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Invited review

Insights in Hodgkin Lymphoma angiogenesis

Christian Marinaccio[a], Beatrice Nico[a], Eugenio Maiorano[b], Giorgina Specchia[c], Domenico Ribatti[a,d,*]

[a] Department of Basic Medical Sciences, Neurosciences, and Sensory Organs, University of Bari Medical School, Bari, Italy
[b] Department of Emergency and Transplantation, Pathology Section, University of Bari Medical School, Bari, Italy
[c] Department of Emergency and Transplantation, Hematology Section, University of Bari Medical School, Bari, Italy
[d] National Cancer Institute "Giovanni Paolo II", Bari, Italy

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ABSTRACT

Angiogenesis is a hallmark of tumor growth and progression in solid and hematological malignancies. Different cellular components of the tumor microenvironment such as macrophages, mast cells, circulating endothelial cells and angiogenic factors, including vascular endothelial growth factor and its receptors are involved in the maintenance of Hodgkin Lymphoma. In this review article, we highlight relevant literature focusing on the relationships between angiogenesis and Hodgkin Lymphoma as well as discussing anti-angiogenic treatments in this malignancy.

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1. Introduction

Angiogenesis is recognized as the process of formation of new blood vessels from pre-existing ones. This process occurs both in physiological conditions such as embryonic development and ovulation, and in pathologic conditions such as wound healing and cancer. The development of a tumor follows two main phases, the first known as the avascular phase, in which tumor growth is limited by the availability of nutrients and oxygen provided by blood vessels, the second known as the vascular phase, in which the tumor cell acquire an angiogenic phenotype to promote the formation of new blood vessels that can support tumor growth [1]. The angiogenic switch that governs the transition from one phase to the another is mediated by a shift in the balance of pro-angiogenic and anti-angiogenic factors by increased gene expression, changing of the bioavailability or activity of the pro-angiogenic molecules, or reduced concentration of anti-angiogenic

* Corresponding author at: Department of Basic Medical Sciences, Neurosciences, and Sensory Organs, University of Bari Medical School, Piazza Giulio Cesare, 11, 70124 Bari, Italy. Tel.: +39 080 5478326; fax: +39 080 5478310.
E-mail addresses: domenico.ribatti@uniba.it, ribatti@anatomia.uniba.it (D. Ribatti).

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mediators. The progression of solid tumors and hematological malignancies is clearly related to their degree of angiogenesis [1]. Lymphomas constitute a large group of lympho-proliferative disorders classified on the basis of morphologic, immunologic, genetic, and clinical criteria. Hodgkin Lymphomas (HL) display distinct morphological hallmarks described for the first time over 100 years ago. They are characterized by mono- and multinucleated Hodgkin–Reed–Stenberg (HRS) cells in classical Hodgkin Lymphoma (cHL) which encompasses mixed cellularity, nodular sclerosis and lymphocyte rich subtypes, and accounts for approximately 95% of HL cases, and lymphocyte predominant (LP) cells in nodular lymphocyte-predominant HL (NLPHL), which represent only 5% of HL cases [2].

Introduction of modern radiotherapy and polychemotherapy in 1960s and 1970s has significantly reduced the number of deaths associated to HL, with the consequent development of modern treatment strategies based on the distinction between cHL and NLPHL and only limited study of new treatments for advanced disease [3]. However, treatment failure persists in a substantial proportion of patients, underscoring the need of novel biomarkers and new therapeutic approaches to treat non-responding and relapsed HL. The growing importance of the tumor microenvironment and its cellular and molecular components might be an important target in developing such novel strategies.

In this review article we will focus on the relationship between angiogenesis in the context of the tumor microenvironment and disease progression in human HL.

2. *In vitro* and *in vivo* experimental models

Expression of angiogenic factors by lymphoid tumor cells was reported in several studies involving both *in vitro* and *in vivo* experimental models. Vascular endothelial growth factor (VEGF) is expressed by HRS cell lines L428 and KM-H2 in conditions of normoxia and hypoxia as demonstrated by FACS analysis and immunoassay of cell culture supernatant, indicating the potential role of HRS cells in enhancing angiogenesis by the secretion of a strong mitogen for endothelial cells as VEGF [4]. Gharbaran et al. [5] reported an overexpression of fibroblast growth factor-2 (FGF-2) and syndecan-1 (SDC-1) in ten HL cell lines and confirmed their increased expression in poor outcome and good outcome groups of HL patients by quantitative RT-PCR.

