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The Role of Angiogenesis in Human Non-Hodgkin Lymphomas[1]

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Abstract

The role of angiogenesis in the growth of lymphomas and survival of patients with leukemias and other hematological malignancies has become evident since 1994. Angiogenic factors, such as vascular endothelial growth factor and its receptors together with other tumor microenvironment components, including myelo-monocytic cell, mast cells, endothelial progenitor cells, and circulating endothelial cells, have been shown to be important in the progression and maintenance of lymphoproliferative disorders. In this review article, we present an overview of the literature focusing on the relationship between angiogenesis and disease progression and the recent advantages in the antiangiogenic treatment in human non-Hodgkin lymphomas.

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Introduction

In the evolution of tumor growth, the avascular phase is followed by a vascular one [1]. Assuming that such growth is dependent on angiogenesis and that this depends on the release of angiogenic factors, the acquisition of an angiogenic ability can be seen as an expression of progression from neoplastic transformation to tumor growth and metastasis. All solid tumors, including those of the colon, lung, breast, cervix, bladder, prostate, and pancreas, progress through these two phases [2]. The role of angiogenesis in the growth and survival of leukemias and other hematological malignancies has become evident since 1994 [3] in a series of demonstrations that the progression is clearly related to their degree of angiogenesis.

Lymphomas constitute a large group of more than 40 lymphoproliferative disorders, classified on the basis of morphologic, immunologic, genetic, and clinical criteria. The importance of angiogenesis in lymphoproliferative disorders has been studied in relation to their impact on the prognosis of patients, suggesting high relevance in different types of lymphomas [4–6]. Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of lymphoproliferative malignancies with different patterns of behavior responses to treatment. B cell lymphomas represent approximately 88%, and T and natural killer (NK) cell lymphomas 12%, respectively, of all NHLs. Among B cell lymphomas, the incidence of diffuse large B cell lymphomas (DLBCLs) is 30%, of follicular lymphoma (FL) 25%, of extranodal marginal zone lymphoma of mucosa-associated lymphatic tissue 7%, of chronic lymphocytic leukemia (CLL) 7%, and of mantle cell lymphoma (MCL) 5%.

Lymphoid tumors are generally divided into one of two categories, namely, indolent lymphomas *versus* aggressive lymphomas, based on the characteristics of the disease at the time of presentation and the patients' life expectancy if the disease is left untreated. Generally, T cell lymphomas have a more aggressive clinical behavior than B cell lymphomas of comparable histology and patients with MCLs or anaplastic large lymphomas have a 5-year survival rate of approximately 30% and 80%, respectively [7].

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In this review article, we present an overview of the literature focusing on the relationship between angiogenesis and disease progression and the recent advantages in the antiangiogenic treatment in human NHL.

In Vitro and *Vivo* Experimental Models

Conditioned media of lymphoma cells induced a five-fold increased proliferation of cultured endothelial cells, suggesting the release of a soluble proangiogenic factor [8]. Human lymphoid tumor cells constitutively produce significant amounts of the extracellular matrix degrading enzymes matrix metalloproteinase-2 (MMP-2) and MMP-9, as demonstrated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis gelatin zymography and *in situ* hybridization [9]. Moreover, human lymphoid tumor cells are able to interact with extracellular matrix components vitronectin and fibronectin and this interaction it is mediated by $\alpha_v\beta_3$ integrin, allowing them to adhere to the substratum and enhancing their proliferation and protease secretion [10].

Lymphoma cells are able to induce an angiogenic response when tested *in vivo* in the hamster check pouch model [11]. Similarly, lymphoma bioptic specimens, when implanted on the chick embryo chorioallantoic membrane (CAM), evoked a strong angiogenic response [12]. The angiogenic response did not correlate with either the malignancy grade or the immunologic phenotype of the tumors. Different human Burkitt's lymphoma cells when inoculated onto the CAM formed solid tumors [13]. However, Epstein-Barr virus–positive cells induced massive recruitment of chick leukocytes at the tumor border and the development of granulation tissue with large number of blood and lymphatic vessels, although all cell lines tested have almost identical vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) expression [13].

