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ONCOLOGY

HODGKIN'S LYMPHOMA: THE ROLE OF CELL SURFACE RECEPTORS IN REGULATION OF TUMOR CELL FATE

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The hallmark of Hodgkin's lymphoma (HL) are mononucleated Hodgkin's cells and multinucleated Reed-Sternberg (HRS) cells, which usually account for only about 1% of cells in the tumor tissue. The majority of HRS cells in classical HL are derived from germinal centre B cells that have acquired disadvantageous Ig variable chain gene mutations and escaped from apoptosis. Due to reprogramming of gene expression, these lymphoma cells have lost the expression of most B-cell specific genes and acquired expression of multiple genes that are typical for other hematopoietic cells. HRS cells attract various cells of immune system into lymphoma tissue resulting in an inflammatory microenvironment. Moreover, HRS cells are dependent on microenvironment, especially on survival signals from other cells. Despite the loss of BCR — the master-regulator of B cell fate, HRS cells express a number of receptors that regulate tumor cell survival. The rescue of HRS cells from apoptosis is a key event in HL pathogenesis. These cells express at least six receptors that belong to TNF receptor family: CD30, CD40, CD95, TACI, BCMA and RANK, co-stimulatory receptors CD80 and CD86, and E-selectins ligand CD15. Due to the mutations in genes encoding proteins of CD95-mediated apoptotic signaling pathway, it is not functional in HRS cells. Ligands of TNF family receptors on cells in HL microenvironment contribute to the activation of canonical and non-canonical NF-κB signaling pathways and survival program of HRS cells. Moreover, in HRS cells a number of multiple mutations in negative NF-κB regulators, and also gains and amplifications of positive regulators, cooperate in deregulating these pathways. All TNF receptors may be linked to the activation of prosurvival gene expression programs via Akt and ERK pathways. HRS cells also express CD150 receptor with specific ITSM motifs in the cytoplasmic tail. Ligation of this receptor on HRS cells induced activation of Akt and ERK pathways, and moreover, it triggered activation of JNK signaling cascade. *Conclusion:* The review presents the current views on the role of cell surface receptors in maintenance of HL microenvironment favorable for HRS cells survival.
Key Words: Hodgkin's lymphoma, microenvironment, surface receptors, HRS cells.

The Hodgkin's lymphoma (HL) is a most striking example of tight tumor-host relationships. The hallmark of HL are mononuclear Hodgkin's cells and multinuclear Reed-Sternberg (HRS) cells, which usually account for only about 1% of cells in the tumor tissue. This disease was first described in 1832 by Thomas Hodgkin that was the first report on lymphoma. HL is one of most frequent lymphoma (account for about 30% of all lymphomas) and is extensively studied for more than 150 years [1]. Nevertheless, only in the last decade studies of individual HRS cells using molecular biology methods and microdissection, as well as studies of antigen expression and signal transduction pathways, revealed the nature of these tumor cells and have provided the major insights in the pathogenesis of Hodgkin's lymphoma.

Patients with HL in general have a favorable prognosis, and about 80% are cured with chemo- and radiotherapy. Treatment schemes for HL have considerable risks of short- and long-term toxicity, which may also

lead to secondary malignancies. Effective therapeutic approaches are still needed for refractory or relapsed patients, as most carry a high morbidity rate. Thus, novel therapies with improved safety and/or efficacy profiles are still needed to be developed for the group of patients with a poor prognosis. Currently, new therapeutic approaches, which were developed based on the biology and molecular profile of HRS cells, are on different stages of clinical trials[2].

HL include two disease entities: nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL) that account for only 5% of cases and classical Hodgkin's lymphoma (cHL), which is further subdivided into nodular sclerosis (60–70%), mixed cellularity (20–25%), lymphocyte rich (5%) and lymphocyte depleted (< 5%) [1]. Neoplastic lymphocytic and/or histiocytic Reed -Sternberg cells in NLPHL have a monoclonal B cell nature. They express multiple B cell lineage markers, such as CD20, CD79a, Ig, and also markers of germinal center cells — transcription factor Bcl- 6 and activation induced cytidine deaminase (AID). Moreover, these cells have rearranged and somatically mutated Ig V genes, sometimes with ongoing somatic hypermutation [3, 4]. Most likely these cells originate from antigen-selected germinal center cells on the intermediate developmental stage between germinal center and memory B cells [4].

ORIGIN OF HRS CELLS

Historically different hypothesis, which tried to explain the origin of HRS cells in cHL, were based on descriptive morphological methods and expression of

surface markers [5]. The ideas about HRS cells origin from endothelial cells, monocytes, megakariocytes, myeloid cells, plasma cells and activated lymphocytes were debated for decades [6]. Experimental evidences suggested that multinuclear Reed-Sternberg cells with limited clonal growth capacity arise from mononuclear Hodgkin's cells by endomitosis [7, 8].

