gland, rectum and breast tissues were CD150–negative. We also did not detect expression of CD150 in tumor cells of lung, stomach, breast, kidney, uterus and colorectal adenocarcinomas. However, tumor cells in squamous cell carcinomas of uterine cervix, esophagus rectum and oral cavity expressed CD150 (Fig.1). All these $CD150^+$ tumor cells have ectodermal origin and demonstrated intracellular localization of CD150. Besides that, 30% of malignant cells in adenocarcinomas of salivary gland were also CD150–positive.

Previous immunohistochemical and flow cytometry studies described CD150 expression in hematopoietic and lymphoid tissues [9]. Studies of lymph nodes, tonsils, spleen, thymus and lymphocytes from peripheral blood of healthy donors revealed CD150 expression on normal cells that have a cell surface phenotype of activated lymphocytes, monocytes and dendritic cells [9–14].

CD150 could bind the SH2–containing adaptor protein SH2D1A, which presumably regulates function of this receptor [1, 3, 15]. Expression of this adaptor protein was found in T–lymphocytes [16–18] , NK–cells [19, 20] and only in small subpopulation of B–cells in

tonsils [5]. At the same time some Burkitt's lymphoma cell lines and B lymphoblastoid cell lines MP–1 and CESS showed high level of SH2D1A both on mRNA and protein levels [5, 21].

One of the tasks of this study was to find out the stage of B–cell differentiation where CD150 and SH2D1A are co–expressed. Malignant cells in non–Hodgkin's lymphomas and Hodgkin's disease represent their normal analogs on the different stages of cell maturation [22, 23]. That is why the focus of this study was on parallel analysis of CD150 and SH2D1A expression in normal lymph nodes and tonsils, and malignant cells in different lymphomas.

In reactive lymph nodes and tonsils (Fig. 2, *a*) CD150 was localized in the cytoplasm and on the surface of germinal center cells and on the surface of the half of mantle zone B cells (Fig. 2, *b*). This receptor was found also on subpopulation of endothelial cells. In germinal center the number of SH2D1A$^+$cells often exceeded the number of CD150$^+$ cells, however only few scattered SH2D1A$^+$ cells were found in mantle zone (Fig. 2, *c*). On the other hand, CD150–negative interfollicular zone contained some SH2D1A$^+$ cells.

Morphological investigation of lymph nodes from patients with small lymphocytic lymphoma of B–cell origin (SLL) showed diffuse effacement of architecture. The lymphoma cells are small, with round nuclei, condensed chromatin, inconspicuous nucleoli, and scanty cytoplasm. Plasma cells are sparse or absent. The mitotic count is usually low (Fig. 3, *a*). From 2 patients on low–power examination the dark–staining lymphoid infiltrates were punctuated by pale, round foci representing proliferation centers ("pseudofollicles") — this is pathognomonic of SLL. Proliferation centers are typically non–expansible and comprise a mixture of prolymphocytes, which are slightly bigger than small lymphocyte, and have more diffuse chromatin, a distinct nucleolus, and a greater amount of pale cytoplasm. Malignant cell in SLL had following phenotype: IgM$^+$, CD5$^+$, CD10$^-$, CD19$^+$, CD20$^+$, CD22$^+$. In all investigated cases the level of surface IgM was low, and often CD20 expression was weak. The level of proliferating cells (IPO–38$^+$) was low, and did not exceed 10% of cells. Immunohistochemical examination of CD150 (Fig. 3, *b*) and SH2D1A (Fig. 3, *c*) in the cells of the lymph nodes from patients with B–SLL did not reveal expression these proteins in malignant cells, however we found CD150$^+$ large cells with long microvillies, probably dendritic cells, that are dispersed through SLL lymphoma tissue.

Mantle cell lymphoma (MCL) was defined morphologically as a neoplasm of monomorphic medium–sized B lymphoid cells of mantle zone of primary follicles. The growth pattern was diffuse without nodular structures. Lymphoma cells appear monotonous and were slightly larger than small lymphocytes. Their nuclei show variable degrees of indentation or sometimes the nuclei were round. The chromatin is fairy condensed and nucleoli are inconspicuous. Immunophenotype of malignant cells was: IgM$^+$, CD5$^+$, CD10$^-$, CD19$^+$, CD20$^+$, CD22$^+$. The mitotic count and the level of proliferating cells (IPO–38$^+$) were very high (80–85% of positive cells). Immunohistochemical investigation disclosed SH2D1A expression only in few scattered cells. Neoplastic cells expressed CD150 on very low level.