Angiogenesis in Normal Lymph Nodes

The lymph node microvasculature consists of arterioles, metarterioles, anastomosing capillaries, small venules, and high endothelial post-capillary venules. Dense plexuses of capillaries arise from arterioles in the medullary cords, in the periphery of the deep cortex units, and in the outermost stratum of the extrafollicular zone of the peripheral cortex. In contrast, the folliculo-nodules and center of the deep cortex units are little vascularized by a loose capillary network, while no vessels occur in the subsinus layer [14–16]. When tissue fragments from normal lymph nodes are grafted *in vivo* on the chick embryo CAM, stereomicroscopic observation of the area around the implant revealed little hyperemia and a small number of growing vessels [12].

Angiogenesis in Benign Lymphadenopathies

In both reactive lymph nodes and lymph nodes with FLs, microvascular density (MVD) is higher in the paracortex than in the follicles and that there is no difference in MVD between reactive germinal centers and neoplastic follicles [17]. Moreover, MVD in the paracortex in reactive lymph nodes is higher than in diffuse large lymphomas [17]. In FL, several studies have recognized an increase in MVD in reactive parts of affected lymph nodes outside the follicles, compared to the neoplastic follicles [18–21].

Other authors [22] have shown that MVD is higher in lymphomas than in reactive lymph nodes and in aggressive than indolent lymphomas or that MVD in reactive lymph nodes is comparable to that observed in lymphomas [21].

Angiogenesis in NHLs

As concerns the morphologic features of tumor blood vessels, two patterns of laminin and type IV collagen expression are recognizable in the perivascular stroma of B-NHL, classified accordingly to the working formulation in low-, intermediate- and high-grade tumors [22]. A granular, speckled, and low-intensity staining was expressed by laminin and more frequently associated with the intermediate- and high-grade tumors. A linear continuous staining was co-expressed by laminin and type IV collagen and was more frequently associated with low-grade tumors. The granular and linear patterns may correspond to different steps in the basement membrane deposition: the granular pattern to the first one, when endothelial proliferation takes place; the linear pattern to the second stage, when basement membrane is completely differentiated [23]. This hypothesis is in agreement with the evidence that endothelial sprouting is associated with a higher concentration of laminin than type IV collagen around the outgrowing capillaries [24].

Moreover, the expression of tenascin in the stroma of B-NHL has been investigated and related to histologic malignancy and angiogenesis [25]. It is well known that tenascin stimulates angiogenesis, and because it forms a long reticulum with long extensions directly from vessels, it could also provide a pathway that favors migration of endothelial cells [26]. The presence of an increased number of immature vessels in DLBCL compared with FL, classified accordingly to the World Health Organization (WHO) classification, has also been demonstrated [17].

At ultrastructural level, the presence of immature capillaries in the stroma of diffuse intermediate-grade and high-grade B-NHLs has been shown [22]. These capillaries lack the basement membrane and generally consisted of two endothelial cells arranged in parallel, with thickened cytoplasm, resulting in slit-like lumen. On the contrary, in follicular intermediate-grade and low-grade B-NHLs, differentiated fenestrated capillaries surrounded by a continuous basement membrane were recognizable. Moreover, a morphologic heterogeneity of tumor blood vessels between histologic subtypes of lymphomas has been shown together with different patterns of neovascularization in both low-grade and high-grade B-NHLs [27,28]. In low-grade B-NHL, the vessel lumen is formed either by endothelial cell body curving or, more frequently, by the fusion of intracellular vacuoles in poorly differentiated endothelial cells. In high-grade B-NHL, however, the prevalent neoangiogenic pattern is the formation of a slit-like lumen [27,28]. Both low-grade and high-grade tumors exhibited development of transluminal bridges, expression of intussusceptive microvascular growth, and alternative mode of tumor vessel growth [29].