Now it is proven that the majority of HRS cells in classical HL are derived from germinal centre B cells that have acquired Ig variable chain disadvantageous gene mutations and escaped from apoptosis [9]. These lymphoma cells have lost the expression of key B-cell specific genes and acquired expression of multiple genes that are typical for other types of hematopoietic cells [10–12]. B cell neoplasias usually retain general phenotypic features of the normal B cells they derive from; however, HRS cells are exception of the rule, since they show a global loss of B cell phenotype. In majority of cHL cases HRS cells have lost their capacity to express a functional B cell receptor (sIg), but these cells express B-lineage maintenance transcription factor PAX5 and carry rearranged and somatically mutated IgV genes [10, 13]. In these cells the expression of B-cell specific surface receptors CD19, CD20 and CD79a are lost or downregulated, however they may express markers of other hematopoietic cell lineages, like CD3 and CD4, CD15, granzyme B and myeloid and dendritic cell markers [14]. While normal germinal center B cells, which have lost their Ig receptors or have destructive somatic mutations that affect Ig function immediately undergo apoptosis, malignant HRS cells survive. This implies that HRS cells are derived from crippled pre-apoptotic germinal center B cells that escape from apoptosis [15].

TRANSCRIPTION FACTORS NETWORK IN HRS CELLS

The germinal center or post-germinal center B cell origin of HRS cells does not exclude the possibility of transforming events on the earlier stages of B cell differentiation. This view is supported by deregulated transcription factors network in HRS cells that contribute to the reprogramming of HRS cells. HRS cells often express transcription factor GATA2, which is required for the proliferation and survival of haematopoietic stem cells (HSC) and mast cell development [16]. High level of T cell transcription factor Notch 1 expression in HRS cells probably is activated by its ligand Jagged 1, which is produced by cells in HL microenvironment. Despite that HRS cells downregulate the inhibitor of Notch 1 — Deltex 1. Notch 1 promote T cell differentiation and inhibit B cell development by reducing expression of key B-lineage commitment transcription factors E12 and E47 (encoded by *E2A*) and EBF1 and at the same time inducing transcription of ABF1 (inhibitor of E12 and E47). Moreover, by binding to B-lineage maintenance transcription factor PAX5, Notch 1 may affect its function [10, 17, 18]. Notch 1 also upregulates expression level of another T-cell specific transcription factor GATA3 in HRS cells [19]. On the

other hand downregulation of B-lineage commitment transcription factor EBF1, which also repress expression of myeloid and T cell genes, may contribute to deregulated expression of myeloid and T cell markers in HRS cells [10, 17]. A number of transcription factors (i.e. BOB1, OCT2, PU.1), which activate expression of B cell specific genes, are not expressed in HRS cells [10, 17, 20, 21]. At the same time these cells express the transcriptional repressor PAX5 essential for the maintaining of B cell commitment, however many of its target genes are downregulated [11, 22].

HRS cells are characterized by high level of nuclear factor kB (NF-κB) activation via both canonical and alternative pathways that is a transient hallmark of germinal center B cells [23–25]. As a result, expression of transcription factor IRF4, which is one of NF-κB downstream targets, is also upregulated in HRS [26]. During B cell differentiation IRF4 expression is elevated on the terminal stages of B cell differentiation — in plasmablasts and plasma cells [25]. STAT transcription factors (STAT3, STAT5A, STAT5B and STAT6) are also activated in HRS cells mainly due to autocrine and/or paracrine signaling events via interleukin receptors and receptor tyrosine kinases [27–29].

All these issues raise the question whether HRS cells are reprogrammed on the stage of pre-apoptotic germinal centre B cells, or transforming events happened on the earlier stages of differentiation?

GENETIC AND EPIGENETIC ALTERATIONS IN HRS CELLS

The simultaneous down-regulation of many B-cell–specific genes in cHL could be also achieved by genetic lesions, and by epigenetic silencing. Moreover, these mechanisms also contribute to deregulation of B-cell transcription factor network.

Genetic instability is a characteristic feature of the malignant HRS cells [30]. HRS cells frequently harbor recurrent but not specific numerical and structural aberrations as detected by classical cytogenetics and fluorescence *in situ* hybridization analysis. Numerical chromosomal aberrations were found in 100% of analyzed cases of CD30$^+$ HRS cells. Chromosome numbers were always in the hyperploid range for HRS cells [31]. Results from molecular genetic studies using comparative genomic hybridization and allelotyping indicate typical genetic patterns in HL with gains and losses of distinct chromosomal regions [30]. Tumor cells of cHL shared common chromosomal imbalances: chromosomal gains most frequently involved chromosomes 2p, 8p, 8q, 9p, 9q, 12q, 16p, 17p, 17q 19p, and 20q, whereas losses primarily affected chromosomes Xp, 6q, 13q [32–34].

