HHS Public Access

Author manuscript

Semin Cancer Biol. Author manuscript; available in PMC 2019 February 14.

Published in final edited form as:

Semin Cancer Biol. 2015 October ; 34: 3–13. doi:10.1016/j.semcancer.2015.03.002.

Shaping of the Tumor Microenvironment: Stromal Cells and Vessels

Marzenna Blonska, PhD[1], **Nitin K. Agarwal, PhD**[2], and **Francisco Vega, MD PhD**[2]

[1]Division of Hematology-Oncology, Department of Medicine, University of Miami and Sylvester Comprehensive Cancer Center, Miami, FL

[2]Division of Hematopathology, Department of Pathology, University of Miami and Sylvester Comprehensive Cancer Center, Miami, FL

Abstract

Lymphomas develop and progress in a specialized tissue microenvironment such as bone marrow as well as secondary lymphoid organs such as lymph node and spleen. The lymphoma microenvironment is characterized by a heterogeneous population of stromal cells, including fibroblastic reticular cells, nurse-like cells, mesenchymal stem cells, follicular dendritic cells, and inflammatory cells such as macrophages, T- and B-cells. These cell populations interact with the lymphoma cells to promote lymphoma growth, survival and drug resistance through multiple mechanisms. Angiogenesis is also recognized as an important factor associated with lymphoma progression. In recent years, we have learned that the interaction between the malignant and non-malignant cells is bidirectional and resembles, at least in part, the pattern seen between non-neoplastic lymphoid cells and the normal microenvironment of lymphoid organs. A summary of the current knowledge of lymphoma microenvironment focusing on the cellular components will be reviewed here.

Keywords

lymphoma microenvironment; stromal cells; angiogenesis; Hedgehog signaling; NF-kB signaling

INTRODUCTION

Lymphomas are malignant neoplasms that typically arise in lymphoid tissues, however extranodal localizations are not uncommon. Recent studies provided compelling evidence that not only the genetic alterations harbored by lymphoma cells themselves but also

Correspondence: Francisco Vega, M.D., Ph.D., Division of Hematopathology, Department of Pathology and Laboratory Medicine, University of Miami/Sylvester Comprehensive Cancer Center, Building UMH, Suite 4061, 1400 NW 12th Ave, Miami, FL 33136, Phone: 305-243-5760, Fax: 305-689-5899 fvega@med.miami.edu.

Conflict of interests

None of the authors have any potential conflicts of interest.

interactions with the surrounding microenvironment are crucial for the growth and survival of malignant cells [1].

The lymphoma microenvironment is composed of a mixture of stromal cells, immune cells and extracellular matrix proteins as well as blood vessels. Cell subtypes that participate in the lymphoma microenvironment include nodal fibroblastic reticular cells (FRC), follicular dendritic cells (FDC), mesenchymal stem/stromal cells (MSC) antigen-presenting cells (APC) and immune cells (macrophages, mast cells, T- and B-cells). It has become increasingly evident that the crosstalk between lymphoma cells and their respective microenvironment is bidirectional and that multiple secreted factors and cell surface molecules contribute to the activation of major signaling pathways in both lymphoma and stromal cells. A better understanding of these complex interactions between lymphoma and microenvironment not only give us insights into the pathogenesis and progression of lymphomas, but also is essential for the development of novel effective treatment strategies. In this review, we will focus on the cellular component of the lymphoma microenvironment and its contribution to the provision of survival and proliferation signals to the lymphoma cells. Finally, we will briefly discuss nuclear factor kB (NF-kB) and Hedgehog (Hh) signaling pathways in the context of lymphoma microenvironment. These two pathways bridge external stimuli to internal cellular events that contribute to lymphomagenesis and lymphoma progression [2-9].

1. Stromal cells

1.1. Fibroblastic reticular cells

Fibroblastic reticular cells (FRCs), called adventitial/perisinusoidal reticular cells (ARC) in the bone marrow, are stromal cells that produce, ensheath and maintain the collagenous reticular fiber network of the paracortex in the lymph node (LN), splenic T-cell zone and hematopoietic bone marrow. Morphologically, FRCs resemble fibroblasts from other sites with long slender cytoplasmic processes, and have variable myofibroblastic features, as shown by electron microscopy and immunoreactivity with vimentin, keratin (8&18), smooth muscle actin, and desmin [10, 11] (Figure 1).

The LN reticular network has been conceptualized by Anderson and Shaw as a concentric arrangement of nested cylinders or “corridors” lined by a monolayer of FRCs that encircle high endothelial venules (HEV) and radiate outwards to the sinuses [12]. FRC provide physical routes or “corridors” for leukocyte trafficking and for the interactions between antigen presenting cells (APC) and lymphocytes (Figure 2). Also, FRCs actively participate in the LN expansion/contractility, in cell trafficking regulation, access of T-cells into the paracortex and promoting chemokinesis of dendritic cells (DC) within LN [13-17]. In addition, FRCs form specialized conduits that transport small molecules and chemokines along the reticular fiber from the nodal subcapsular sinuses to the abluminal surface of the HEV located deep in the LN paracortex [15, 18, 19]. FRC are in intimate contact with lymph-derived cytokines (e.g. tumor necrosis factor, TNF), and are subjected to the same dynamic cytokine regulation as sinus endothelium and HEV [14].

