

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

M. Yurchenko, S.P. Sidorenko*

Department of Cell Regulation, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine, Vasylykivska str. 45, 03022 Kyiv, Ukraine

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- κ B signaling pathways and survival program of HRS cells. Moreover, in HRS cells a number of multiple mutations in negative NF- κ B 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

Received: October 10, 2010.

*Correspondence: Fax: +380442581656;

E-mail: svetasad@onconet.kiev.ua

Abbreviations used: BCMA – B-cell maturation antigen; cHL – classical Hodgkin's lymphoma; GC – germinal centre; HL – Hodgkin's lymphoma; HRS – Hodgkin's and Reed-Sternberg; HSC –hematopoietic stem cells; IL – interleukin; NF- κ B – nuclear factor κ B; NLPHL – nodular lymphocyte predominant Hodgkin's lymphoma; RANK – receptor activator of NF- κ B; TACI – transmembrane activator and calcium modulator and cyclophilin ligand interactor; TNF – tumor necrosis factor.

surface markers [5]. The ideas about HRS cells origin from endothelial cells, monocytes, megakaryocytes, 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 (slg), 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 κ B (NF- κ B) 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- κ B 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 center 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- κ B, Jak-STAT, p53 and CD95 pathways. For example, gains and amplifications of *REL*, a member of NF- κ B family, is the most frequent genetic alteration in cHL (near 50% cases). In addition, point mutations and deletions were found for inhibitors of NF- κ B signaling pathways *NFKBIA*, *NFKBI*, *TNFAIP3* [23, 24, 35, 36]. All these contribute to activation of NF- κ B 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 roles 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 (GM-CSF), lymphotoxin- α (LT- α), transforming growth factor- β (TGF β), 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 (TNF α , 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 GM-CSF, 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 TGF β , 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 TGF β , PD-L1 may promote the *de novo* generation of T_{Reg} s and enhance their immunosuppressive 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- κ B (RANK), transmembrane activator and calcium modulator and cyclophilin 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- κ B 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 proapoptotic 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- κ B-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- κ B 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- κ B expression and activation could be achieved not only in consequence of genomic alterations of signaling components of NF- κ B 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- κ B signaling pathways [82, 83]. Therefore, TNF family receptors on HRS and microenvironment cells contribute to activation of canonical

and alternative NF- κ B 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- κ B and Jak-STAT pathways. All these receptors are also linked to activation of prosurvival 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-3 β — was phosphorylated upon CD150 ligation on lymphoblastoid 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, neutrophils and eosinophils, macrophages and histiocytes, stromal and endothelial cells