Sporadic Burkitt's lymphoma (BL) was characterized by monotonous infiltrate of medium–sized cells with round nuclei, coarse chromatin, 2–5 basophilic nucleoli and basophilic vacuolated cytoplasm. Although most nuclei were round, some show small nuclear protrusions. A "starry sky" pattern at low power was pathognomonic of Burkitt's lymphoma. The proliferative rate, as measure by IPO–38, was higher than 80% of cells. Malignant cell in BL were CD150 and SH2D1A negative. In one case, where lymphoma was associated with gut tissue, epithelial cells expressed the high level of SH2D1A.

Diffuse large B–cell lymphoma (DLBCL) is an aggressive, fast growing neoplasm. Diffuse large B–cell lymphoma typically replaced the normal architecture of the lymph node in a diffuse pattern. Lymph node involvement was complete, partial, interfollicular or seldom sinusoidal. The perinodal soft tissue was often infiltrated.

Gene expression profiling has revealed two distinct DLBCL subtypes: germinal center (GC–DLBCL) and activated B cell (ABC–DLBCL) [24, 25]. Malignant cells in GC–DLBCL had immunophenotype of GC B–cells (Table). B–lineage markers such as CD19, CD20 and CD22 were positive. In some cases malignant cell expressed CD5 and CD10. All studied GC–DLBCL cases were CD150$^-$, however majority of malignant cells showed SH2D1A expression. SH2D1A expression varied from low to moderate level depending on case.

Table. CD150 and SH2D1A expression in non–Hodgkin's lymphomas

B–cell NHL	Immunophenotype of malignant cells	IPO–38	CD150	SH2D1A
SLL (n = 7)	IgM$^+$(weak), CD5$^+$, CD10$^-$, CD19$^+$, CD20$^+$, CD22$^+$, CD23$^+$	< 10%	—	—
MCL (n = 2)	IgM$^+$, CD5$^+$, CD10$^-$, CD19$^+$, CD20$^+$, CD22$^+$	80–85%	+ (low)	—
BL (n = 3)	IgM$^+$, CD5$^-$, CD10$^-$, CD19$^+$, CD20$^+$, CD22$^+$	> 80%	—	—
GC–DLBCL (n = 7)	IgM$^{+/-}$, CD5$^{+/-}$, CD10$^{+/-}$, CD19$^+$, CD20$^+$, CD22$^+$	> 50%	—	+
ABC–DLBCL (n = 2)	IgM$^+$, CD5$^-$, CD10$^-$, CD19$^-$, CD20$^-$, CD22$^{+/-}$, CD30$^{+/-}$, CD38$^{-/+}$	> 70%	++	++
LPL (n = 2)	cyIgM$^+$, CD5$^-$, CD10$^-$, CD19$^+$, CD20$^+$, CD22$^+$	< 10%	—	—

Both studied ABC–DLBCL cases showed CD150 and SH2D1A coexpression in this subtype of DLBCL. Case 46 was classified as anaplastic variant of diffuse large B–cell lymphoma. Tumor was composed of very large round, oval or polygonal cells with voluminous cytoplasm and large bizarre nuclei (Fig. 3, *d*). Some Reed — Sternberg–like cells were present. Immunophenotype was: sIgM$^+$, CD5$^-$, CD10$^-$, CD19$^-$, CD20$^-$, CD22$^+$, and CD30$^+$. Malignant cells showed high level of surface and cytoplasmic CD150 expression with moderate level of SH2D1A in cytoplasm (Fig. 3, *e, f*). In diffuse large B–cell lymphoma with plasma cell differentiation (case 126) neoplastic cells were engorged with some globular inclusions of IgM. These cells were IgM$^+$ and CD38$^+$, but lack other B–cell markers (CD5$^-$, CD10$^-$, CD19$^-$, CD20$^-$, CD22$^-$). These malignant cells showed

very high level of surface CD150 expression as well as high level of cytoplasmic SH2D1A.