FRCs provide homing and pro-survival signals to non-neoplastic lymphocytes and to lymphoma cells. The homing of naïve T-, B-cells and migratory DCs to the LN paracortex is mediated by CC-chemokine receptor 7 (CCR7), a homeostatic chemokine receptor expressed in T-, B- and dendritic cells, and by the ligands CCL19 and CCL21 expressed by FRCs and HEVs. Several studies demonstrated that CCR7 is highly expressed in lymphomas with widespread LN dissemination including chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma (MCL) and follicular lymphoma (FL). On the other hand, the low expression of CCR7 in primary mediastinal large (thymic) B-cell lymphoma might contribute to the tendency of this lymphoma to remain localized [20-22]. In a Myc-driven B-cell lymphoma model, it has been shown that CCR7 regulates homing of the lymphoma cells to the nodal paracortex and to the splenic T-cell zone [23]. Lymphoma cells expressing CCR7 rapidly infiltrate LNs, spleen and bone marrow of the recipient mice. By contrast, the absence of CCR7 expression on malignant cells delayed the onset of disease and reduced involvement in these organs [23].

Experimental data has shown that the interaction between lymphoma cells with the FRC network is reciprocal. For example, lymphoma cells secrete lymphotoxin-α (LTα) that stimulates LTαR-expressing FRC to enhance the release of CCL19/CCL21 [23]. It has been shown that FL cells interact with FRC in the LN and with ARC in the bone marrow to upregulate the expression of tissue transglutaminase (protein involved in extracellular matrix production and integrin adhesion) and low-affinity nerve growth factor receptor (LNFGR) [14, 24].

1.2. Follicular dendritic cells and other dendritic cells

Follicular dendritic cells (FDC) are stromal cells that form a network of processes in primary follicles and germinal centers (GCs) of secondary lymphoid follicles. FDC have the ability to capture large amounts of antigen in the form of immune complexes in highly ordered units designated as iccosomes [25]. In GCs, FDCs are more densely concentrated in the light zone as demonstrated by monoclonal antibodies to CD21 or CD23.

The origin of FDC has not been established definitely but they are not derived from hematopoietic precursors and it is believed that FDC develop from local mesenchymal cells under the influence of signals provided by B- and T-cells. FDC are important for the development of GCs and FDC contribute to the migration and positioning of GC cells by secreting chemokines and survival factors. CXCL13 is one the most important chemokine secreted by FDC as it is the major chemoattractant for B- and follicular T-cells. VCAM-1, the major ligand for VLA-4, is highly expressed on the surface of FDC and the pair VCAM-1/VLA-4 acts not only as an initial tether for the B-cells allowing antigen recognition by the B cell receptor (BCR), but also enhancing B-cell activation and survival.

FDC contributes to the pathobiology of lymphomas. FDCs deliver angiogenesis, adhesion, migration and survival signaling to FL cells [26] and gene profiling array studies have showed that high expression of macrophage and activated FDC related genes was associated with shorter survival in FL patients [27]. FDCs protect lymphoma cells against apoptosis, in part through upregulation of MDR1, an ATP binding cassette (ABC) transporter triggering multidrug resistance [28], and through activation of a miR-181a-dependent mechanism

involving down-regulation of Bim expression [29]. Similar survival roles for the FDC in MCL and CLL have been documented [29, 30].

In angioimmunoblastic T cell lymphoma (AILT) there is a characteristic proliferation of arborizing HEV that are often surrounded by an expanded meshwork of cells expressing FDC markers as highlighted by immunohistochemical studies (very useful pathologic diagnostic clue for the diagnosis of this lymphoma type) (Figure 3). It has been suggested that these expanded networks of cells are not true FDC but activated FRC with upregulation of dendritic cell markers after interacting with the neoplastic T cells [31] (Figure 3).

The innate immune system is integrated by a heterogeneous group of DCs that function as antigen presenting cells. Based on their immunophenotype four major subsets of DCs have been identified: conventional (migratory and lymphoid tissue-resident) DCs, Langerhans cells, plasmacytoid DCs, and monocyte-derived DCs [32]. Potentially, anti-inflammatory cytokines and immunosuppressive signals from tumor cells or other stromal cells from the microenvironment can program DCs to accrue tolerogenic properties to induce T-cell anergy and thus facilitate lymphoma growth and progression [33, 34].

1.3. Mesenchymal stem/stromal cells (MSC)

Mesenchymal stem/stromal cells (MSC) are another stromal subset that contributes to development and progression of malignant neoplasms [35]. The bone marrow is the main reservoir of MSCs. It is believed that these cells migrate to various organs in the context of tissue remodeling and represent a source of pluripotent cells for the repairs of damaged tissues [36].

Ame-Thomas and collaborators [37] have found that *in vitro*, bone marrow derived MSC acquire a complete FRC phenotype in response to a combination of TNFα and lymphotoxinα1α2. Moreover, these authors also showed that MSCs, recruit primary FL cells that, in turn, trigger their differentiation into FRCs contributing to lymphoma survival [37].

Co-culture of CLL or MCL cells with MSC results in migration of a fraction of the lymphoma cells underneath the MSCs (pseudoemperipolesis) inducing a cobblestone-like appearance [38]. This migration depends on CXCR4 and VLA-4 expression by the tumor cells and exemplifies the migratory potential and adhesive interactions of lymphoma cells with the microenvironment. Ligation of the G-protein-associated receptor CXCR4 with the homeostatic chemokine CXCL12/stromal cell derived factor 1 (SDF-1) modulates intracellular signaling pathways related to chemotaxis, proliferation and survival [39]. MCL adhesion to stromal cells confers adhesion-mediated drug resistance, common feature in CLL, acute leukemias and plasma cell myeloma as well [40]. Natalizumab, a VLA-4 antibody, and plerixafor, a CXCR4 antagonist, both drugs are effective in inhibiting adhesion of MCL cells to MSCs, indicating these drugs are potential candidates for clinical trials [41]. MSC also support the viability of several T cell subsets (follicular helper and regulatory T cells) in the context of FL [42].