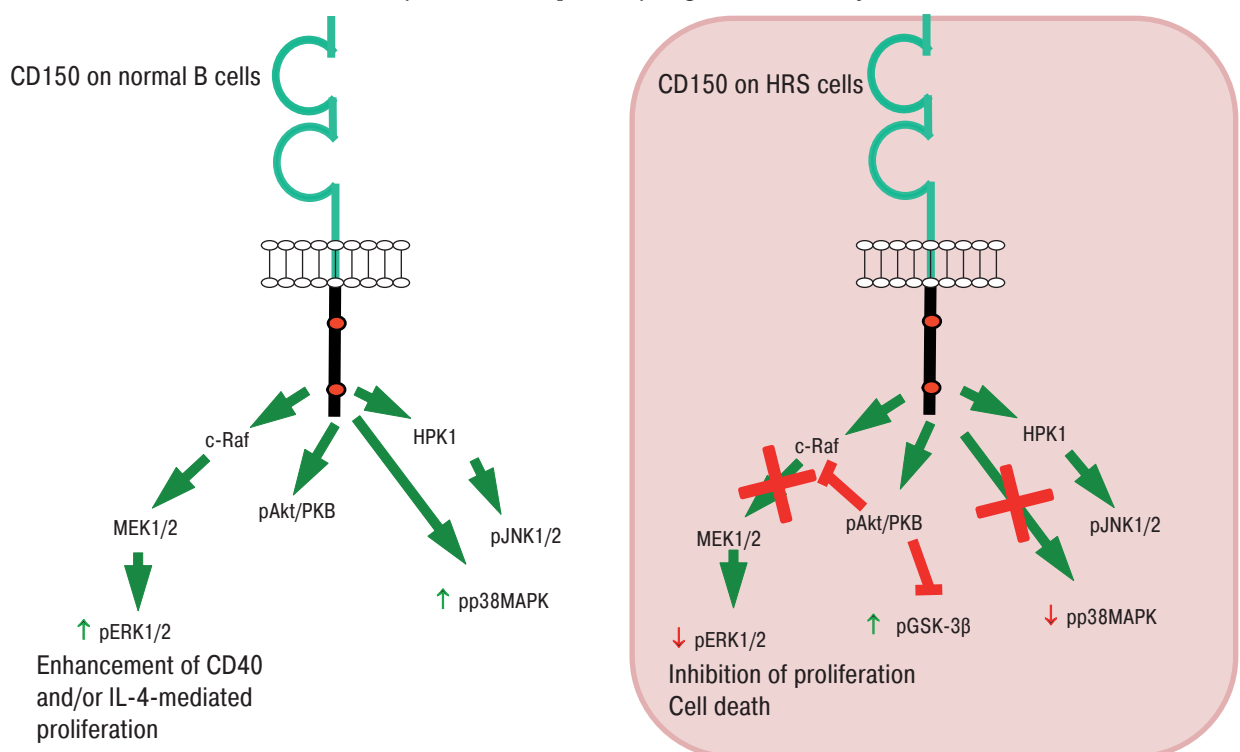


Fig. 1. Ligation of CD150 receptor by monoclonal antibodies (IPO-3) induce multiple signaling events, and overall have different impact on the proliferation and survival of normal tonsillar B cells and malignant cells in HL

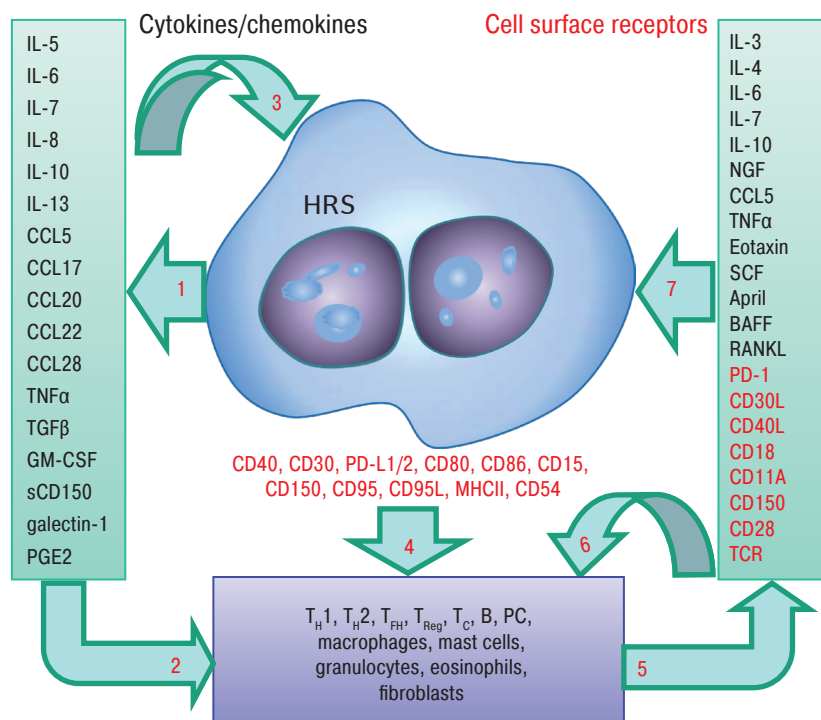


Fig. 2. The microenvironment maintenance in HL. Arrows: 1 — cytokines/chemokines secreted by HRS cells; 2 — cells of microenvironment, attracted by cytokines, paracrine effect; 3 — autocrine effect of some HRS cell secreted cytokines; 4 — receptors and ligands on HRS cells that contribute to the regulation 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.