The nodal architecture of lymphoplasmacytic lymphoma (LPL) was totally obliterated with diffuse infiltration of neoplastic lymphocytes, proliferation centers were absent. Small lymphocytes were admixed with large activated lymphoid cells, lymphoplasmacytoid cells and plasma cells. The plasma cells often contain abundant crystalline immunoglobulin inclusions. B–lineage markers were positive (cyIgM, CD19, CD20, CD22). CD5 and CD10 were negative. Malignant cells in LPL did not show expression either CD150 or SH2D1A.

Anaplastic large cell lymphoma from null cells (primary systemic type, 1 patient) was characterized by large neoplastic cells with bizarre looking nuclei and abundant cytoplasm that expressed high level of CD30. $CD30^+$ cells disseminated within lymph node sinuses. Neoplastic cells did not express CD150, nevertheless, majority of these cells showed high cytoplasmic reaction on SH2D1A. On the other hand, 2 cases of peripheral T–cell lymphoma (unspecified) had high CD150 expression with very low level of SH2D1A.

In Hodgkin's lymphoma (HL) the malignant Hodgkin/Reed — Sternberg (HRS) cells characteristically constitute only a minority of the tumor load. Their origin was debated for decades, but now it is clear that in most instances HRS cells represent clonal population of transformed germinal centre B cells. There are evidences that HRS cells in classical HL originate from pre–apoptotic GC B cells, while in lymphocyte predominant type of HL malignant cells resemble mutating and antigen–selected GC B cells [23, 26]. Using immunohistochemical approach we were able to reveal both CD150 and SH2D1A expression in Hodgkin's and Reed–Sternberg cells in classical as well as in lymphocyte predominant type HL.

Our tissue screening studies revealed CD150 expression outside of hematopoietic system: in basalioma and squamous carcinomas of uterine cervix, esophagus, rectum and oral cavity. These tumors have ectodermal origin and their normal counterparts did not show expression of CD150. CD150 is upregulated on hematopoietic cells in response to antigenic and mitogenic stimulation, with interleukins and via toll–like receptors [1, 10–13, 27]. Possibly, CD150 expression in tumors of ectodermal origin reflects activation state of tumor cells in response to bacterial/viral antigens and/or cytokines in the site of inflammation. We will test this hypothesis in our future studies.

CD150 was found on the surface of mantle zone cells and germinal center cells. Detailed studies in lymphomas of B cell origin showed CD150 expression in MCL (very low level) and in ABC–DLBCL that is in accordance with CD150 expression in their normal counterparts. We also found CD150 expression in T–cell lymphoma.

Parallel analysis of CD150 and SH2D1A expression in lymphoma cells reveal stages of cell differentiation where these molecules are coexpressed. SH2D1A was found in GC–DLBCL and anaplastic large cell lymphoma from null cells that did not express CD150. More mature cells in T cell lymphoma and ABC–DLBCL coexpress CD150 with SH2D1A. Presumably, SH2D1A is expressed on earlier stages of differentiation than CD150 in cells of both T– and B–cell lineage, and CD150 is upregulated later, especially after cell activation. This is also in line with CD150 and SH2D1A coexpression in HRS cells in HL. Only MCL (normal counterpart pre–GC B cell) with low level of CD150 expression did not fit in this scheme.

Taken together, this study showed expression of CD150 in both T cell and B cell lymphomas that originate from mature T and B cell with activated phenotype. We also found SH2D1A expression in HRS cell in HL, GC–DLBCL and anaplastic lymphomas of null–, T– and B–cell origin. Moreover, we revealed SH2D1A and CD150 coexpression in malignant cell in HL, ABC–DLBCL and T–cell lymphoma, where normal counterparts are mature T and B cells with activated phenotype. SH2D1A is involved in regulation of CD150 signaling and function especially in CD150–mediated activation of Akt/PKB kinase [1, 4, 6, 15], Akt/PKB is a key anti–apoptotic serine/threonine kinase that delivers cell survival signals via different pathways [28]. Since ABC–DLBCL are agressive NHL with poor prognosis, coexpression of SH2D1A and CD150 in ABC–DLBCL and other NHL could contribute to pathogenesis of these types of lymphomas. We will adress this question in our future studies.

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