The interaction between leukemia/lymphoma cells and MSC is bi-directional and in the presence of lymphoma cells or medium collected from lymphoma cells, the MSC in turn also become activated [43, 44]. For example, CLL cell supernatants activate platelet-derived growth factor receptors (PDGFRs) in MSC, and contact with CLL cells causes expression of protein kinase C (PKC)-αII and subsequent activation of nuclear factor κB (NF-κB) in MSC [44].

2. Vessels and endothelial cells

The lymphocyte-endothelial recognition plays a central role in controlling access of specialized lymphocytes to lymphoid and non-lymphoid tissues, process regulated by adhesion molecules and chemokines. Lymphoma dissemination patterns often reflect basic rules of lymphocyte homing, explaining the strikingly tissue-specific dissemination of a number of lymphoid neoplasms including mucosal/cutaneous lymphomas and plasma cell myeloma. Expression of α4α7 integrins, a mediator of lymphocyte rolling and adhesion in the gut-associated lymphoid tissues, explain the gastrointestinal homing of a subset of lymphomas such as MCL, extranodal MALT lymphomas, FL, and enteropathy-associated T-cell lymphomas. Understanding the molecular mechanisms underlying lymphoma dissemination may provide novel targets for treatment of lymphoma patients.

Angiogenesis is increasingly recognized as an important prognostic factor associated with lymphoma progression as well as an attractive target for novel treatments [45, 46]. Lymphoma cells produce vascular endothelial growth factor-A (VEGF-A) and other angiogenic factors such as placental growth factor (PIGF) and VEGF-C which promote neo-angiogenesis via at least two mechanisms: sprouting angiogenesis for resident endothelial cells and vasculogenesis from recruitment of bone marrow progenitors cells [47, 48]. VEGF-A also supports the survival, proliferation and migration of lymphoma cells which express VEGFR1 and VEGFR2. Both VEGF and VEGF receptors are expressed by a number of lymphomas including CLL, MCL, classical Hodgkin lymphoma and diffuse large B cell lymphoma (DLBCL), implicating both autocrine and paracrine survival mechanisms [49-53]. High pretreatment serum levels of VEGF have been found associated with poor clinical outcomes in non-Hodgkin lymphomas [54].

Other pro-angiogenic growth factors may contribute to the angiogenesis and lymphoma progression; for example, one of the best-characterized pro-angiogenic cytokine is fibroblastic growth-factor-2 (FGF-2) also known as basic FGF (bFGF). Increased serum levels of bFGF have been reported in CLL, and plasma cell myeloma and expression of bFGF was found in tumor cells in CLL/SLL, FL, DLBCL, MCL, Burkitt and lymphoblastic lymphomas [55]. Interestingly, our recent study revealed that the direct interaction of DLBCL cells with mesenchymal cells strongly enhanced the production of bFGF and VEGF by lymphoma cells [56].

By analyzing gene expression profiles, Lenz and colleagues demonstrated in DLBCL that two gene signatures unrelated to the lymphoma cells had prognostic significance [57]. The first signature named “stromal 1” was associated with a favorable prognosis and was enriched with genes encoding extracellular matrix proteins, such as vitronectin receptor

ITGAV and integrin genes encoding extracellular matrix proteins, such as vitronectin receptor ITGAV and integrin α2 (ITGB2). The second signature, “stromal 2” was enriched in genes attributed to endothelial cells and genes involved in the regulation of angiogenesis and correlated with blood vessels density [57]. Ruan and colleagues further showed that the blockage of PDGFRα impairs lymphoma growth by affecting tumor angiogenesis (depleting perivascular pericytes and vascular smooth muscle cells) [58].

Interestingly, the presence of lymphoma-specific chromosomal translocations in endothelial cells in B-cell lymphomas has been reported [59]. In this study the authors suggested that endothelial cells in B-cell lymphomas are part of the neoplastic clone; although the nature of this relationship is unclear [59].

Mast cells support tumor growth and neovascularization by producing a wide array of angiogenic factors. In CLL involving bone marrow, there is an association between the number of mast cells and microvascular density. The number of mast cells and vascular density increase as the disease progresses [60]. Tryptase together with other angiogenic factors stored in the secretory granules of the mast cells contribute to angiogenesis in lymphomas.

3. Immune cells

3.1. Macrophages

Although macrophages are present in virtually any tumor in general, there is a group of lymphomas that are highly enriched with macrophages/histiocytes such as classical Hodgkin lymphoma, T-cell/histiocyte rich large B cell lymphoma, and lymphoepitheloid variant of peripheral T cell lymphoma among others (Figure 4).

In general, macrophages have been subdivided at least into two types based on their immunophenotype, M1 and M2. Macrophages with a M1 phenotype are considered to prevent the growth of tumors, whereas M2 macrophages are associated with angiogenesis and tumor progression and referred as tumor (lymphoma)-associated macrophages (LAM).