The clinical significance of increased MVD is not clear and difficult to establish because most of the studies describe heterogeneous populations including a wide variety of histologic subtypes of NHL and different treatment regimens.

As concerns the evaluation of MVD in bioptic specimens, a correlation between MVD and histologic subtype in NHL has not been established [30], or differences in MVD in patients with chemotherapy-resistant DLBCL and those with chemosensitive lymphomas has not been found [31]. Other studies in NHL and in DLBCL found no correlation between MVD and VEGF expression [32–34]. On the contrary, an increased vascularity pretreatment predicted favorable outcome in terms of progression-free and overall survival in patients with FL who received chemotherapy in association with interferon-α2b [18], or in FL, a high MVD predicted progressive disease and overall survival and correlated with transformation to DLBCL [32].

MVD is highest in aggressive subtypes including Burkitt's lymphoma and peripheral T cell lymphoma, compared with intermediate in DLBCL and lower in indolent FL [22]. In DLBCL, the average MVD correlates with the intensity of VEGF tumor cell immunoreactivity [35]. On the contrary, another study of the same group on patients affected by DLBCL treated with anthracycline-based chemotherapy showed no correlation between increased MVD and lymphoma cell VEGF expression [36]. In cutaneous T cell and B cell lymphomas, MVD is higher than in normal skin of a benign cutaneous lymphoproliferative disorder [37–39].

Several studies have demonstrated that high levels of VEGF in lymphoma samples correlate with advanced tumor stage and higher risk for relapsed/refractory disease after standard chemotherapy.

In NHL, high pretreatment levels of serum VEGF was a prognostic factor for survival in multivariate analysis [40]. In both T and B cell lymphomas, a negative correlation between the overall survival rate, respectively, 5-year disease-free survival and the pretreatment serum level of VEGF has been established [41], while in patients with DLBCL treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone, high serum level of VEGF was associated with adverse outcome, having lower values in survivors than in nonsurvivors [42].

VEGF expression was also demonstrated in peripheral T cell lymphoma, DLBCL, MCL, primary effusion lymphoma, and CLL/small lymphocytic lymphoma [43–46]. An adverse outcome associated with an increased VEGF tissue expression in aggressive and indolent lymphomas of B cell and T cell origin [47] has been demonstrated. In angioimmunoblastic T cell lymphoma, *VEGF-A* gene is overexpressed in both tumor and endothelial cells in comparison with reactive lymph nodes in association with a short survival time [48], and a high expression of *VEGF-A* in aggressive T cell lymphomas compared to indolent B cell lymphomas has been found [44]. In contrast, only a minority of indolent FLs show variable expression of VEGF-A [18,32], and transformation from indolent B cell lymphoma to aggressive DLBCL and poor prognostic subgroups within DLBCL are associated with increased VEGF expression [49].

In primary diffuse central nervous system lymphomas (PCNSLs), VEGF expression is correlated to MVD and VEGF expression is associated with a longer survival and blood-brain barrier alteration [50]. In 24 human diffuse large B cell PCNSL studied by means of immunocytochemistry and confocal laser microscopy, it has been demonstrated that 1) Aquaporin 4 (AQP4) expression was directly correlated with Ki-67 index, while AQP4 expression was low in tumor areas with a low Ki-67 index. 2) Different cells participated to vessel formation: $CD20^+$ tumor cells and factor $VIII^+$ endothelial cells; $AQP4^+$ tumor cells and $CD31^+$ endothelial cells; $CD20^+$ and $AQP4^+$ tumor cells; glial fibrillary acidic protein positive endothelial cells surrounded by glial fibrillary acidic protein positive tumor cells. Overall, these data suggest the importance of AQP4 in PCNSL due to its involvement in pathogenesis and resolution of cerebral edema. AQP4 is also involved in migration of tumor cells. It was also documented that tumor microvasculature in PCNSL is extremely heterogeneous, confirming the importance of neoangiogenesis in their pathogenesis [51].