Notably, there is no report available about the evaluation of the angiogenic potential of HL cells with *in vivo* models such as the chick embryo chorioallantoic membrane (CAM). Xenotransplantation in NOD/SCID mouse strain has been evaluated for several myeloid and lymphoid tumor cell lines [6–8], while increased tumor vascularity was observed when HL cell lines KMH2, L428 and HDLM2 were injected in conjunction with bone marrow mast cells in NOD/SCID mouse [9].

3. Angiogenesis in HL

There are no literature data concerning the morphological and ultrastructural features of tumor vessels in HL.

Korkolopoulou et al. [10] investigated angiogenesis in 286 HL patients using a morphometric approach. Parameters of the vessels such as caliber showed a gradual increase through Ann Arbor stages I–IV supporting the notion that the larger the lumen of the microvessel the higher the chance of neoplastic cells to have access into the circulation. Conversely, microvascular density (MVD) declined with stage progression. The disparity between these parameters may be a consequence of the prevalence of vessels differentiating factors upon vascular growth factors. Moreover, a prognostic relevance of the morphometric variables was established through multivariate analysis [10].

Several studies investigated the expression of angiogenic cytokines in biopsy specimens of different HL subtypes. VEGF, matrix metalloproteinases-2 and -9 (MMP-2 and MMP-9) and tissue inhibitor of MMP-1 (TIMP1) are expressed in HRS cells in childhood HL. MMP-9 was significantly correlated with B symptoms while TIMP-1 in reactive lymphocytes correlated with advanced stage. However MVD was not correlated with expression of this cytokines in HRS cells [11]. Kuittinen et al. [12] also found a correlation between MMP-9 in reactive lymphocytes and B symptoms but no correlation between extent of neovascularization and level MMP-2 expression in malignant cells or reactive lymphocytes. Expression of hepatocyte growth factor (HGF) and its receptor c-met was found respectively in the reactive cellular background and in HRS cells from HL biopsy specimens [13] but there is no report of its correlation with potential stimulation of the angiogenic response by this cytokine in HL. Hypoxia Inducible Factor 1 alpha (HIF-1α) was investigated as well by immunohistochemistry in HL and found to be expressed moderately in the nuclei of HRS cells but not correlated with increased MVD suggesting that HL may utilize other angiogenic pathways relatively independent from HIF-1α [14]. Expression of VEGF-D was also evaluated by immunohistochemistry both in non-HL (NHL) and HL and found to be strongly expressed by HRS cells in line with a high number of tumor microvessels suggesting a role for this cytokine in angiogenesis [15]. Khnykin et al. [16] studied the FGF cytokines family and their receptors in HRS cells both at the protein and gene expression levels, but again no direct relationship was established between the expression of these cytokines and neovascularization.

Relevance of circulating serum levels of angiogenic factors has been demonstrated in several studies. In a retrospective study involving 67 patients of NHL and 37 patients of HL pre-treatment serum levels of VEGF, FGF-2, HGF and angiogenin were measured and compared to post-therapy levels. Patients with HD had abnormally elevated pre-treatment VEGF and HGF levels which were significantly reduced post-therapy and notably both pre-therapy and post-therapy VEGF levels were predictive of survival [17]. Elevated VEGF serum levels present in pre-treatment HL patients undergo a reduction in patients with prolonged complete remission, but these levels are still elevated when compared to healthy subjects' serum VEGF levels [18]. The post-treatment reduction of VEGF serum levels was also observed in a group of 36 patients with pediatric lymphomas in addition to a significant increase of post-treatment serum endostatin in the same cohort of patients [19]. These studies further support a role of angiogenesis in hematological malignancies in which the net balance of angiogenic and anti-angiogenic stimulus is shifted toward the angiogenic phenotype.

Vascular endothelial growth factor receptor-1 (VEGFR-1) and VEGFR-2 are two highly related tyrosine kinase receptors that bind VEGF-A and promote survival of endothelial cells through the Raf-MEK-MAP kinase pathway [20,21]. Dimtsas et al. [22] evaluated the expression pattern of VEGF-A, VEGFR-1 and VEGFR-2 retrospectively in cHL and NLPHL for a total of 194 cases and found these angiogenic cytokines were expressed in the majority of the cases by HRS cells and lymphocytic and histiocytic cells. Moreover, a significant correlation between these markers and vessel branching was observed while no correlation was found between other microvascular parameters and the expression of these angiogenic markers.

4. Macrophages and mast cells in HL angiogenesis

Inflammatory infiltrate in tumors consists of different types of cells capable of supporting tumor growth and neovascularization by the production of several angiogenic factors. Tumor associated