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Bone marrow vascular niche and the control of angiogenesis in multiple myeloma

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1. ABSTRACT

Bone marrow contains hematopoietic stem cells (HSCs) and non hematopoietic cells. HSCs are able to give rise to all types of mature blood cells, while the non hematopoietic component includes osteoblasts/osteoclasts, endothelial cells, endothelial progenitor cells and mesenchymal stem cells. All of these cells form specialized "niches" which are close to the vasculature ("vascular niche") or to the endosteum ("osteoblast niche"). The "vascular niche" is rich in blood vessels where endothelial cells and mural cells (pericytes and smooth muscle cells) create a microenvironment that affects the behavior of several stem and progenitor cells. The vessel wall serves as an independent niche for the recruitment of endothelial progenitor cells, mesenchymal stem cells and HSCs. The activation by angiogenic factors and inflammatory cytokines switch of the "vascular niche" promote tumor growth. This review article will focus on the description of the mechanisms involved in the generation of signals released by endothelial cells in the "vascular niche" that promote tumor growth in multiple myeloma.

2. INTRODUCTION

A specialized microenvironment, termed 'niche' could be required to support cell subpopulations characterized by stem cell potential (1). Stem cell niches or bone marrow niches have been described as anatomic sites that specifically enable stem cells to self-renew (2).

Bone marrow (BM) microenvironment comprises hematopoietic stem cells (HSCs) and non-hematopoietic cells. HSCs are multipotent stem cells that give rise to all the blood cell types from the myeloid and lymphoid lineages (3). The non-hematopoietic cells are composed of endothelial cells, pericytes, fibroblasts, osteoblasts, osteoclasts, mast cells, macrophages, and mesenchymal stem cells (4). These cells contribute to formation of specialized niches, which are closed to the endosteum, named 'osteoblast or endosteal niche', or to the BM vasculature, named 'vascular niche' (5).

Quiescent HSCs reside in the endosteal niche, where their interaction is mediated by several factor,

including N-cadherins, integrins, Jagged-1, Notch, bone morphogenetic proteins (BMPs), transforming growth factor beta (TGF-β), angiopoietin-1 (Ang-1), Wnt, and fibroblast growth factor-1 (FGF-1) (6, 7, 8).

The vascular niche is a site rich in blood vessels whereas endothelial cells, pericytes, and smooth muscle cells create a microenvironment that recruits endothelial progenitor cells (EPCs), mesenchymal stem cells and HSCs, and is important for stem cell mobilization, proliferation, and differentiation (9-11) Osteoblasts and vascular niches are adjacent and intimately related establishing several interactions between hematopoietic and non-hematopoietic cells (12, 13). There is evidence of a close relationship occurring between hematopoietic tumor cells and BM niches, through modulation of expression of growth factors, cytokines and adhesion molecules (14-16).

In the BM vascular niche, sinusoidal endothelial cells, HSCs, and EPCs trigger several complex interactions mediated by cell-cell and cell-basement membrane contacts and release of specific signal molecules, named angiocrine factors, favoring tumor growth (17). Angiocrine factors include interleukins (IL)-1, -3, -6, granulocyte-colony stimulation factor (G-CSF), granulocyte-macrophage-CSF (GM-CSF), and nitric oxide (NO). The bone marrow is a hypoxic space with low oxygen tension and this hypoxic environment contributes to maintain HSCs in the endosteal niche in a quiescent state (18).

Osteoblasts and HSCs are closely associated in the endosteal niche, leading to the secretion of different growth factors, including receptor activator of NF-kappa B ligand (RANKL) and Notch activation (11). HSCs detach from the endosteal niche and migrate in the vascular niche, where they come in contact with endothelial cells to re-establish hematopoiesis (11).

3. MULTIPLE MYELOMA NICHE AND ANGIOGENESIS

Endothelial cells and bone marrow stromal cells (BMSCs), including HSCs, fibroblasts, osteoblasts/osteoclasts, adipocytes, EPCs, T lymphocytes, macrophages, and mast cells, and extracellular matrix (ECM) proteins play an important role in creating a beneficial microenvironment for multiple myeloma (MM) cell growth and survival, which can be described as a "MM niche" (19). Reciprocal positive and negative interactions between plasma cells and BMSCs, are mediated by an array of cytokines, receptors and adhesion molecules. Interactions between these components determine the proliferation, migration and survival of plasma cells, as well as their acquisition of drug resistance and the development of the disease (Figure 1)(20).

MM cells preferentially engraft at the metaphyseal region of the bone marrow endosteum due to its rich vascularization, and form a complex with osteoblasts and osteoclasts (21). During MM progression, an increased number of osteoclasts are localized near to MM cells in the metaphyseal region. The vascular component of the MM niche plays an important role in supporting MM cell growth, in particular regard to chemoresistance.