The degree of VEGF expression correlated with the expression level of VEGFR-1 and VEGFR-2 in DLBCL lymphoma cells [35], and VEGFR-1, VEGFR-2, and VEGFR-3 are expressed in CLL, suggesting the possibility that VEGF acts as an autocrine/paracrine factor [52]. Moreover, VEGF prevents apoptosis and increases phosphorylation of VEGFR-1 and VEGFR-2, further supporting the existence of an autocrine prosurvival loop in CLL [53]. Blocking of VEGF and VEGFRs, by using neutralizing antibodies or tyrosine kinase inhibitors, resulted in decreased levels of p-STAT-3 and apoptosis of CLL cells [54]. High VEGF and VEGFR-1 expression identified a subgroup of patients affected by DLBCL with improved overall survival and progression-free survival when treated with anthracycline-based chemotherapy, suggesting that the autocrine signaling through VEGFR-1 may be susceptible to this therapeutic approach [36]. When immunodeficient mice engrafted with human DLBCL were treated with antibodies against human or murine VEGFR-1 or VEGFR-2, a significant tumor reduction of 50% was observed after treatment with human anti–VEGFR-1 but not with murine anti–VEGFR-1. By contrast, inhibition of murine VEGFR-2 resulted in a similar tumor reduction, but inhibition of human VEGFR-2 had no antitumor effect [55]. Anti–VEGFR-2 antibody was as effective as rituximab, and when combined, tumor volume was reduced even more to 75% [55]. A lesser expression of hypoxia-inducible factor-1 and hypoxia-inducible factor-2 and VEGF in indolent lymphomas, consisting mainly of FL, than in aggressive lymphomas has been observed [56]. Accordingly, only a minority of indolent lymphomas, showing histologic transformation to aggressive lymphoma, expressed VEGF-A in contrast to aggressive lymphomas [57].

Inhibition of autocrine or paracrine VEGFR-mediated loops with receptor-specific antibodies suppresses the growth of lymphomas by increasing tumor apoptosis and decreasing vascularization, respectively. These results confirm the role of VEGF in lymphomagenesis and support the targeting of VEGFRs as a therapeutic approach for aggressive lymphomas.

Other angiogenic growth factors may contribute to the angiogenic process and tumor progression in NHL. Among these, fibroblast growth factor-2 (FGF-2) is one of the best characterized proangiogenic cytokines. Because of its pleiotropic activity that may affect both tumor vasculature and tumor parenchyma, FGF-2 may contribute to cancer progression by inducing neovascularization, as well as by acting directly on tumor cells.

Various lymphoblastoid cell lines secrete FGF-2 [58]. Pazgal et al. [59] measured FGF-2 serum concentration in patients with NHL before and after treatment, conducted an immunohistochemical study to determine the expression of FGF-2 and FGF receptor-1 (FGFR-1) and MVD, and evaluated the prognostic significance of FGF-2 and FGFR-1 expression. They demonstrated that FGF-2 expression was correlated with poor survival and progression-free survival, while FGFR-1 expression was correlated with decreased rate of achievement of complete remission. Moreover, they did not detect a significant change in serum FGF-2 levels after two to three cycles of chemotherapy, nor they did find a correlation between MVD and NHL histology or grade or between MVD and prognosis. Moreover, in malignant lymphoma, high pretreatment levels of FGF-2 were a prognostic factor for survival in multivariate analysis, independently of other risk factors, including serum lactate dehydrogenase and number of extranodal sites [40]. Soluble VEGF, FGF-2, and platelet-derived growth factor-β levels decline after radiotherapy in NHL, suggesting that may have predictive significance for response to treatment and recurrence [60].

The Role of Myelo-monocytic Cells

At least three categories of proangiogenic bone marrow–derived circulating cells have been implicated in tumor angiogenesis: 1) cells that

contribute directly to the structural components of angiogenesis, including endothelial progenitor cells (EPCs) and pericyte progenitors; 2) myeloid progenitor subsets that can differentiate into endothelial-like cells and incorporate into the tumor neovessels; 3) a large heterogeneous group of cells of monocytic lineage that functions as vascular modulators that are not physically part of the vasculature [61].