Microarray and immunohistochemical studies have suggested a central role of LAM in the pathogenesis and prognosis of several lymphomas. Dave and colleagues demonstrated in FL that two gene signatures unrelated to the lymphoma cells had prognostic significance [61]. The first signature “immune response 1” composed by mostly expressed by T-cells was associated with a favorable prognosis. The second signature, “immune response 2” was enriched in genes attributed to macrophages and FDCs and was associated with an inferior clinical course. Note that CD68 positive macrophages are present in FL but are less conspicuous than in reactive GCs and are not phagocytic but have upregulated mannose receptors that could facilitate survival of neoplastic cells via binding of the glycosylated VH regions of the BCR molecule [62].

The presence of high number of LAMs has been also associated with aggressive clinical course in FL, MCL, classical Hodgkin lymphoma, DLBCL and AITL. However, some studies have suggested that the prognostic significance is therapy type-dependent. For

example, in DLBCL patients treated with chemotherapy alone (without rituximab), a high number of CD68 positive LAMs was associated with poor prognosis; however, in DLBCL patients treated with chemotherapy plus rituximab, the prognostic effect of a high number of CD68 positive LAMs was opposite [63]. Similar findings were reported in FL [64, 65]. These data provide the possibility that rituximab can switch the function of LAMs, from tumor promotion to inhibition.

LAMs have also influence on tumor angiogenesis as they produce angiogenic factors such as VEGF-A, VEGF-C and MMP-9, among others, to support endothelial proliferation [66].

3.2. Nurse-like cells

Burger and colleagues found that some cultured mononuclear cells might differentiate into large, adherent cells that promote CLL survival *in vitro*. These cells were named nurse-like cells (NLCs) because of the similarities with thymic nurse cells that nurture developing thymocytes in a contact-dependent fashion [67]. Pseudofollicles or proliferation centers are a hallmark of CLL/SLL as they constitute truly proliferation and survival centers for CLL cells. The proliferation centers contain CD68 positive myeloid cells that are believed to represent the tissue counterpart of NLCs [68]. NLCs are of myeloid origin, differentiate from CD14-positive mononuclear cells, have a gene expression profile that resembles LAM (in particular those of M2 subtype), are positive for CD68, IL-10, CD11b, CD14, and IL-8 but not IL-12 [69].

NLCs attract CLL cells by secreting CXCL12 (SDF-1) and CXCL13 and contribute to survival of CLL cells by producing CXCL12, BAFF (B-cell activating factor or CD257), CD31 APRIL (CD256) and plexin B1 among others and by direct cell-to-cell contact [67, 70]. In CLL cells NOTCH1 is constitutively expressed and increases cell survival. Microenvironment interactions appear critical in activating NOTCH1 pathway; for example, NSCs express Jagged 1 and contribute to sustain NOTCH1 activity in CLL cells over time [71].

NLCs produce cytokines that can contribute to the expression of VCAM-1, ligand for CD49d, by the stromal/endothelial compartment. Specific VCAM-1/CD49d interactions can increase survival of CD49d-expressing CLL cells. This circuitry may represent the cellular basis explaining the aggressive and accelerated clinical course of CLL expressing CD49d [72]. VCAM-1/CD49d interactions may be targeted using anti-CD49d monoclonal antibodies. Natalizumab (Tysabri™) is a humanized antibody against CD49a already approved for the treatment of multiple sclerosis and Crohn’s disease. Lenalidomide is an immunomodulatory agent that is clinically active in CLL patients. It has been found that lenalidomide interferes with the nurture properties of NSCs in CLL cells reducing the expression of prosurvival signals such as CCL2, IGF1, and CXCL12 [73, 74].

The relationship between CLL cells and NLCs is reciprocal and recruitment of NLC can be pursued by CLL cells through secretion of specific chemokines (e.g., CCL3 and CCL4, previously called MIP-1A and MIP-1B) and by CD38 [75, 76]. In fact, plasma levels of CCL3 are associated with established prognostic markers of CLL and therefore they can become useful for risk assessment in patients with CLL [77].

3.3. T- and B-cells

Most lymphomas have a population of T cells mixed with the neoplastic cells that in some cases can be numerous (Figure 5). In FL, the neoplastic follicles contain T cells, predominantly CD4+, which are usually less numerous than in reactive follicles and lack the typical distribution seen in reactive GCs. The CD4:CD8 ratio is higher in low-grade FL than in FL grade 3. FoxP3+ T cells (Treg) are more numerous in FL than in reactive GCs and decreased in areas with transformation to DLBCL [78].

The role of T cells in lymphoma progression is complex. Some T cell subsets probably favor tumor progression. For example, follicular T helper cells (T_{FH}) (CD3+, CD4+, CD57+, PD1+, CXCL13+) express high levels of IL-4 and CD40L and are involved in promoting survival of FL cells. In addition, IL-4 and CD40L induce production of CCL17 and CCL22 by FL cells and facilitate the recruitment of Tregs contributing to the generation of an immunosuppressive tumor microenvironment [79]. FL cells express the ligand programmed cell death protein 1 (PD-L1) (one of two PD-1 ligands) and interactions with PD1 expressed on intratumoral T_{FH} results in immune suppression. In FL, the number and distribution of Tregs and T_{FH} cells have been reported to predict clinical prognosis in some [78], but not all [80], studies.

In CLL, proliferation centers contain activated CD4+ T cells that express CD40L and interact with CD40+ CLL cells, rescuing them from apoptosis. Lucatumumab (HCD122) is an anti-CD40 monoclonal antibody that inhibits the interaction between CD40L with CD40 and is currently in phase I clinical trials in CLL [81].