Molecular analysis of microdissected HRS cells provided further insight into the copy number imbalances affecting small chromosomal regions. Since escape from apoptosis is the main strategy of HRS cells, it could be expected activating aberrations for anti-apoptotic/survival genes and inhibiting alterations of pro-apoptotic genes. Indeed, more often alterations

are linked to NF-κB, Jak-STAT, p53 and CD95 pathways. For example, gains and amplifications of *REL*, a member of NF-κB family, is the most frequent genetic alteration in cHL (near 50% cases). In addition, point mutations and deletions were found for inhibitors of NF-κB signaling pathways *NFKBIA*, *NFKBI*, *TNFAIP3* [23, 24, 35, 36]. All these contribute to activation of NF-κB signaling pathways. Similarly, frequent genomic gains of *STAT6* and *JAK2*, as well as point mutations or deletions in *SOCS1*, aimed at activation of Jak-STAT pathway [30, 37].

Several gained small chromosomal regions also included genes constitutively expressed in HRS cells like *NOTCH1* (9q34) and *JUNB* (19p13), encoding transcription factors that negatively regulate B cell program and regulate proliferation, respectively [34].

Most recent study of microdissected HRS cells (53 cases) from cHL identified new and recurrent changes defining regions of chromosomal gain or loss harboring potential oncogenes and tumor suppressor genes involved in the pathogenesis of HL: *CD40, MAP3K14*, and *TNFRSF14*. Copy number alterations were found in more than 20% cHL cases. It was also demonstrated that gains of 16p, inducing the overexpression of the multidrug resistance gene *ABCC1*, may contribute to the drug-resistance phenotype identified in the cell line KM-H2 derived from a patient with relapsed cHL [36].

Epigenetic regulation also is actively involved in formation of HRS cell phenotype. Two main processes are involved in epigenetic gene silencing: DNA methylation and histone modification [38]. In cHL DNA methylation is involved in silencing the tumor suppressor genes *p16INK4a*, *p15INK4b* [39], *RASSF1A* (RAS-associated domain family 1) [40] and *p18INK4c* [41]. It was shown that silencing of the B-cell–specific genes (*PU.1, BOB.1/OBF.1, CD19, SYK*, and *CD79B*) correlated with promoter methylation of cHL cell lines and in HRS cells of cHL primary cases. Consequently, it was assumed that down-regulation of a few master transcription factors in cHL results in silencing of numerous target genes [42]. Inhibition of immunoglobulin transcription in HRS cells may at least in part be explained by epigenetic silencing [43]. At the same time, DNA demethylation alone or in conjunction with histone acetylation is not able to reconstitute the B-cell gene expression program in cultured HRS cells. Instead, combined DNA demethylation and histone acetylation of B-cell lines could induce an almost complete extinction of their B-cell-expression program and a tremendous upregulation of numerous Hodgkin-characteristic genes [44], suggesting one of the central role of epigenetics in the development of HRS phenotype.

HL MICROENVIRONMENT

HRS phenotype also depends on tumor microenvironment. HRS cells attract various types of immune system cells into lymphoma tissue resulting in typical inflammatory microenvironment. The stromal background of HL include non-malignant T and B lymphocytes, plasma cells, histiocytes/macrophages, granulocytes, eosinophils, mast cells, interdigitating reticulum cells and fibroblast cells in collagen bands [45]. Fibrosis is a common feature of cHL especially for nodular sclerosis variant [45]. For the recruitment of different cell types into tumor tissue HRS cells are using a broad array of cytokines and chemokines and also their cell surface receptors. Secondary symptoms in the HL patient, such as fever, weight loss, and night sweats, are consistent with a pathological pattern of cytokine/chemokine secretion. HRS cells, as well as their cellular environment, contribute to this process [46]. It is shown that HRS cells can express and secrete a variety of cytokines, including interleukin-1 (IL-1), IL-2 — IL-10, IL-13, IL-15, IL-21, granulocyte-macrophage colony-stimulating factor (GMCF), lymphotoxin-alpha (LF-α), transforming growth factor-beta (TGFβ), members of CC subfamily of chemokines: CC chemokine ligand 5 (CCL5/RANTES), CCL17/TARC, CCL20, CCL22; and also a number of tumor necrosis factor (TNF) family cytokines (TNFα, BAFF, APRIL, RANKL, NGF) [2, 29, 45, 46]. Expression of 140 genes of chemokines, cytokines and their receptors was analyzed by laser capture microdissection followed by cDNA microarray technique, and the expression of 17 genes was > 2.5-fold higher and six genes was < 0.4-fold lower in HRS cells than in germinal centre (GC) cells in more than half of HL cases [47]. At the same time, cells of HL microenvironment also secrete cytokines that influence HRS cells biology. According to their functional role, cytokines in HL may be grouped in (1) attracting cells into microenvironment, (2) suppressing immune cells and (3) autocrine and/or paracrine regulators of HRS survival.