REFERENCES

1. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4 ed, ed. Swerdlow SH, Campo E, Harris NL, *et al.* Lyon: IARC, 2008. 439 p.

2. Gerber HP. Emerging immunotherapies targeting CD30 in Hodgkin's lymphoma. *Biochem Pharmacol* 2010; **79**: 1544–52.

3. Marafioti T, Hummel M, Anagnostopoulos I, *et al.* Origin of nodular lymphocyte-predominant Hodgkin's disease from a clonal expansion of highly mutated germinal-center B cells. *N Engl J Med* 1997; **337**: 453–8.

4. Brune V, Tiacchi E, Pfeil I, *et al.* Origin and pathogenesis of nodular lymphocyte-predominant Hodgkin lymphoma as revealed by global gene expression analysis. *J Exp Med* 2008; **205**: 2251–68.

5. Gluzman DF, Sidorenko SP, Poludnenko L, *et al.* Current theories on the origin of Reed-Sternberg cells in lymphogranulomatosis. *Hematol Transfusiol* 1990; **35**: 24–7 (in Russian).

6. Gluzman DF, Abramenko IV, Sklyarenko LM, *et al.* Laboratory diagnostics of oncohematological diseases. Kiev: Morion, 1998. 335 p (in Russian).

7. Drexler HG, Gignac SM, Hoffbrand AV, *et al.* Formation of multinucleated cells in a Hodgkin's-disease-derived cell line. *Int J Cancer* 1989; **43**: 1083–90.

8. Marafioti T, Hummel M, Foss HD, *et al.* Hodgkin and Reed-Sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. *Blood* 2000; **95**: 1443–50.

9. Kuppers R. Clonotypic B cells in classic Hodgkin lymphoma. *Blood* 2009; **114**: 3970–1; author reply 3971–2.

10. Hertel CB, Zhou XG, Hamilton-Dutoit SJ, *et al.* Loss of B cell identity correlates with loss of B cell-specific transcription factors in Hodgkin/Reed-Sternberg cells of classical Hodgkin lymphoma. *Oncogene* 2002; **21**: 4908–20.

11. Schwering I, Brauning A, Klein U, *et al.* Loss of the B-lineage-specific gene expression program in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood* 2003; **101**: 1505–12.