Angiogenesis is enhanced in the bone marrow of MM in parallel with tumor progression (22). Angiogenesis is induced by plasma cells via angiogenic factors with the transition from monoclonal gammopathy of undetermined significance (MGUS) to MM, and probably with loss of angiostatic activity on the part of MGUS. The pathophysiology of MM-induced angiogenesis is complex and involves both direct production of angiogenic cytokines by plasma cells and their induction within the microenvironment. The latter are secreted by stromal cells, endothelial cells and osteoclasts, and promote plasma cell growth, survival and migration, as well as paracrine cytokine secretion and angiogenesis in the BM milieu (20). Angiogenesis is also supported by inflammatory cells following their recruitment and activation by MM plasma cells (23). Finally, circulating endothelial cells and EPCs contribute to the neovascularization, and the presence of EPCs suggests that vasculogenesis may also contribute to the full MM vascular tree (24).

4. ROLE OF DIFFERENT CELLS OF THE MM NICHE IN INDUCING ANGIOGENESIS

The deregulated interactions between MM cells, BMSCs and endothelial cells is important in MM pathogenesis and drug resistance. These interactions are at the basis of the clinical manifestations of the disease, including osteolytic bone lesions, hypercalcemia, suppressed hematopoietic functions, and increased angiogenesis.

Disruption of the normal balance between osteoblasts and osteoclasts is a crucial event in MM pathogenesis and progression (25). Myeloma plasma cells stimulate secretion of RANKL and inhibit expression of osteoprotegerin (OPG; the decoy receptor for RANKL) by osteoblasts, resulting in promotion of bone resorption by osteoclasts (26).

Osteoclastogenesis and angiogenesis progress in parallel to the growth of MM cells. In fact, inhibition of osteoclasts reduces angiogenesis and tumor burden in MM (27). Osteoclasts secrete the pro-angiogenic factor osteopontin (OPN), a ligand of αvβ3 integrin, which cooperates with vascular endothelial growth factor (VEGF) from MM cells to enhance angiogenesis and induce osteoclastogenic activity by endothelial cells (28). Moreover, in combination with IL-6, OPN enhances MM plasma cell growth (29). OPN knockout mice display minimal bone resorption compared with wild type and decreased osteoclast association at the bone surface (30). Secretion of matrix metalloproteinases-9 (MMP-9) by osteoclasts enhances angiogenesis through the release of VEGF from the ECM (31). BM endothelial cells, in their turn, secrete hepatocyte growth factor (HGF) that stimulates MMP-9 secretion in MM plasma cells, enhancing their invasive capacity (32).

