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Microenvironment and multiple myeloma spread

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ABSTRACT

In patients with multiple myeloma (MM), the bone marrow (BM) 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 mesenchymal stem cells (MSCs), fibroblasts, osteoblasts, osteoclasts, chondroclasts, endothelial cells, endothelial progenitor cells (EPCs), B and T lymphocytes, NK cells, erythrocytes, megakaryocytes, platelets, macrophages and mast cells. All of these cells form specialized "niches" in the BM microenvironment 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, MSCs and HSCs. The activation by angiogenic factors and inflammatory cytokines switch the "vascular niche" to promote MM tumor growth and spread.
This review will focus on the mechanisms involved in the generation of signals released by endothelial cells in the "vascular niche" that promote tumor growth and spread in MM.

The role of bone marrow microenvironment in multiple myeloma progression

Multiple myeloma (MM) mainly progresses in the bone marrow (BM), and signals from this microenvironment play a critical role in maintaining plasma cell growth, spread, and survival [1]. The MM microenvironment is formed by clonal plasma cells, extracellular matrix (ECM) proteins, including collagen, fibronectin and laminin, and BM stromal cells, including haematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), fibroblasts, osteoblasts, osteoclasts, chondroclasts, endothelial cells, endothelial progenitor cells (EPCs), B and T lymphocytes, NK cells, erythrocytes, megakaryocytes, platelets, macrophages and mast cells, which are intimately involved in all biological stages of intramedullary growth.

The BM microenvironment is organized in a complex three-dimensional architecture of sub-microenvironments (niches, which are close to the endosteum, named 'osteoblast or endosteal niche', or to the BM vasculature, named 'vascular niche') [2]. The osteoblastic niche is composed by osteoblasts, reticular cells, fibroblasts and adipocytes, which create a supportive environment for stem cells [3].

Interactions between these components determine the proliferation, spread, and survival of plasma cells, as well as their acquisition of drug resistance and the development of relapse [4-6]. Very late activating antigen-4 (VLA-4), leukocyte function-associated antigen (LFA-1), mucin-1 antigen (MUC-1), and $\alpha_v\beta_3$ integrin, expressed by plasma cells, and vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expressed by bone marrow stromal cells (BMSCs) mediate their heterotypic and homotypic interactions, resulting in enhanced expression and release of cytokines and growth factors needed for the plasma cell survival [7].

Plasma cells secrete cytokines, such as tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), angiopoietin-1 (Ang-1) and matrix metalloproteinases (MMPs) [8-11]. Moreover, binding of plasma cells to BMSCs triggers transcription and secretion of cytokines by the latter, such as interleukin-6 (IL-6), insulin-like growth factor-1 (IGF-1), VEGF and CXCL12/stromal cell derived factor-1α (SDF-1α) [12-15] that mediate plasma cell growth, survival, and drug resistance, as well as BM angiogenesis.

The vascular niche is comprised of vasculature forming a conduit which enables MM cells both to leave the osteoblastic niche and enter the vascular system via transendothelial migration, hence to return to the BM via homing mechanisms. In this context, endothelial cells, pericytes, and smooth muscle cells create a microenvironment that recruits EPCs, MSCs and HSCs. The vascular niche is a site required for the differentiation

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and maturation of HSCs via both the production and secretion of various cytokines and growth factors, as well as via direct cell-cell contact. HSCs in turn prolong survival of BM endothelial cells by secreting endothelial cell growth factors [16-18].

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. HSCs detach from the endosteal niche and migrate into the vascular niche, where they come in contact with endothelial cells to re-establish hematopoiesis [17]. Disruption of the balance between osteoblasts and osteoclasts is a crucial event in MM pathogenesis and progression [19]. MM 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 [20].

The role of endothelial cells

Endothelial cells of the myeloma BM exhibit enhanced expression of specific angiogenic factors and their receptors such as VEGF and VEGF receptor-2 (VEGFR-2), FGF-2 and FGF-2 receptor-2 (FGF-2R-2), Ang-2 and Tie-2, by an increased *in vitro* and *in vivo* angiogenic activity [21]. Moreover, MM endothelial cells express more mRNA and secrete larger amounts of the CXC-chemokines CXCL8/IL-8, CXCL11/interferon-inducible T-cell alpha chemoattractant (I-TAC), CXCL12/SDF-1α and CCL2/monocyte chemotactic protein-1 (MCP-1) than umbilical vein endothelial cells. In their turn, paired plasma cells express cognate receptors to a variable extent, suggesting that paracrine loops between MM endothelial cells and plasma cells involving the CXC-chemokines and their receptors mediate plasma cell proliferation, chemotaxis, and spread [15].