12. **Kuppers R, Klein U, Scherling I, et al.** Identification of Hodgkin and Reed-Sternberg cell-specific genes by gene expression profiling. *J Clin Invest* 2003; **111**: 529–37.
13. **Kanzler H, Hansmann ML, Kapp U, et al.** Molecular single cell analysis demonstrates the derivation of a peripheral blood-derived cell line (L1236) from the Hodgkin/Reed-Sternberg cells of a Hodgkin's lymphoma patient. *Blood* 1996; **87**: 3429–36.
14. **Kuppers R.** The biology of Hodgkin's lymphoma. *Nat Rev Cancer* 2009; **9**: 15–27.
15. **Kuppers R, Rajewsky K.** The origin of Hodgkin and Reed/Sternberg cells in Hodgkin's disease. *Annu Rev Immunol* 1998; **16**: 471–93.
16. **Schneider EM, Torlakovic E, Stuhler A, et al.** The early transcription factor GATA-2 is expressed in classical Hodgkin's lymphoma. *J Pathol* 2004; **204**: 538–45.
17. **Mathas S, Janz M, Hummel F, et al.** Intrinsic inhibition of transcription factor E2A by HLH proteins ABF-1 and Id2 mediates reprogramming of neoplastic B cells in Hodgkin lymphoma. *Nat Immunol* 2006; **7**: 207–15.
18. **Jundt F, Acikgoz O, Kwon SH, et al.** Aberrant expression of Notch1 interferes with the B-lymphoid phenotype of neoplastic B cells in classical Hodgkin lymphoma. *Leukemia* 2008; **22**: 1587–94.
19. **Stanelle J, Doring C, Hansmann ML, et al.** Mechanisms of aberrant GATA-3 expression in classical Hodgkin lymphoma and its consequences for the cytokine profile of Hodgkin and Reed/Sternberg cells. *Blood* 2010; **116**: 4202–11.
20. **Stein H, Marafioti T, Foss HD, et al.** Down-regulation of BOB.1/OBF.1 and Oct2 in classical Hodgkin disease but not in lymphocyte predominant Hodgkin disease correlates with immunoglobulin transcription. *Blood* 2001; **97**: 496–501.
21. **Garcia-Cosio M, Santon A, Martin P, et al.** Analysis of transcription factor OCT.1, OCT.2 and BOB.1 expression using tissue arrays in classical Hodgkin's lymphoma. *Mod Pathol* 2004; **17**: 1531–8.
22. **Foss HD, Reusch R, Demel G, et al.** Frequent expression of the B-cell-specific activator protein in Reed-Sternberg cells of classical Hodgkin's disease provides further evidence for its B-cell origin. *Blood* 1999; **94**: 3108–13.
23. **Emmerich F, Meiser M, Hummel M, et al.** Overexpression of I kappa B alpha without inhibition of NF-kappaB activity and mutations in the I kappa B alpha gene in Reed-Sternberg cells. *Blood* 1999; **94**: 3129–34.
24. **Emmerich F, Theurich S, Hummel M, et al.** Inactivating I kappa B epsilon mutations in Hodgkin/Reed-Sternberg cells. *J Pathol* 2003; **201**: 413–20.
25. **Klein U, Dalla-Favera R.** Germinal centres: role in B-cell physiology and malignancy. *Nat Rev Immunol* 2008; **8**: 22–33.
26. **Carbone A, Gloghini A, Aldinucci D, et al.** Expression pattern of MUM1/IRF4 in the spectrum of pathology of Hodgkin's disease. *Br J Haematol* 2002; **117**: 366–72.
27. **Baus D, Pfitzner E.** Specific function of STAT3, SOCS1, and SOCS3 in the regulation of proliferation and survival of classical Hodgkin lymphoma cells. *Int J Cancer* 2006; **118**: 1404–13.
28. **Scheeren FA, Diehl SA, Smit LA, et al.** IL-21 is expressed in Hodgkin lymphoma and activates STAT5: evidence that activated STAT5 is required for Hodgkin lymphomagenesis. *Blood* 2008; **111**: 4706–15.
29. **Lamprecht B, Kreher S, Anagnostopoulos I, et al.** Aberrant expression of the Th2 cytokine IL-21 in Hodgkin lymphoma cells regulates STAT3 signaling and attracts Treg cells via regulation of MIP-3alpha. *Blood* 2008; **112**: 3339–47.