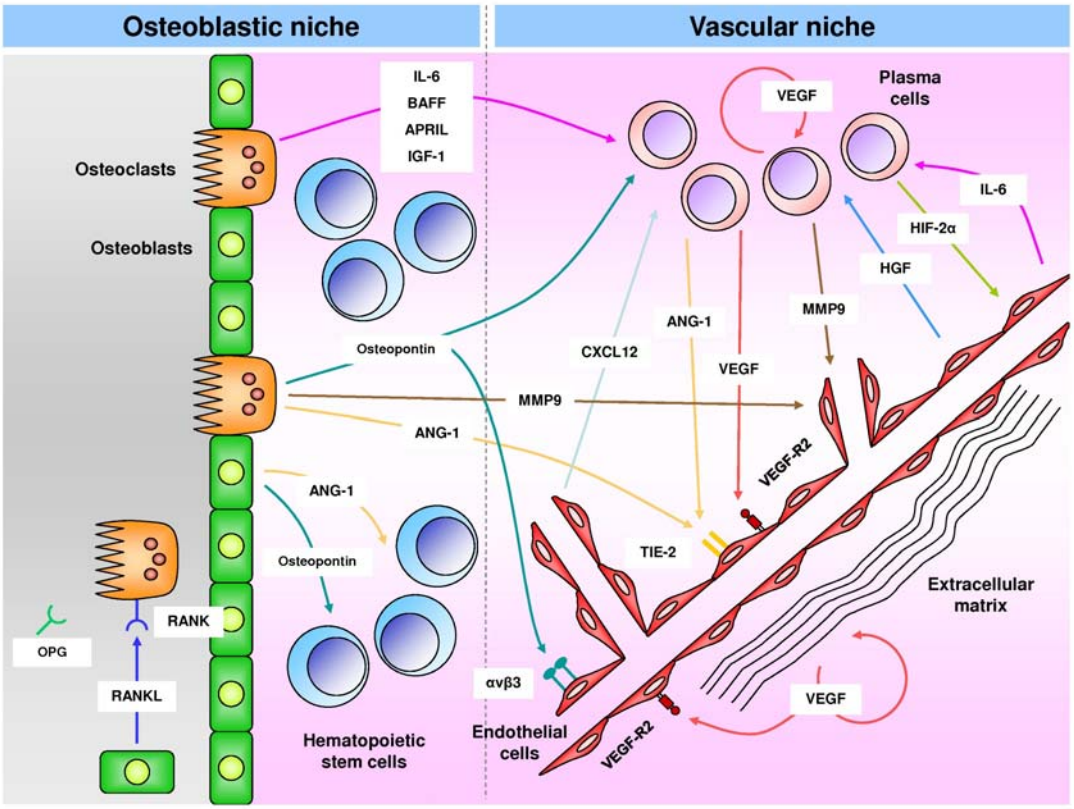

Figure 1. Interactions occurring in the bone marrow niches in multiple myeloma. The non-hematopoietic cells contribute to formation of two specialized niches: an "osteoblastic niche" which is closed to endosteum, and a "vascular niche" which is closed to the bone marrow vasculature. Osteoblasts express osteopontin and Ang-1 that mediate hematopoietic stem cells maintenance. Multiple myeloma cells stimulate RANKL secretion by osteoblasts, causing bone resorption by osteoclasts. In turn, osteoclasts secrete osteopontin, IL-6, BAFF, APRRIL and IGF-1, that favour angiogenesis and myeloma cell proliferation. Plasma cells synthesize VEGF, HIF-2α, MMP-9, and Ang-1, which support angiogenesis. In turn, endothelial cells secrete CXCL12, HGF, and IL-6 that are able to stimulate tumor cell proliferation.

Among myeloma growth factors and cytokines, insulin like growth factor-1 (IGF-1) and IL-6 are the most potent. Activation of the MEK/ERK pathway by IGF-1 and IL-6 leads to VEGF secretion, that promotes angiogenesis and tumor cell growth and survival by inducing IL-6 secretion (33). The elevated IL-6 levels stimulate paracrine and autocrine secretion of VEGF, that promotes further secretion of IL-6 by BMSCs (34). MM plasma cells VEGF can support osteoclast formation by RANKL. In turn, osteoclasts secrete anti-apoptotic cytokines for MM plasma cells, including IL-6, B-cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL) (35, 36). Finally, IL-6 expression by BMSCs is required for correct hematopoiesis and for differentiation of B cells into plasma cells, and protection of plasma cells from apoptosis (37, 38).

Angiogenesis favored by the hypoxic bone marrow environment increases the oxygen tension in the bone marrow and stimulates MM tumor growth (39). In this context, hypoxia inducible factor 2 alpha (HIF-2α) is expressed by $CD138^+$ MM plasma cells, resulting in enhanced angiogenesis (40). Moreover, levels of CXCL12 in the peripheral blood of MM patients positively correlate with the degree of BM angiogenesis, and BM endothelial cells isolated from MM patients express higher levels of CXCL12 compared with those derived from healthy donors, stimulating MM plasma cell proliferation (41).

Osteoblasts express OPN and Ang-1 at the bone surface that are required for osteoblast-mediated HSCs maintenance (42). Moreover, Ang-1 is expressed by MM plasma cells and is associated with up-regulated expression of its specific receptor Tie-2 on BM endothelial cells, increasing angiogenesis (43).

5. TARGETING ANGIOGENESIS IN THE MM NICHE

In contrast to traditional chemotherapeutics, the new compounds target not only the MM plasma cells, but also the microenvironment, that allows the plasma cells to

survive and proliferate. New agents that target tumor and stromal cells include: 1) agents targeting proteins dynamics (HSP90 and ubiquitin-proteasome system); 2) agents targeting intracellular signaling kinases (JAK/STAT, PI3K/Akt/mTOR, MAP pathways); 3) agents targeting cell cycle molecular machinery [cycline dependent kinase (CDKIs) and aurora kinase inhibitors]; 4) agents targeting membrane-bound receptors (IGF-1, VEGF, and CD40); 5) epigenetic modulators [DNA methyltransferase, histone deacetylase (HDAC)]; 6) agents targeting tumor vasculature; 7) immunomodulatory drugs (IMiDs).

The anti-angiogenic properties of thalidomide led to the consideration of its use in MM (44). Furthermore, in addition to its anti-angiogenic activity, thalidomide enhances T-cell- and NK-cell-mediated immunological responses, induces caspase-8 mediated apoptosis, and down-regulates IL-6 production within the BM microenvironment (45, 46).