It is well established that neoangiogenesis and growth of murine lymphomas is dependent on the recruitment of bone marrow–derived proangiogenic hematopoietic cells [62]. Increased hematopoietic infiltration by myeloid progenitors $CD68^+$ and VEGFR-1^+ and producing VEGF-A has been correlated with histologic subtypes of lymphoma, suggesting their involvement in the development of a proangiogenic phenotype [19,34,63]. In aggressive subtypes of Burkitt's lymphoma and DLBCL, VEGF-A–producing $CD68^+$ $VEGFR1^+$ myelo-monocytic cells are closely associated to new-formed blood vessels [34]. Genetic depletion of this subpopulation of $CD68^+$ $VEGFR1^+$ myelo-monocytic cells was sufficient to inhibit angiogenesis in various tumor experimental models, including lymphoma [62].

The Role of Macrophages and Mast Cells

It is well known that, among inflammatory cells found in tumors, tumor-associated macrophages and mast cells support tumor growth and neovascularization by producing a wide array of angiogenic cytokines. Tumor-associated macrophages have profound influence on the regulation of tumor angiogenesis. In fact, the degree of macrophage infiltration is positively correlated with tumor stage and angiogenesis in several human tumors in which a relationship between MVD and tumor progression has been clearly demonstrated [64].

Angiogenesis extent and macrophage density increase simultaneously with pathologic progression in B cell NHL, suggesting that an increase number of macrophages may be recruited and activated locally by malignant B cells and that angiogenesis associated with B-NHL may be induced, at least, partly, by angiogenic factors secreted by macrophages [19]. A high number of intratumoral macrophages correlate with poor prognosis in FL treated with chemotherapy alone, and rituximab appears to circumvent the unfavorable prognosis associated with high number of macrophages [65,66].

The extent of angiogenesis has been correlated with the number of mast cells in B-NHL and both counts increase in step with the increase of malignancy grade [64,67]. Tryptase together with other angiogenic factors stored in mast cell secretory granules may contribute to angiogenesis in B-NHL [64,67]. In an ultrastructural study of samples of B cell NHL, the presence of a heterogeneous population of mast cells characterized by the presence of granules with semilunar aspect and containing scrolls has been demonstrated [28,68]. Semilunar granules are the expression of a slow but progressive release of angiogenic factors due to chronic and progressive stimulation of mast cell degranulation, while, in the granules containing scrolls is stored tryptase, an angiogenic factor [69]. In B cell CLL, there is a striking association between the number of mast cells and MVD in bone marrow and both increase as the disease progresses [70]. Moreover, the consistent decrease of bone marrow angiogenesis after sequential fludarabine induction and alemtuzumab consolidation therapy in advanced CLL parallels the reduction of mast cells [71].

The Role of EPCs and Circulating Endothelial Cells

Circulating EPCs (CEPCs) have been detected within the blood flow during tumor growth and several evidences indicate that bone marrow–derived CEPCs contribute to tumor growth and tumor angiogenesis [72]. An increased number of $CD133^+$ $CD34^+$ VEGFR-2^+ CEPC has been found in younger patients and those with aggressive NHL, and the levels of CEPC decreased following complete response to treatment [73]. Moreover, lymph node EPCs were detected in vascular structure and in the stroma and correlated with an increased angiogenesis in indolent lymphoma [73]. In angiogenesis-defective Id-mutant mice, VEGFR-2^+ EPCs constitute >90% of tumor vessels following wild-type bone marrow rescue in a murine xenograft model of aggressive B cell lymphoma [74].

Within immunodeficient mice engrafted with lymphoma cells, a significant increase in the number of circulating endothelial cells (CECs) was observed after a period of 21 days, they being correlated with tumor size and serum level of VEGF [75]. An increase in CEC in patients with lymphoma compared with the control cases has been reported [76]. In those patients achieving complete remission after chemotherapy, the number of CECs was similar to healthy controls [76]. Accordingly, an increased number of CEC has been recognized in younger patients and those with aggressive NHL and the levels of CEC decreased following complete response to treatment [73].