The characteristic morphological features of HL are rosettes that form CD40L expressing T cells around HRS cells [48]. These CD4$^+$ T cells with considerable fraction of Treg cells may be attracted to HRS cells by CCL5, CCL17, CCL22, CCL28 and macrophage inflammatory protein 3a [14, 29]. Eosinophils are probably recruited to the HRS cells by GMCF, IL-5, CCL28, and CCL5, which also attract mast cells, and neutrophils — by IL-8 [46]. Moreover, activated by tumor cells microenvironment cells also secrete cytokines that attract additional cells in the tumor. In turn, HRS cells are also dependent of microenvironment, especially on growth and survival signals from other cells that are mediated via receptors by cell surface and soluble ligands.

Several immune suppression strategies are used by HRS cells in HL. Secretion of soluble factors such as TGFβ, IL-10, galectin 1 and prostaglandin E2 (PGE2), have been shown to inhibit the activation of cytotoxic T lymphocytes and antigen-presenting cells [49–51]. HRS cells modulate their cellular microenvironment by shifting the T_H response from an anti-cellular T_H1 response to a humoral T_H2 response. HL patients have defective cellular immunity as they are susceptible to bacterial, fungal, and viral infections, and *in vitro* stud-

ies show inhibited T-cell proliferation and low level of T_H1 cytokines [52].

Cell-to-cell interactions might also lead to immune inhibition. For example, HRS cells express CD95 ligand, which induces apoptosis of activated T_H1 and CD8$^+$ T cells [52, 53]. There are several evidences that programmed cell death protein 1 (PD-1) interactions with ligands might lead to immune inhibition of tumor infiltrating T cells. PD-1, a member of the CD28 family within Ig superfamily negatively regulates T-cell antigen receptor signaling. To date, two PD-1 ligands that belongs to B7 family have been identified: B7-H1 (PD-L1, CD274) and B7-DC (PD-L1, CD273) [54]. The PD-1-PD-L pathway delivers inhibitory signals that regulate the balance among T-cell activation and tolerance, and directly contributes to T-cell exhaustion and suppressive tumor microenvironment [54]. Either one or both of these ligands are expressed on HRS cells *in situ* and on HL cell lines [55, 56]. In the presence of TGFβ, PD-L1 may promote the *de novo* generation of T_{Regs} and enhance their immunosupressive activity on HL-infiltrating cytotoxic T cells [14, 54]. That is why by ligation of PD-1 on T lymphocytes HRS cells may inhibit effector functions of tumor-infiltrating lymphocytes and contribute to permissive microenvironment.

HRS cells express also other receptors and ligands, which they are using to maintain tumor environment. Among them are co-stimulatory members of B7 family — CD80 and CD86, which by binding to CD28 activate CD4$^+$ T_H lymphocytes [57, 58].

Also HRS cells express CD15, which serves as a diagnostic marker of HL. CD15 molecules are a group of fucosylated carbohydrate structures that are expressed on a protein or lipid backbone [59]. CD15 functions as a ligand for E-selectins on endothelial cells and promotes cell adhesion. Addition of sialic acid enhances the affinity of CD15 to selectins [60]. Due to this fact expression of nonsialylated CD15 molecules on HRS cells are likely to have positive prognostic value for patients with HL, while presence of sialylated CD15 may correlate with a poor outcome [60, 61]. Triggering of CD15 by antibodies and selectins can induce the activation of HL-derived cell lines with the involvement of c-Cbl and c-Jun [61]. CD15–selectins interactions play important role in the development and maintenance of tumor microenvironment and together with receptor-ligand pair CD54–LFA-3 could promote tumor cell migration.

CELL SURFACE RECEPTORS IN REGULATION OF HRS CELL SURVIVAL PROGRAM

Despite the loss of BCR — the master-regulator of B cell fate, HRS cell express a number of receptors that may regulate tumor cell survival. The rescue of HRS cells from apoptosis is a key event in HL pathogenesis. And the strategy of tumor cells is to block apoptotic pathways with constitutive activation of pro-survival pathways. Tumor necrosis factor (TNF) family receptors could have dual functions in cells — stimulating

apoptosis or *vice versa* — inducing cell proliferation and protecting cells from apoptosis-inducing stimuli. The outcome of receptor stimulation depends on the pattern of intracellular signaling molecules expression by cells or the capability of cells to upregulate expression of such molecules in response to stimulation. HRS cells express at least six receptors that belong to TNF receptor family: CD95, CD40, CD30, receptor activator of NF-κB (RANK), transmembrane activator and calcium modulator and cyclophylin ligand interactor (TACI), and B-cell maturation antigen (BCMA). It is important to note that all these receptors, except CD95, were shown to contribute to NF-κB activation in HRS cells.