30. **Re D, Zander T, Diehl V, et al.** Genetic instability in Hodgkin's lymphoma. *Ann Oncol* 2002; **13 Suppl 1**: 19–22.
31. **Weber-Matthiesen K, Deerberg J, Poetsch M, et al.** Numerical chromosome aberrations are present within the CD30+ Hodgkin and Reed-Sternberg cells in 100% of analyzed cases of Hodgkin's disease. *Blood* 1995; **86**: 1464–8.
32. **Joos S, Menz CK, Wrobel G, et al.** Classical Hodgkin lymphoma is characterized by recurrent copy number gains of the short arm of chromosome 2. *Blood* 2002; **99**: 1381–7.
33. **Chui DT, Hammond D, Baird M, et al.** Classical Hodgkin lymphoma is associated with frequent gains of 17q. *Genes Chromosomes Cancer* 2003; **38**: 126–36.
34. **Hartmann S, Martin-Subero JI, Gesk S, et al.** Detection of genomic imbalances in microdissected Hodgkin and Reed-Sternberg cells of classical Hodgkin's lymphoma by array-based comparative genomic hybridization. *Haematologica* 2008; **93**: 1318–26.
35. **Lake A, Shield LA, Cordano P, et al.** Mutations of NFKBIA, encoding I kappa B alpha, are a recurrent finding in classical Hodgkin lymphoma but are not a unifying feature of non-EBV-associated cases. *Int J Cancer* 2009; **125**: 1334–42.
36. **Steidl C, Telenius A, Shah SP, et al.** Genome-wide copy number analysis of Hodgkin Reed-Sternberg cells identifies recurrent imbalances with correlations to treatment outcome. *Blood* 2010; **116**: 418–27.
37. **Weniger MA, Melzner I, Menz CK, et al.** Mutations of the tumor suppressor gene SOCS-1 in classical Hodgkin lymphoma are frequent and associated with nuclear phospho-STAT5 accumulation. *Oncogene* 2006; **25**: 2679–84.
38. **Jones PA, Baylin SB.** The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; **3**: 415–28.
39. **Garcia MJ, Martinez-Delgado B, Cebrian A, et al.** Different incidence and pattern of p15INK4b and p16INK4a promoter region hypermethylation in Hodgkin's and CD30-Positive non-Hodgkin's lymphomas. *Am J Pathol* 2002; **161**: 1007–13.
40. **Murray PG, Qiu GH, Fu L, et al.** Frequent epigenetic inactivation of the RASSF1A tumor suppressor gene in Hodgkin's lymphoma. *Oncogene* 2004; **23**: 1326–31.
41. **Sanchez-Aguilera A, Delgado J, Camacho FI, et al.** Silencing of the p18INK4c gene by promoter hypermethylation in Reed-Sternberg cells in Hodgkin lymphomas. *Blood* 2004; **103**: 2351–7.
42. **Ushmorov A, Leithauser F, Sakk O, et al.** Epigenetic processes play a major role in B-cell-specific gene silencing in classical Hodgkin lymphoma. *Blood* 2006; **107**: 2493–500.
43. **Ushmorov A, Ritz O, Hummel M, et al.** Epigenetic silencing of the immunoglobulin heavy-chain gene in classical Hodgkin lymphoma-derived cell lines contributes to the loss of immunoglobulin expression. *Blood* 2004; **104**: 3326–34.
44. **Ehlers A, Oker E, Bentink S, et al.** Histone acetylation and DNA demethylation of B cells result in a Hodgkin-like phenotype. *Leukemia* 2008; **22**: 835–41.
45. **Aldinucci D, Gloghini A, Pinto A, et al.** The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape. *J Pathol* 2010; **221**: 248–63.
46. **Skinninger BF, Mak TW.** The role of cytokines in classical Hodgkin lymphoma. *Blood* 2002; **99**: 4283–97.
47. **Karube K, Ohshima K, Suzumiya J, et al.** Gene expression profile of cytokines and chemokines in microdissected primary Hodgkin and Reed-Sternberg (HRS) cells: high expression of interleukin-11 receptor alpha. *Ann Oncol* 2006; **17**: 110–6.
48. **Carbone A, Gloghini A, Gruss HJ, et al.** CD40 ligand is constitutively expressed in a subset of T cell lymphomas and on