Classical Hodgkin lymphoma is another lymphoma type where CD4+ memory T-cells dominated the microenvironment, along with a variable number of macrophages, B-cells, eosinophils, plasma cells, and neutrophils. The tumor cells in classical Hodgkin lymphoma use multiple immune suppressive mechanisms that provide multiple immune escape strategies for the tumor cells. Recently, an activated functional and proinflammatory TH1-biased infiltrate has been demonstrated in classical Hodgkin lymphoma [82].

The role of non-neoplastic B-cells in lymphomas remains to be elucidated. However, there is evidence that, at least in other neoplasms, tumor-associated non-neoplastic B-cells have activated STAT3 and contribute to tumor development by promoting tumor angiogenesis [83].

4. Pro-survival signals in B-cell lymphoma

Cytokines/chemokines and growth factors have a crucial role in promoting proliferation and survival of neoplastic cells [1, 84]. Among them IL-6, IL-7, IL-4, and SDF1/CXCL12, are commonly released in the lymphoma microenvironment and efficiently activate the major pro-survival signaling pathways in neoplastic cells [85-87].

IL-7 is produced by FRC and lymphatic endothelial cells, and controls development and activation of different immune cells [88]. In bone marrow, IL-7 contributes to the development of B-cells regulating immunoglobulin gene rearrangement [89]. In the thymus,

IL-7 contributes to thymocyte survival and maturation and in the lymph nodes provides antiapoptotic and proliferative signals to T cells *via* activation of the PI3K/AKT pathway resulting in downregulation of p27 and upregulation of BCL-2. It has been demonstrated that IL-7 contributes to the progression of T-cell neoplasms and has oncogenic potential *in vivo* [90, 91].

In contrast, interleukin 6 (IL-6) is a multifunctional cytokine secreted by FDC, macrophages, bone marrow stromal cells and T-cells that remain the prototypal survival factor in B-cell neoplasms, including plasma cell myeloma [92]. It is also frequently released by lymphoma cells and plays an autocrine role [93]. IL-6 receptors consist of 2 glycoproteins, a soluble IL-6 receptor (gp80) and a transmembrane IL-6 receptor (gp130). IL-6 signals via a heterodimeric gp80/gp130 complex, and 2 trimeric IL-6/gp80/gp130 complexes initiate the signaling and trigger activation of Janus (JAK) kinases, and the downstream effectors STAT3, SHP-2/Ras, and PI3K/Akt [92]. Zhang and colleagues demonstrated that autocrine and/or paracrine IL-6 is involved in the growth and survival of MCL and resistance of MCL to chemotherapy [94]. Moreover, IL-6-mediated Jak/STAT3 signaling is activated in the activated subtype of DLBCL [95-97].

4.1. NF-κB signaling pathway

NF-κB pathway is an essential and tightly regulated signaling cascade that mediates proliferation and survival of normal lymphocytes [98]. However, the numerous studies indicate that deregulated NF-κB is a hallmark of various lymphoid malignancies, including plasma cell myeloma, MALT lymphoma, classical Hodgkin lymphoma, and the activated subtype of DLBCL [2, 3, 99-102].

NF-κB family of transcription factors consists of five proteins (RelA, RelB, c-Rel, p52, and p50) that form homo- or heterodimers. The term NF-κB traditionally refers to the RelA/p50 heterodimer activated through the classical (canonical) NF-κB pathway [103]. In resting cells, the canonical NF-κB activation is engaged by a large series of stimuli, including proinflammatory cytokines, T-cell receptor, B-cell receptor, and Toll-like receptors (TLRs). In addition, in mature B cells, NF-κB can be engaged by CD40, the lymphotoxin α receptor, and BAFF receptor through the non-canonical (NIK and IKKα-mediated) pathway [104]. Both NF-κB pathways initiate the transcription of anti-apoptotic genes (including BCL-2 family members, c-FLIP, c-IAP1, and c-IAP2), positive regulators of cell cycle (cyclin D1, cyclin D2), numerous inflammatory and immunoregulatory cytokines, cytokine receptors and others [105]. Therefore, it is not surprising that the NF-κB signaling is the most common pathway utilized by lymphoid malignances to avoid apoptosis.

Although constitutive NF-κB activity is usually linked to various oncogenic events [106-109], some lymphoma types (e.g. MCL) do not carry oncogenic mutations or chromosomal abnormalities that can explain aberrant NF-κB. Indeed, several studies indicate that microenvironmental stimuli potently induce NF-κB signaling and expression of NF-κB-dependent genes [110-112]. Also, our work indicates that co-culturing NF-κB-negative DLBCL cell lines with human stromal cells, activates NF-κB and enhances secretion of NF-κB target cytokines in the lymphoma cells [9]. These results can be explained, at least in part, by the release of B-cell activating factor (BAFF) [110] and/or Hh

ligand [9] by the stromal cells that activate multiple pathways including NF-kB. BAFF is produced by MSC, monocytes, macrophages and DCs and it has been demonstrated before that it protects lymphoma cells from spontaneous and drug-induced apoptosis [110]. Furthermore, BAFF seems to act as a B-cell survival factor by activating NF-κB [113, 114].

Accumulating experimental and preclinical data validates the NF-κB pathway as a promising therapeutic target in lymphomas. Inhibition of NF-κB signaling could be most effective in lymphomas that solely depend on NF-κB for survival, such as the activated-subtype of DLBCL [100, 115]. However, blocking NF-κB could also be useful in combination with chemotherapy in other types of lymphoma [116].