CD95 is a marker of activated T and B lymphocytes, however is also broadly expressed outside of hematopoietic system [62, 63]. Moreover, almost all human tumors express CD95, and it was reported to act as a tumor promoter in lung, thyroid and ovarian cancer [64]. CD95 expression was detected in 90.5% of the cHL cases, and the expression was observed in a 50–100% of the HRS cells, exhibiting strong cytoplasmic and membrane staining [65, 66]. CD95L is expressed by T cells in HL microenvironment. Apoptosis-triggering function is well described for CD95 receptor [67], but HRS cells are using specific mechanisms to avoid CD95-mediated apoptosis. CD95 machinery appears to be up-regulated on HRS cells [53]. At the same time, structural alterations of the CD95 in HRS cells are rare [30, 68] despite the reports on CD95 death domain (DD) somatic mutations in primary cHL cases [69]. However, not only mutations in CD95 would switch off apoptosis induction. Protection from CD95-induced apoptosis in HRS cells could be also provided by mutations in genes encoding crucial proteins in CD95-mediated apoptotic signaling pathway.

HRS cells were shown to highly express activated caspase-3 and other components of the TNFR-associated signal transduction machinery [70]. Caspases can be inhibited by a family of molecules, called inhibitors of apoptosis proteins (IAP). It was shown that several members of this family, such as XIAP, cIAP1, and cIAP2 are able to directly inhibit the effector caspase-3 [71]. Since cIAP2 was strongly expressed in HRS cells in majority of examined cHL cases, it could be important for silencing CD95-mediated pro-apoptotic signals and for the survival of HRS cells by blocking caspase-3 [72].

Resistance of HRS tumor cells to death receptor stimulation could be as well explained by functional inhibition mediated by a strong, NF-κB-dependent up-regulation of c-FLIP proteins. It was shown that FLICE inhibitory protein c-FLIP was overexpressed in 55 out of 59 studied cases of cHL [73]. FLIPs structurally resemble caspases, but lack proteolytic activity, and were shown to function as antiapoptotic proteins. Specific down-regulation of c-FLIP proteins by small interfering RNA oligoribonucleotides (siRNAs) was sufficient to render HRS cell lines sensitive to CD95 stimulation [73, 74]. Thus, c-FLIP could play important role

in protection of HRS cells from CD95 death receptor stimuli, and may be also from pro-apoptotic stimuli via other TNFR family members.

It is important to point out that CD95 could mediate a variety of nonapoptotic signaling events [64], and induce proliferation and differentiation of cells, as well as cytokine production [75, 76]. Moreover, CD95 signaling could activate NF-κB binding to its specific target genes, which was not abrogated by the deletion of a portion of death domain in CD95 receptor [77]. CD95 is highly expressed and was shown to mediate nonapoptotic signals in such tissues as heart, pancreas, and colon [64]. However, these CD95 functions were not examined in HL.

High level of NF-κB expression and activation could be achieved not only in consequence of genomic alterations of signaling components of NF-κB pathways, but also due to high expression of TNF receptors transmitting activation signals in HRS cells. In total of 93.9% of the examined cHL cases almost all the HRS cells expressed pan-B cell antigen CD40 [65] that is also expressed on epithelial cells, cells of monocyte-macrophage origin, dendritic cells and others. CD40L found on activated T cells and endothelial cells [48]. CD30 expression was demonstrated in almost 100% of the HRS cells. In non-pathological conditions, CD30 expression is generally limited to activated B and T lymphocytes and NK cells and generally lower levels of expression were reported for activated monocytes and eosinophils [2]. CD30L is broadly expressed by cells in HL microenvironment: T and B lymphocytes, neutrophils and eosinophils, and also mast cells [2]. RANK messenger RNA (mRNA) is ubiquitously expressed in human tissues, but RANK protein expression has been detected only in DCs, $CD4^+$ and $CD8^+$ T lymphocytes, and osteoclast hematopoietic precursor cells [78]. An average of 75% of HRS cells expressed RANK in all studied cases of cHL, and it was rarely and weakly expressed by the HL microenvironment cells. In HRS cells RANK could be activated in an autocrine fashion, as expression of the RANK ligand was reported for HL cell lines [79]. TACI is a transmembrane receptor protein found predominantly on the surface of B cells, mainly within the GC, on memory cells and plasma cells [80, 81]. B-cell maturation antigen (BCMA) is preferentially expressed on plasma cells and subpopulation of mature B lymphocytes [80–82]. TACI and BCMA are expressed in 93% and 67% of cHL cases, respectively, and could be activated both in paracrine and autocrine fashion, since one of their ligands APRIL is secreted by neutrophils, and another ligand — BAFF — is expressed by HRS cells as well as by cells in HL microenvironment [80]. CD30, CD40, RANK, TACI and BCMA may associate with different sets of TNF receptor-associated factors (TRAF2, TRAF3, TRAF5 or TRAF6), which link to the classical and/or alternative NF-κB signaling pathways [82, 83]. Therefore, TNF family receptors on HRS and microenvironment cells contribute to activation of canonical

and alternative NF-κB signaling pathways and survival program of HRS cells.