the microenvironmental reactive T cells of follicular lymphomas and Hodgkin's disease. *Am J Pathol* 1995; **147**: 912–22.

49. **Gandhi MK, Moll G, Smith C, et al.** Galectin-1 mediated suppression of Epstein-Barr virus specific T-cell immunity in classic Hodgkin lymphoma. *Blood* 2007; **110**: 1326–9.

50. **Newcom SR, Gu L.** Transforming growth factor beta 1 messenger RNA in Reed-Sternberg cells in nodular sclerosing Hodgkin's disease. *J Clin Pathol* 1995; **48**: 160–3.

51. **Chemnitz JM, Driesen J, Classen S, et al.** Prostaglandin E2 impairs CD4+ T cell activation by inhibition of I κ B: implications in Hodgkin's lymphoma. *Cancer Res* 2006; **66**: 1114–22.

52. **Poppema S, van den Berg A.** Interaction between host T cells and Reed-Sternberg cells in Hodgkin lymphomas. *Semin Cancer Biol* 2000; **10**: 345–50.

53. **Metkar SS, Naresh KN, Redkar AA, et al.** Expression of Fas and Fas ligand in Hodgkin's disease. *Leuk Lymphoma* 1999; **33**: 521–30.

54. **Francisco LM, Sage PT, Sharpe AH.** The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* 2010; **236**: 219–42.

55. **Chemnitz JM, Eggle D, Driesen J, et al.** RNA fingerprints provide direct evidence for the inhibitory role of TGF β and PD-1 on CD4+ T cells in Hodgkin lymphoma. *Blood* 2007; **110**: 3226–33.

56. **Yamamoto R, Nishikori M, Kitawaki T, et al.** PD-1-PD-1 ligand interaction contributes to immunosuppressive microenvironment of Hodgkin lymphoma. *Blood* 2008; **111**: 3220–4.

57. **Van Gool SW, Delabie J, Vandenberghe P, et al.** Expression of B7-2 (CD86) molecules by Reed-Sternberg cells of Hodgkin's disease. *Leukemia* 1997; **11**: 846–51.

58. **Younes A.** Novel treatment strategies for patients with relapsed classical Hodgkin lymphoma. *Hematology Am Soc Hematol Educ Program* 2009; 507–19.

59. **Crocker PR, Feizi T.** Carbohydrate recognition systems: functional triads in cell-cell interactions. *Curr Opin Struct Biol* 1996; **6**: 679–91.

60. **Lasky LA.** Selectin-carbohydrate interactions and the initiation of the inflammatory response. *Annu Rev Biochem* 1995; **64**: 113–39.

61. **Ohana-Malka O, Benharroch D, Isakov N, et al.** Selectins and anti-CD15 (Lewis x/a) antibodies transmit activation signals in Hodgkin's lymphoma-derived cell lines. *Exp Hematol* 2003; **31**: 1057–65.

62. **Hamann KJ, Dorscheid DR, Ko FD, et al.** Expression of Fas (CD95) and FasL (CD95L) in human airway epithelium. *Am J Respir Cell Mol Biol* 1998; **19**: 537–42.

63. **Reinehr R, Haussinger D.** Hyperosmotic activation of the CD95 system. *Methods Enzymol* 2007; **428**: 145–60.

64. **Peter ME, Budd RC, Desbarats J, et al.** The CD95 receptor: apoptosis revisited. *Cell* 2007; **129**: 447–50.

65. **Kim LH, Eow GI, Peh SC, et al.** The role of CD30, CD40 and CD95 in the regulation of proliferation and apoptosis in classical Hodgkin's lymphoma. *Pathology* 2003; **35**: 428–35.

66. **Sidorenko SP, Vetrova EP, Yurchenko OV, et al.** Monoclonal antibodies of IPO series against B cell differentiation antigens in leukemia and lymphoma immunophenotyping. *Neoplasma* 1992; **39**: 3–9.

67. **Strasser A, Jost PJ, Nagata S.** The many roles of FAS receptor signaling in the immune system. *Immunity* 2009; **30**: 180–92.

68. **Maggio EM, Van Den Berg A, de Jong D, et al.** Low frequency of FAS mutations in Reed-Sternberg cells of Hodgkin's lymphoma. *Am J Pathol* 2003; **162**: 29–35.