Lenalidomide is a 4-amino-glutarimide analogue of thalidomide with anti-angiogenic proprieties. It inhibits the interaction between cadherin 5, beta-catenin, CD31 and adherens junction proteins interaction, critical for angiogenesis. Furthermore, lenalidomide inhibits VEGF-induced PI3K-Akt pathway signalling and HIF-1α expression (47), exerts an anti-tumor necrosis factor alpha (TNFα) activity, modulates the immune response stimulating T cells and NK cells activities, induces apoptosis of tumor cells, and decreases the binding of MM cells to BMSCs (46, 48-51). Moreover, lenalidomide alter the balance of bone resorption by inhibiting osteoclast formation (52, 53). Lenalidomide received FDA approval for treatment of MM patients who have received at least one prior therapy. A retrospective analysis of clinical trials, with previously treated relapsed/refractory MM, demonstrated an improved response rate and increased median for patients treated with lenalidomide and dexamethasone, compared to the those treated only with dexamethasone (54, 55, 56). Lenalidomide may sensitize MM plasma cells to bortezomib (57). In a phase 2 study, lenalidomide/bortezomib /dexamethasone produced responses in 84% of relapsed/refractory patients, including complete response or near complete response in 21% (58), and produced responses in 98-100% of newly diagnosed MM patients (59).

Bortezomib is a proteasome inhibitor, which induces endothelial cell apoptosis (60), inhibits VEGF, IL-6, Ang-1 and Ang-2 and IGF-1 secretion in BMSCs and endothelial cells derived from MM patients (61, 62), HIF-1α activity (63), downregulates caveolin-1 tyrosine phosphorylation, which is required for VEGF-mediated MM cell migration, and also blocks the caveolin-1 phosphorylation induced by VEGF in endothelial cells, thereby inhibiting ERK-dependent cell proliferation (64). The use of bortezomib in pre-transplant induction therapy revealed a higher response rate, compared to other induction regimens (65). Bortezomib and zoledronic acid display distinct and synergistic activities on bone marrow macrophages in MM patients (66). They inhibited macrophage proliferation, adhesion, migration, and expression of angiogenic cytokines [i.e. VEGF, FGF-2, HGF and platelet derived growth factor (PDGF)], and angiogenesis on Matrigel. Moreover, VEGFR-2 and ERK1/2 phosphoactivation as well as nuclear factor kB (NF-kB) were also inhibited (66). A reverse correlation between osteoblast differentiation and MM tumor growth has been reported in patients treated with bortezomib (67). Bortezomib induces the differentiation of mesenchymal stem cells into osteoblasts and induces apoptosis of osteoclasts (68, 69).

The administration of inhibitors of osteoclasts activity, including bisphosponates, not only prevents MM-induced bone destruction, but also exterts an anti-angiogenic activity. Therapeutic doses of zoledronic acid markedly inhibit *in vitro* proliferation, chemotaxis and angiogenesis of MM endothelial cells and *in vivo* angiogenesis in the chorioallantoic membrane (CAM) assay (70). These effects are partly sustained by gene and protein inhibition of VEGF and VEGFR-2 in an autocrine loop. Mevastatin, a specific inhibitor of the mevalonate pathway, which prevents prenylation of several proteins leading to cellular apoptosis, anti-angiogenesis and activation of gamma/delta T-cells, reverts the zoledronic acid anti-angiogenic effect, indicating that the drug halts this pathway. Overall, these data suggest that the zoledronic acid antitumoral activity in MM is also sustained by anti-angiogenesis, which would partly account for its therapeutic efficacy in MM (71, 72).

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Abbreviations: angiopoietin (Ang); a proliferation-inducing ligand (APRIL); B-cell activating factor (BAFF); bone marrow (BM); bone marrow stromal cells (BMSCs); bone morphogenetic proteins (BMPs); chorioallantoic membrane (CAM); cycline dependent kinase (CDKIs); endothelial progenitor cells (EPCs); extracellular matrix (ECM); fibroblast growth factor (FGF); granulocyte-colony stimulation factor (G-CSF); granulocyte-macrophage-CSF (GM-CSF); hematopoietic stem cells (HSCs); hepatocyte growth factor (HGF); histone deacetylase (HDAC); hypoxia inducible factor alpha (HIF-α); interleukin (IL); immunomodulatory drugs (IMiDs); insulin like growth factor-1 (IGF-1); matrix metalloproteinases-9 (MMP-9); monoclonal gammopathy of undetermined significance (MGUS); multiple myeloma (MM); nitric oxide (NO); nuclear factor kB (NF-kB); osteopontin (OPN); osteoprotegerin (OPG); platelet derived growth factor (PDGF); receptor activator of NF-kappa B ligand (RANKL); transforming growth factor beta (TGF-β); tumor necrosis factor alpha (TNFα).

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