Activation of the phosphatidylinositol 3-kinase (PI3K) pathway has been linked with tumor cell growth, survival and resistance to therapy in several cancer types [84]. The main downstream PI3K effector, which control cell survival is Akt/PKB [85]. Ligation of CD30, CD40, or RANK could induce Akt phosphorylation/activation in HRS cells [86]. Other TNF receptors also could contribute to Akt activation in these cells, as it was shown that TNFRs are linked to PI3K/Akt pathway [82]. The phosphorylated form of Akt (pAkt S473) was found to be aberrantly expressed in HL derived cell lines and in HRS cells in 64% of primary lymph node sections of HL [86]. Several downstream effectors of Akt signalling, including glycogen synthase kinase 3 (GSK-3) α and β, mTOR substrates 4E-BP1 and p70 S6 kinase, were phosphorylated in primary HL cells [87]. The MEK/ERK pathway is also aberrantly active in HL, and is involved in regulation of HRS cell proliferation and survival shared between CD30, CD40, and RANK signaling pathways [88].

HRS cells express a number of receptor tyrosine kinases, like platelet-derived growth factor receptor- α(PDGFRA), macrophage-stimulating protein receptor (MSPR), epithelial discoidin domaincontaining receptor 2 (DDR2), tyrosine kinase receptor A (TRKA) and TRKB, which may contribute to survival via activation of NF-kappa B and Jak-STAT pathways. All these receptors are also linked to activation of prosuvival gene expression programs via Akt and ERK pathways [89, 90].

Among receptors that could play role in regulation of HRS survival and/or tumor cell microenvironment maintenance is CD150/SLAM, which is expressed on B and T cells, activated macrophages and dendritic cells [91, 92]. CD150 is differentially expressed on $CD4^+$ T cells: T_H1 cells have higher level of CD150 on their surface in comparison with T_H2 cells [93, 94]. Activation of T and B cells results in upregulation of CD150 expression [95–97].

CD150 expression is found on different stages of B cell differentiation starting on naïve B cells, and the highest level of CD150 expression is detected on terminal stages of B cell differentiation — on plasmablasts and plasma cells [98]. It could be expected that CD150 expression would be found on malignant cells at different B-cell leukemias and lymphomas. But in fact CD150 expression was found only on hairy cell leukemia tumor cells, diffuse large B cell lymphoma with activated phenotype and classical Hodgkin's lymphoma [97, 99]. The expression of CD150 was shown both for HL cell lines and primary tumors in 100% of studied HL cases [99, 100]. CD150 could be expressed both in soluble and full transmembrane isoforms in normal B cells and HL cell lines [101, 102], which makes it present both on cell surface and in tissue matrix, surrounding CD150-expressing cells. As CD150 was shown to be homophilic receptor, being a self-ligand, its expression in soluble isoform

(sCD150) could contribute to autocrine regulation of secreting cells. Both membrane-bound CD150 receptor (mCD150) and sCD150 may bind CD150 on HRS cells and cells in tumor microenvironment. Upon self binding or ligation with antibodies, CD150 mediates signaling events due to the presence of two specific immunoreceptor tyrosine-based switch motifs (ITSM) in the cytoplasmic tail. The consequence of CD150 self binding depends on whether CD150 is either ligated or blocked. Thus, CD150 could target HRS cells as well as T cells (especially T_{Reg} and T_c), B cells, PC, and macrophages surrounding HRS.

What signaling pathways are linked to CD150 receptor in HRS cells and lymphocytes in microenvironment? CD150 mediates Akt kinase activation not only in normal naïve and activated human B cells and lymphoblastoid cell line MP-1 [103], but also in HL cell lines [99, 102, 104].

CD150 also could regulate ERK activity in HL cells [98, 102]. Moreover, CD150 was found to be linked to all three MAPKs signaling pathways in normal human B cells and HL cell lines — ERK1/2, p38MAPK and JNK1/2 [98]. In all studied cases of classical HL more than 75% of malignant HRS cells demonstrated high level of pJNK1/2. Signaling via CD150 could play important role in maintaining JNK activation in HRS cells as CD150 ligation was shown to induce prolonged JNK activation in all studied HL cell lines [98].