4.2. Hedgehog signaling

Recently, compelling evidence suggests that Hh proteins mediate the transduction of signals between stromal and lymphoma cells. Hh signaling is an evolutionary conserved pathway involved in organogenesis, embryogenesis and homeostasis of adult tissues [117]. Sonic Hh (Shh), Indian Hh, and Desert Hh were identified as the ligands, whereas, Patched 1 (PTCH1) and smoothened (SMO) serve as the cellular receptors. PTCH1 is the ligand receptor subunit and, in the absence of Hh ligands, inhibits SMO. In the presence of Hh ligands, the inhibition of PTCH1 over SMO is diminished, resulting in SMO activation. Upon activation, SMO transduces the signal to the cytoplasm using glioma-associated oncogene homolog (GLI) proteins as major transcriptional effectors [118].

FRCs produce and secrete Ihh and the suppression of Ihh secretion by the stromal cells substantially decreased lymphoma cell growth in mice models as well as in human lymphomas [23, 119]. Some studies have documented the paracrine survival role of Hh signaling in CLL/SLL, DLBCL and plasma cell myeloma [7, 120, 121]. In addition, it has been also shown that DLBCL cells (in contrast to CLL cells) are capable of synthesizing and secreting Hh ligands, supporting the presence of an autocrine Hh signaling loop in DLBCL [6] (Figures 6 and 7). The gain of cell-autonomous activation of the Hh pathway seen in DLBCL cells might represent a survival and/or proliferative advantage for the lymphoma cells and suggest, at least to some degree, stromal independence.

Recently, it has been also shown that Hh ligands secreted by stromal cells contribute to augment the activation of NF-κB in DLBCL in cell lines of germinal center and activated cell type [9]. A role for Hh signaling in the pathobiology of other hematopoietic neoplasms has been reported including ALK-positive anaplastic large cell lymphoma (ALCL), chronic myelogenous leukemia, and acute leukemias [122-125].

5. Concluding remarks

In summary, the lymphoma microenvironment is characterized by a heterogeneous population of accessory stromal cells and immune cells. The interaction between stroma and lymphoma cells is bidirectional, promotes cell survival, lymphoma growth, contributes to chemotherapy resistance and activates multiple major oncogenic pathways including PI3K/AKT, STAT3, Hh, and NF-κB among others. Numerous drugs are demonstrating effects in disrupting the microenvironment-lymphoma interaction resulting in a decrease in the

transduction of prosurvival signals in the tumor cells. It is expected that these drugs will have a growing role in the treatment of lymphomas in the close future.

Acknowledgments

Funds

Some of the work described in this article was supported by funds from University of Miami, CTSI Pilot Award Program (to FV and NA), The Translational Grant of The Leukemia & Lymphoma Society (to FV), and K08 Physician-Scientist Award 1 K08 CA143151-01 (NIH) (to FV).

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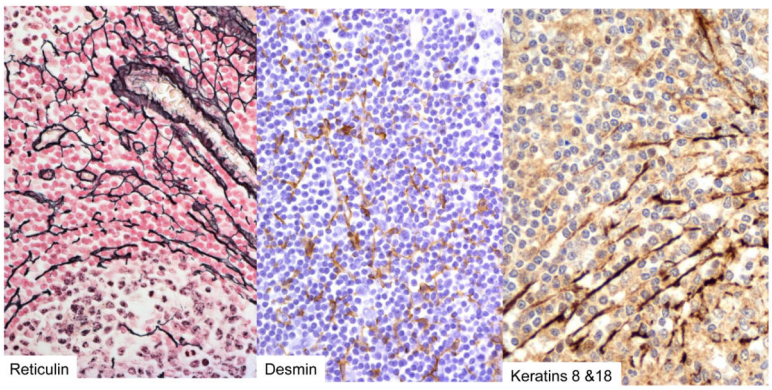

Figure 1. T-cell paracortex

Left, reticulin stain highlights the reticular fiber network that delineates the “corridors” of the T-cell paracortex. The reticular network is produced by fibroblastic reticular cells (FRC). FRC are variable positive for desmin and cytokeratins 8 &18 among other markers (center and right).

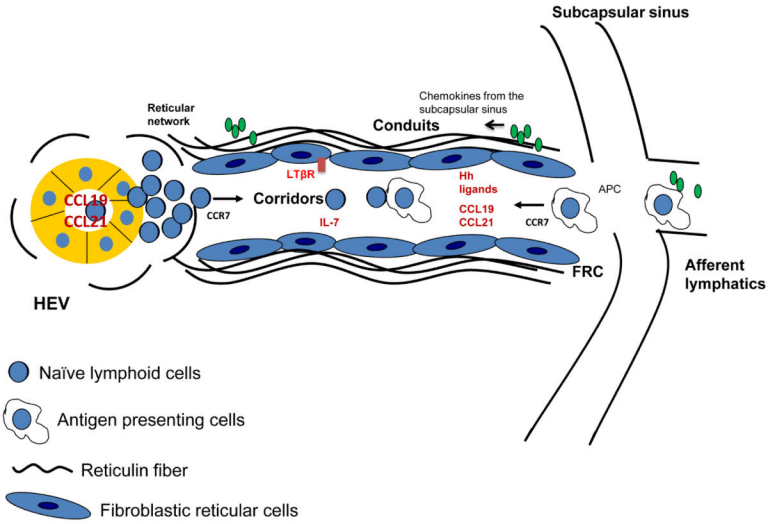

Figure 2. Schematic representation of the "corridors and conduits" of the paracortex