Genetically Modified Lymphoma Endothelial Cells

Chromosomal abnormalities involving all chromosomes may occur in lymphomas, and characterization of genetic abnormalities, while not an absolute requirement, can be essential to the diagnosis of many lymphomas [77].

The presence of lymphoma-specific chromosomal translocations in endothelial cells in B cell lymphomas has been demonstrated, suggesting that microvascular endothelial cells in B cell lymphomas are, in part, tumor related [78]. Moreover, 15% to 85% of microvascular endothelial cells harbored lymphoma-specific genetic alterations consisting not only of B cell–specific translocation of immunoglobulin heavy locus (IGH) but also secondary genetic alterations in FL [78]. As suggested by Streubel et et al. [78], four mechanisms may be involved: lymphoma cells and endothelial cells may be derived from a multipotent hemangioblastic precursor cell targeted by neoplastic transformation that can differentiate in tumor cells or endothelial cells sharing the same genetic abnormalities; the endothelial cells carrying the genetic alterations of the lymphoma may arise from a cell that was already committed to the lymphoid lineage; fusion of lymphoma cells and endothelial cells with formation of hybrid vessels or gene transfer by means of the uptake of apoptotic bodies from tumor cells by neighboring cells may be alternative mechanisms.

The expression of a transcript called T cell Ig and mucin-containing molecule 3 has been identified in microvessels of DLBCL but not in reactive lymph nodes, suggesting that the lymphoma endothelium may act as a functional barrier facilitating the establishment of lymphoma immune tolerance [79].

Antiangiogenesis in NHL

Antiangiogenesis is a promising therapeutic approach in cancer. Preclinical studies with various angiogenesis inhibitors have produced remarkable antitumor effects in animal models and inhibition of angiogenesis is a major area of therapeutic development for the treatment of hematological malignancies.

Endostatin

Endostatin is an endogenous inhibitor of angiogenesis, which inhibits endothelial cell proliferation and migration, induces apoptosis, and causes a G_1 arrest of endothelial cells. Moreover, endostatin inhibits

MMP-2 activity, blocks the binding of VEGF to VEGFR-2, and stabilizes cell-cell and cell-matrix adhesions, preventing the breakage of these junctions required during angiogenesis [80].

Various endogenous inhibitors of angiogenesis may be found in the bloodstream, and a circulating form of human endostatin has been identified [81]. In a subgroup of patients with large cell and immunoblastic lymphoma, patients with high serum endostatin levels had a significantly better survival as compared with those with lower levels [82]. In a mouse model of B cell lymphoma, a delay in tumor growth has been shown after administration of endostatin [83] and continuous infusion of endostatin inhibits tumor growth and the mobilization and differentiation of EPC in mice bearing an angiogenic human lymphoma [84].

Treatment of lymphoma-bearing mice with endostatin caused an increase in the frequency of apoptotic cells in the endothelial cell compartment and most of the CECs were apoptotic or dead, while cyclophosphamide had no such effect. This difference probably occurred because most of the circulating apoptotic cells were hematopoietic and not endothelial in nature [75]. Endostatin administration in advanced stages of tumor growth led to tumor regression even in cyclophosphamide- and rituximab-resistant cases [83]. This effect was induced by inhibition of proliferation and stimulation of apoptosis in endothelial cells.

Immunomodulatory Drugs

Thalidomide exerts its antiangiogenic action through the inhibition of various cytokines, including tumor necrosis factor–α (TNF-α) and VEGF [85]. Thalidomide as single agent demonstrated a low overall response rate in patients with relapsed/refractory indolent NHL [86] and in heavily pretreated patients with recurrent lymphoma [87]. In combination with fludarabine, thalidomide was associated with significant therapeutic efficacy in CLL [88].