It is important to note that CD150 ligation could have different impact on signaling and cell fate of HRS cells and B cells in tumor microenvironment (Fig. 1). Prolonged stimulation of normal B cells via CD150 alone did not affect cell proliferation, but enhanced CD40 and IL-4 induced proliferation [97,

101]. At the same time stimulation of HL cell lines by anti-CD150 mAb IPO-3 for 48 h resulted in inhibition of proliferation for all three studied cell lines (KM-H2, L428, L1236), and even in cell death of L1236 cells [98].

As to the CD150-mediated signals, Akt kinase target — GSK-3β — was phosphorylated upon CD150 ligation on lymphoblasoid cell line MP-1 and HL cell line L1236, but not in normal tonsillar B cells [104]. It was shown that CD150 ligation up-regulates phosphorylation/activation of ERK1/2 and p38 MAPKs in normal tonsillar B cells but down-regulates — in HL cell line L1236 [98]. The signaling events linked to CD150 receptor in HRS cells and normal B cells clarified up to date, are presented on Fig. 1. It is possible that using sCD150 (with low affinity binding) HRS cells protect themselves from signaling via CD150 receptor mediated by membrane-bound CD150 on microenvironment cells. So, high affinity anti-CD150 monoclonal antibodies could be of interest as potential therapeutic agent for HL treatment. Thus, CD150 can be regarded as one of the receptors that is involved in regulation of tumor cell maintenance in low-rate proliferating Hodgkin's lymphoma and may be a promising target for development of new therapeutic approaches.

CONCLUSION

HL is an example of how tumor cells use the immune system signaling machinery to create and maintain favorable for tumor cell survival microenvironment (Fig.2). HRS cells secrete a number of cytokines and chemokines (Fig. 2, arrow 1) that attract T and B lymphocytes, neutorphils and eosinophils, macrophages and histiocytes, stromal and endothelial cells
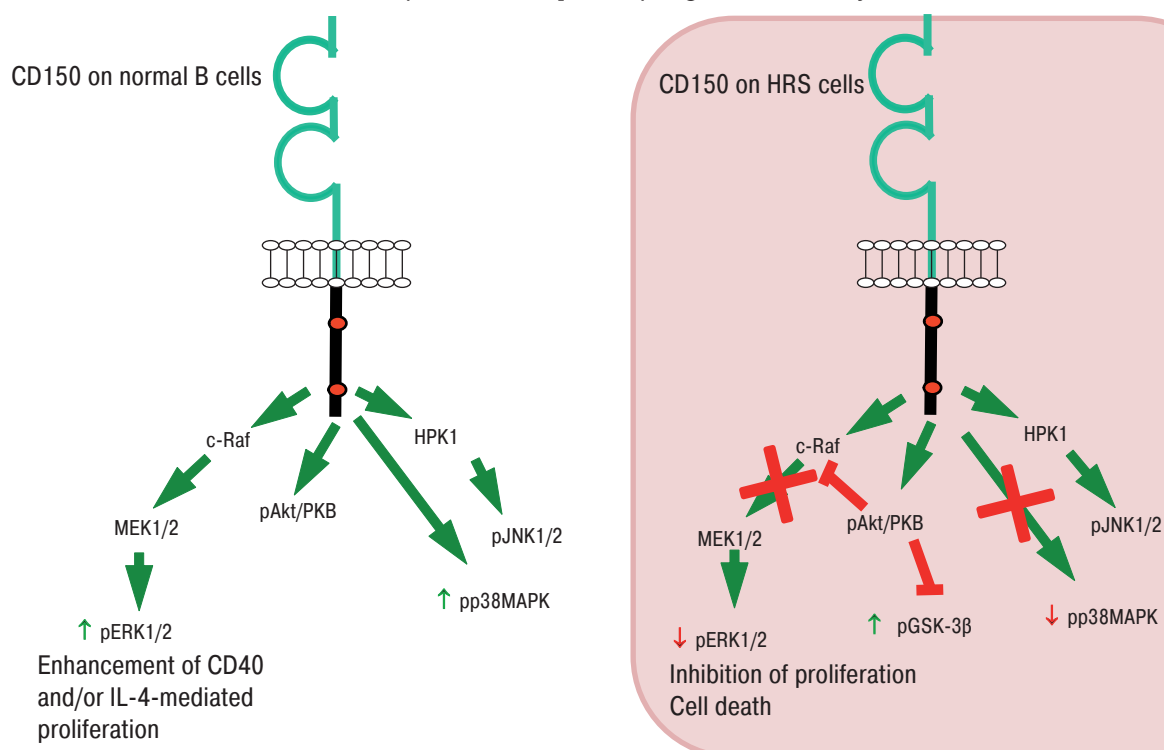