69. **Muschen M, Re D, Brauninger A, et al.** Somatic mutations of the CD95 gene in Hodgkin and Reed-Sternberg cells. *Cancer Res* 2000; **60**: 5640–3.

70. **Chhanabhai M, Krajewski S, Krajewska M, et al.** Immunohistochemical analysis of interleukin-1 β -converting enzyme/Ced-3 family protease, CPP32/Yama/Caspase-3, in Hodgkin's disease. *Blood* 1997; **90**: 2451–5.

71. **Vaux DL, Silke J.** IAPs, RINGs and ubiquitylation. *Nat Rev Mol Cell Biol* 2005; **6**: 287–97.

72. **Durkop H, Hirsch B, Hahn C, et al.** cIAP2 is highly expressed in Hodgkin-Reed-Sternberg cells and inhibits apoptosis by interfering with constitutively active caspase-3. *J Mol Med* 2006; **84**: 132–41.

73. **Mathas S, Lietz A, Anagnostopoulos I, et al.** c-FLIP mediates resistance of Hodgkin/Reed-Sternberg cells to death receptor-induced apoptosis. *J Exp Med* 2004; **199**: 1041–52.

74. **Dutton A, O'Neil JD, Milner AE, et al.** Expression of the cellular FLICE-inhibitory protein (c-FLIP) protects Hodgkin's lymphoma cells from autonomous Fas-mediated death. *Proc Natl Acad Sci U S A* 2004; **101**: 6611–6.

75. **Desbarats J, Birge RB, Mimouni-Rongy M, et al.** Fas engagement induces neurite growth through ERK activation and p35 upregulation. *Nat Cell Biol* 2003; **5**: 118–25.

76. **Kennedy NJ, Kataoka T, Tschoep J, et al.** Caspase activation is required for T cell proliferation. *J Exp Med* 1999; **190**: 1891–6.

77. **Ponton A, Clement MV, Stamenkovic I.** The CD95 (APO-1/Fas) receptor activates NF- κ B independently of its cytotoxic function. *J Biol Chem* 1996; **271**: 8991–5.

78. **Wong BR, Josien R, Choi Y.** TRANCE is a TNF family member that regulates dendritic cell and osteoclast function. *J Leukoc Biol* 1999; **65**: 715–24.

79. **Fiumara P, Snell V, Li Y, et al.** Functional expression of receptor activator of nuclear factor κ B in Hodgkin disease cell lines. *Blood* 2001; **98**: 2784–90.

80. **Chiu A, Xu W, He B, et al.** Hodgkin lymphoma cells express TACI and BCMA receptors and generate survival and proliferation signals in response to BAFF and APRIL. *Blood* 2007; **109**: 729–39.

81. **Benson MJ, Dillon SR, Castigli E, et al.** Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. *J Immunol* 2008; **180**: 3655–9.

82. **Mackay F, Schneider P.** Cracking the BAFF code. *Nat Rev Immunol* 2009; **9**: 491–502.

83. **Elgueta R, Benson MJ, de Vries VC, et al.** Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev* 2009; **229**: 152–72.

84. **Courtney KD, Corcoran RB, Engelman JA.** The PI3K pathway as drug target in human cancer. *J Clin Oncol* 2010; **28**: 1075–83.

85. **Nicholson KM, Anderson NG.** The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal* 2002; **14**: 381–95.

86. **Georgakis GV, Li Y, Rassidakis GZ, et al.** Inhibition of the phosphatidylinositol-3 kinase/Akt promotes G1 cell cycle arrest and apoptosis in Hodgkin lymphoma. *Br J Haematol* 2006; **132**: 503–11.

87. **Dutton A, Reynolds GM, Dawson CW, et al.** Constitutive activation of phosphatidylinositol 3 kinase contributes to the survival of Hodgkin's lymphoma cells through a mechanism involving Akt kinase and mTOR. *J Pathol* 2005; **205**: 498–506.