The corridors are lined by an epithelium-like monolayer of FRCs and are filled with lymphocytes and antigen presenting cells (APC). The lymphocytes trafficking inside of the corridors enter to the lymph node through the high endothelial venules (HEV) and the APC enter through the afferent lymphatics. The conduits are postulated to be located between the FRC monolayer of cells and the basal membrane. The conduits transport cytokines and chemokines (represented in green) from the sinuses and afferent lymphatics to the HEV. The homing of naïve T cells and migratory dendritic cells to the nodal paracortex is mediated by the homeostatic chemokine receptor CCR7. The CCR7 ligands, CCL19 and CCL21, are produced by FRCs. IL-7 is another cytokine produced by FRCs and FRCs express LTαR that after stimulation contributes to enhance the secretion of CCL19 and CCL21 by FRCs. Hh ligands are also produced and secreted by FRC.

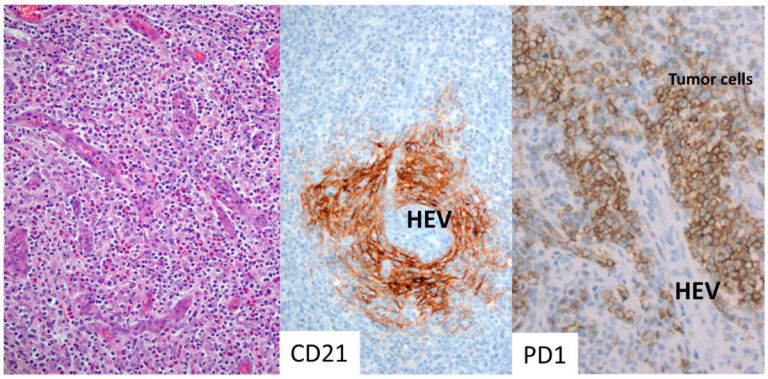

Figure 3. Angioimmunoblastic T cell lymphoma

Left, characteristic proliferation of arborizing HEV and numerous eosinophils in angioimmunoblastic T cell lymphoma. Center, a characteristic pathologic feature is the presence of a network, variable in size, of cells expressing the follicular dendritic cell (FDC) marker (CD21) surrounding the HEV. It has been suggested that these expanded networks of dendritic cells are not true FDC but activated perivascular FRC with upregulation of dendritic cell markers after interacting with the neoplastic T cells [31]. Right, PD1 highlights large aggregates of neoplastic follicular helper T cells in close proximity to the HEV, same areas where the perivascular networks of CD21 positive cells are noted.

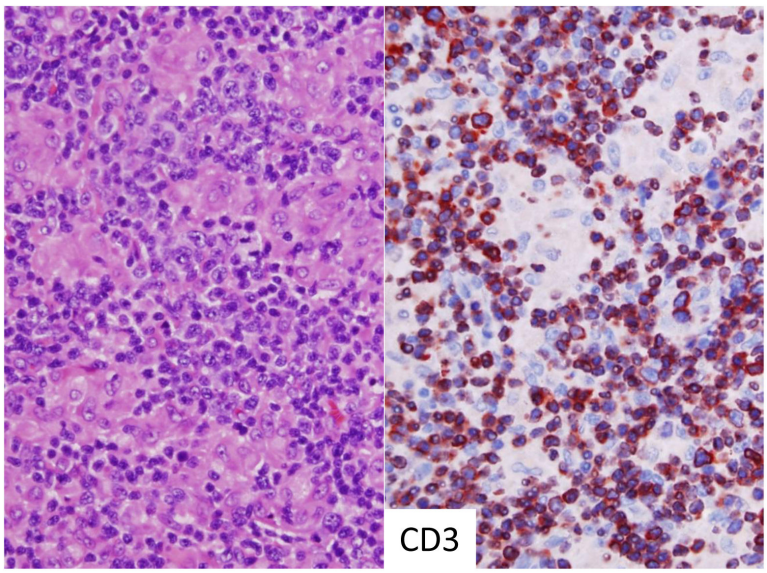

Figure 4. Peripheral T cell lymphoma, not otherwise specified (NOS), lymphoepitheloid variant (Lennert lymphoma)

In this T-cell lymphoma the tumor cells are admixed with clusters of epithelioid histiocytes.

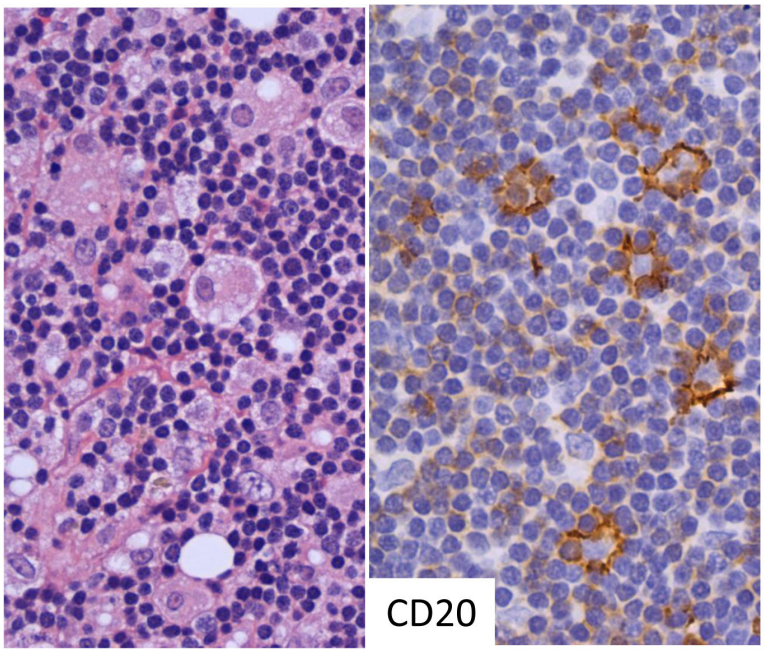

Figure 5. T-cell/histiocyte-rich large B-cell lymphoma

This lymphoma type is characterized by a small number of scattered large neoplastic cells (highlighted by CD20) in a background of abundant T cells and histiocytes.