Fig. 1. Ligation of CD150 receptor by monoclonal antibodies (IPO-3) induce multiple signaling events, and overall have different impact on the proliferation and survival of normal tonsillar B cells and malignant cells in HL
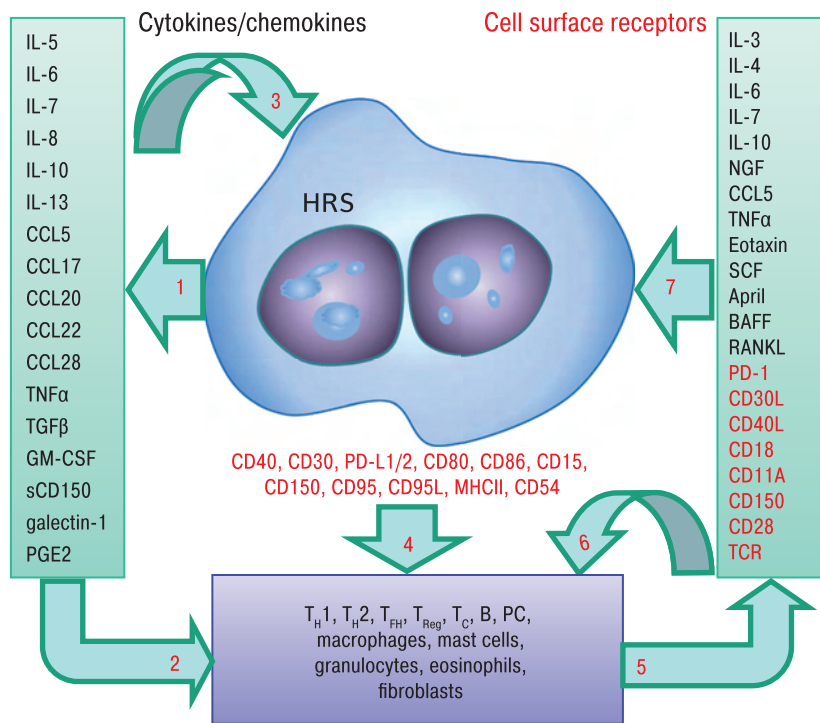

Cytokines/chemokines — **Cell surface receptors**

Left box: IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, CCL5, CCL17, CCL20, CCL22, CCL28, TNFα, TGFβ, GM-CSF, sCD150, galectin-1, PGE2

Center (HRS): CD40, CD30, PD-L1/2, CD80, CD86, CD15, CD150, CD95, CD95L, MHCII, CD54

Right box: IL-3, IL-4, IL-6, IL-7, IL-10, NGF, CCL5, TNFα, Eotaxin, SCF, April, BAFF, RANKL, PD-1, CD30L, CD40L, CD18, CD11A, CD150, CD28, TCR

Bottom box: T$_H$1, T$_H$2, T$_{FH}$, T$_{Reg}$, T$_C$, B, PC, macrophages, mast cells, granulocytes, eosinophils, fibroblasts

Fig. 2. The microenvironment maintenance in HL. Arrows: 1 — cytokines/chemokines secreted by HRS cells; 2 — cells of microenvironment, attracted by cytokines, paracrine effect; 3 — autocrine effect of some HRS cell secreted cytokines; 4 — receptors and ligands on HRS cells that contribute to the regulation of cell functions in microenvironment; 5 — soluble factors and receptors expressed by cells in microenvironment, which could bind to their counterparts both on cells in microenvironment (6) and HRS cells (7)

(Fig. 2, arrow 2). Some of these soluble factors could regulate effector functions of microenvironmental immune cells (Fig. 2, arrow 2), and other may function as autocrine factors (Fig. 2, arrow 3). HRS cells express a number of receptors and ligands that interact with their counterparts creating receptor-ligand pairs, which may transmit signals to HRS cells as well as to cells in microenvironment (Fig. 2, arrow 4). These signals support prosurvival program in tumor cells and inhibit effector functions of cells involved in innate and adaptive immune reactions. As a result of receptor interactions with soluble and membrane-bound ligands cells in microenvironment are changing their surface phenotype expressing new array of receptor/ligand machinery, producing and secreting a broad spectrum of soluble factors (Fig. 2, arrow 5). Using this fortified signaling machinery these cells attract more cells in the environment and stimulate formation of extracellular matrix (Fig. 2, arrow 6). Moreover, the microenvironment provides growth factors and stimuli via receptor-ligand interactions (mainly via TNFRs) that regulate HRS cell phenotype and support survival of HRS cells by protecting from apoptosis (Fig. 2, arrow 7). Taking together, receptors on HRS cells, which are involved in regulation of their survival, could be considered as promising therapeutic targets.

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