88. **Zheng B, Fiumara P, Li YV, et al.** MEK/ERK pathway is aberrantly active in Hodgkin disease: a signaling pathway shared by CD30, CD40, and RANK that regulates cell

proliferation and survival. *Blood*. 2003; **102**: 1019–27. Epub 2003 Apr 10.

89. **Renne C, Willenbrock K, Kuppers R, et al.** Autocrine- and paracrine-activated receptor tyrosine kinases in classic Hodgkin lymphoma. *Blood* 2005; **105**: 4051–9.

90. **Renne C, Minner S, Kuppers R, et al.** Autocrine NGF-beta/TRKA signalling is an important survival factor for Hodgkin lymphoma derived cell lines. *Leuk Res* 2008; **32**: 163–7.

91. **Sidorenko SP, Clark EA.** The dual-function CD150 receptor subfamily: the viral attraction. *Nat Immunol* 2003; **4**: 19–24.

92. **Schwartzberg PL, Mueller KL, Qi H, et al.** SLAM receptors and SAP influence lymphocyte interactions, development and function. *Nat Rev Immunol* 2009; **9**: 39–46.

93. **Castro AG, Hauser TM, Cocks BG, et al.** Molecular and functional characterization of mouse signaling lymphocytic activation molecule (SLAM): differential expression and responsiveness in Th1 and Th2 cells. *J Immunol* 1999; **163**: 5860–70.

94. **Hamalainen H, Meissner S, Lahesmaa R.** Signaling lymphocytic activation molecule (SLAM) is differentially expressed in human Th1 and Th2 cells. *J Immunol Methods* 2000; **242**: 9–19.

95. **Aversa G, Carballido J, Punnonen J, et al.** SLAM and its role in T cell activation and Th cell responses. *Immunol Cell Biol* 1997; **75**: 202–5.

96. **Cocks BG, Chang CC, Carballido JM, et al.** A novel receptor involved in T-cell activation. *Nature*. 1995; **376**: 260–3.

97. **Sidorenko SP, Clark EA.** Characterization of a cell surface glycoprotein IPO-3, expressed on activated human B and T lymphocytes. *J Immunol* 1993; **151**: 4614–24.

98. **Yurchenko MY, Kovalevska LM, Shlapatska LM, et al.** CD150 regulates JNK1/2 activation in normal and Hodgkin's lymphoma B cells. *Immunol Cell Biol* 2010; **88**: 565–74.

99. **Mikhailap SV, Shlapatska LM, Yurchenko OV, et al.** The adaptor protein SH2D1A regulates signaling through CD150 (SLAM) in B cells. *Blood* 2004; **104**: 4063–70.

100. **Kis LL, Nagy N, Klein G, et al.** Expression of SH2D1A in five classical Hodgkin's disease-derived cell lines. *Int J Cancer* 2003; **104**: 658–61.

101. **Punnonen J, Cocks BG, Carballido JM, et al.** Soluble and membrane-bound forms of signaling lymphocytic activation molecule (SLAM) induce proliferation and Ig synthesis by activated human B lymphocytes. *J Exp Med* 1997; **185**: 993–1004.

102. **Yurchenko MY, Kashuba EV, Shlapatska LM, et al.** The role of CD150-SH2D1A association in CD150 signaling in Hodgkin's lymphoma cell lines. *Exp Oncol* 2005; **27**: 24–30.

103. **Mikhailap SV, Shlapatska LM, Berdova AG, et al.** CDw150 associates with src-homology 2-containing inositol phosphatase and modulates CD95-mediated apoptosis. *J Immunol* 1999; **162**: 5719–27.

104. **Yurchenko MY, Ganshevskiy DV, Shlapatska LM, et al.** CD150-mediated Akt signaling in normal and malignant B cells. *Exp Oncol* 2011; **33**: in press.