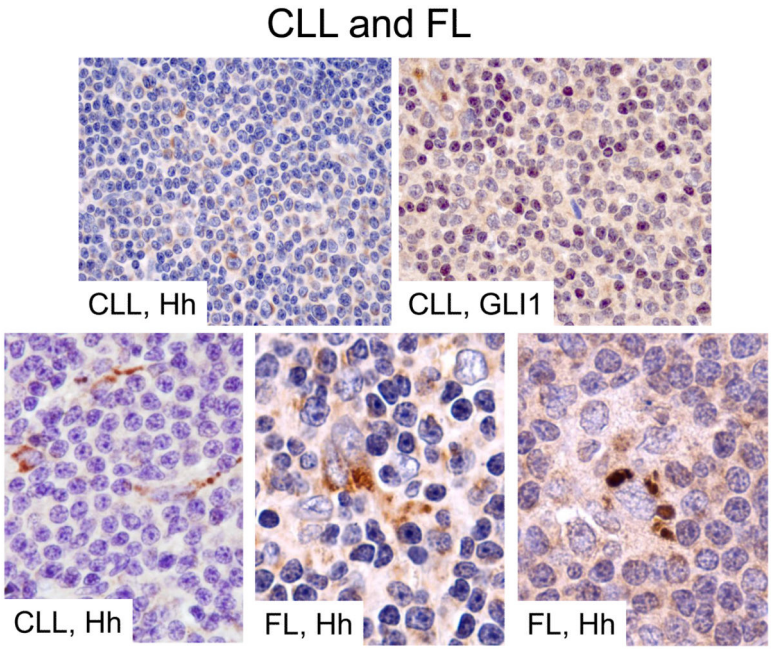

Figure 6. Hedgehog (Hh) signaling in lymphomas

Hh ligands are soluble ligands secreted by bone marrow, nodal and splenic stromal cells. The expression levels of Hh-related proteins (Hh ligands and the transcription factors GLI1, GLI2, and GLI3) were explored in low grade and high grade B cell lymphomas using immunohistochemistry (for Hh ligands, the polyclonal antibody that recognizes all three Hh ligands was used). In low-grade lymphomas, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and follicular lymphoma (FL), Hh ligands were detected in the cytoplasm of FRCs (lower left), follicular dendritic cells (lower center), endothelial cells, and macrophages (lower right), but not in the tumor cells. Only in CLL/SLL, a subset of the tumor cells, the proliferative component (prolymphocytes and paraimmunoblasts inside the proliferation centers), were positive for Hh ligands. Hh pathway was active in CLL as documented by the nuclear expression of GLI1 by a subset of the tumor cells.

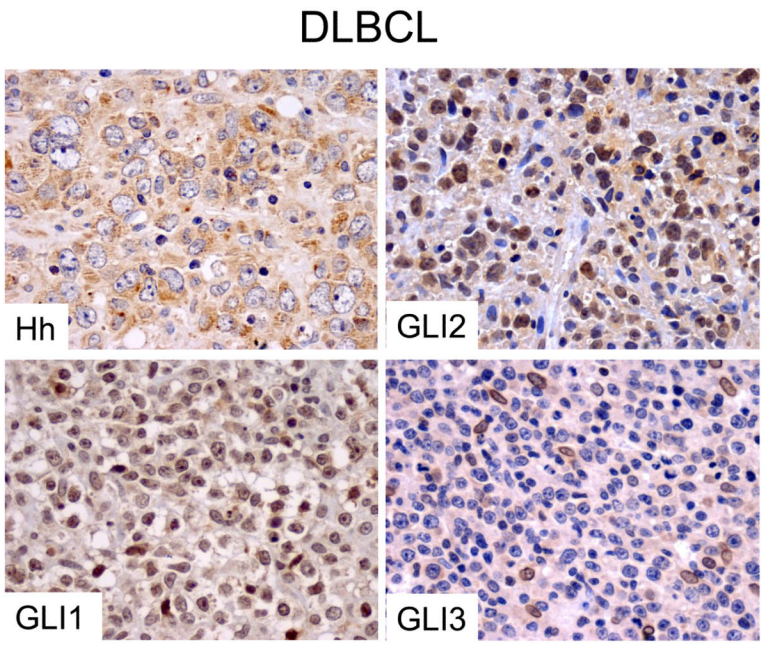

Figure 7. Hedgehog (Hh) signaling in lymphomas

In diffuse large B-cell lymphomas, in contrast to low-grade B cell lymphomas, expression of Hh ligands was detected in the cytoplasm of the lymphoma cells. Hh signaling was active, as documented by the detection of nuclear expression of GLI1 and GLI2, but not GLI3. These findings suggested that Hh ligands are produced and secreted by the DLBCL lymphoma cells. These results were confirmed by *in vitro* studies [5].