Lenalidomide is a more potent analog of thalidomide with preferentially TNF-α inhibitory properties and weaker antiangiogenic effect. Lenalidomide has been used as monotherapy in the treatment of both aggressive (DLBCL and transformed) and indolent relapsed/refractory NHL, MCL, and angioimmunoblastic T cell lymphoma [89–94]. Lenalidomide has also been studied in relapsed/refractory CLL, inducing complete and partial remissions, and has considerable activity in both heavily pretreated CLL patients and patients with unfavorable prognostic factors [95,96]. Moreover, thalidomide has shown antitumor activity in combination with rituximab in patients with relapsed or refractory MCL [97].

Bortezomib, a proteasome inhibitor, exerts anticancer activity mainly by inhibiting nuclear factor-κB (NF-κB), which has a pivotal role in the synthesis of antiapoptotic and angiogenic factors [98]. Clinical studies using bortezomib in relapsed or refractory B cell NHL, MCL, or marginal zone B cell lymphoma have shown promising results [99–104].

Anti-VEGF Neutralizing Antibodies and VEGFR Inhibitors

Stimulation of VEGFRs and other receptor tyrosine kinases causes activation of signaling pathways in endothelial cells. Many of the processes involved in tumor growth, progression, and metastasis are mediated by signaling molecules acting downstream from activated receptor tyrosine kinases. The VEGF/VEGFR pathway is considered a key regulator of angiogenesis and most of the agents currently in preclinical and clinical development focus on the inhibition of this pathway.

Bevacizumab (Avastin), a recombinant humanized monoclonal antibody directed against VEGF-A, has been the first antiangiogenic agent to be approved by the US Food and Drug Administration. Bevacizumab inhibit tumor growth, either alone or in combination with chemotherapy in untreated DLBCL [33,105]. A long disease-free survival in patients with aggressive NHL subtypes treated with bevacizumab as single agent has been reported [105]. Anti-VEGF neutralizing antibodies and VEGFR inhibitors blocked the pro-survival effect of CD154 (CD40 ligand) on CLL cells and decreased the migration of CLL cells through the endothelium [106,107].

Histone Deacetylase Inhibitors

Acetylation and deacetylation of histone proteins are important mechanisms for the regulation of gene expression. The interest in histone deacetylases (HDACs) as antineoplastic drugs originated with the observation that these agents could reverse the malignant phenotype of transformed cells [108]. HDACs represent an emerging class of therapeutic agents effective in hematological malignancies [109] that induce tumor cell cytostasis, differentiation, and apoptosis, in part due to an angiostatic effect, through an inhibition of VEGFR expression in endothelial cells [110,111].

Vorinostat, panobinostat, and MGCD0103 have been evaluated in NHL [112,113]. Successful therapy with vorinostat was associated with a reduced MVD and an increase of the antiangiogenic molecule thrombospondin-1 following treatment [111]. Panobinostat was evaluated in cutaneous T cell lymphoma (CTCL), and microarray analysis of skin biopsies showed a consistent down-regulation of proangiogenic gene *guanylate cyclase 1A3* and angiopoietin-1 [114]. Two HDAC inhibitors, sodium butyrate and suberoylanilide hydroxamic acid, reduced VEGF production and induced growth suppression and apoptosis in human MCL cell lines [115].

Concluding Remarks

Over the last 20 years, the importance of angiogenesis in human lymphoma is now well recognized, and several factors involved in its control are being identified. Within the different types of B cell NHL, angiogenesis may be prominent in aggressive rather than indolent subtypes. In addition to the demonstration that lymph node bioptic specimens involved with NHL contain high number of MVD and high number of inflammatory cells secreting angiogenic cytokines, several studies reported high levels of soluble angiogenic factors in sera of patients with NHL.

The antiangiogenic therapy is an important tool for the treatment of human lymphoma. However, a significant number of patients are resistant, whereas those who respond have minimal benefits. A tumor resistance and also significant side effects including toxicity can occur.

Further research should provide new useful therapeutic approaches and increase options for patients with resistant or refractory disease.

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