Furthermore, platelet-derived growth factor (PDGF)-receptor beta (PDGFRβ) and pp60-Src as constitutively shared tyrosine kinases (TKs) are expressed in endothelial cells isolated from MM patients [22]. PDGF-BB/PDGFRβ kinase axis promotes MM vessel sprouting by activating ERK1-2, AKT, and the transcription of MM endothelial cells-released VEGF and IL-8. This activity was selectively induced by VEGF and the use of small interfering (si)RNAs validated pp60c-Src as a key signaling effector of the VEGF loop required for the MM endothelial cell survival, spread, and angiogenesis. It was inhibited by dasatinib, a novel orally bioactive PDGFRβ/Src TK-inhibitor.

A comparative gene expression profiling of endothelial cells derived from patients with MM and with monoclonal gammopathy of undetermined significance (MGUS) has been carried out [23]. Twenty-two genes were found differentially expressed (14 down-regulated and 8 up-regulated) at relatively high stringency in MM endothelial cells. Deregulated genes were mostly involved in ECM formation and bone remodeling, cell adhesion, chemotaxis/spread, angiogenesis, resistance to apoptosis, and cell-cycle regulation. Validation was focused on *DIRAS3*, *SERPINF1*, *SRPX*, *BNIP3*, *IER3*, and *SEPW1* genes, which were not previously found to be functionally correlated to the angiogenic phenotype of MM endothelial cells. The siRNA for three up-regulated genes (*BNIP3*, *IER3*, and *SEPW1*) affected endothelial cell proliferation, apoptosis, adhesion/spread, and capillary tube formation.

The role of circulating endothelial cells and endothelial precursor cells

Circulating endothelial cells (CECs) and EPCs in peripheral blood were 6 fold higher in MM patients compared to controls, and correlated positively with serum M protein and β2-microglobulin [24]. Moreover, EPCs displayed late colony formation/outgrowth and capillary-like network formation on Matrigel (an ECM-mimicking support), and these processes were inhibited by thalidomide treatment. Co-expression of VEGFR-2 and CD133 characterized EPCs and VEGFR-2 mRNA elevations were correlated with the M protein levels.

EPCs are closed associated with HSCs with a typical phenotype including the CD133, CD34 and VEGFR-2 molecules [25,26]. In patients with active MM, plasma cells and stromal cells in the BM microenvironment recruit HSCs, and induce their transformation into mature endothelial cells [27]. When patients' HSCs were incubated with VEGF, FGF-2 and IGF, cells differentiated into endothelial cell-like cells expressing typical endothelial markers, such as factor VIII-related antigen (FVIII-RA), VEGFR-2 and vascular endothelial (VE)-cadherin, and form capillary-like networks *in vitro* [27].

The role of macrophages and mast cells

When BM macrophages from MM patients are exposed to VEGF and FGF-2, they transform into cells similar to paired endothelial cells, and generate capillary-like networks overlapping those of endothelial cells [28]. Moreover, endothelial cell-like macrophages and apparently typical macrophages contribute sizeably to the formation of the neovessel wall in patients with active MM (i.e., those at diagnosis, at relapse or on refractory phase) [28]. In these patients, fluorescent activated cell sorter (FACS) analyses on freshly isolated BM mononuclear cells revealed higher percentages of $CD14^+/CD68^+$ cells than in those with non-active disease (i.e., those in complete/partial remission) and MGUS. Furthermore, in active MM patients BM biopsies displayed macrophages with both endothelial cell-like (i.e. $CD68^+/FVIII\text{-}RA^+$) and apparently typical (i.e. $CD68^+/FVIII\text{-}RA^-$) features located in the microvessel wall. Overall, these data suggest that in active MM, macrophages contribute to neovascularization through a vasculogenic pathway (Figure 1). Accordingly, Chen et al. [29] demonstrated that monocytes induce vascular endothelial cell gene expression and develop tube-like structures when they were cultured with BM from patients with MM that express pleiotrophin, effect specifically blocked with anti-pleiotrophin antibodies. Moreover, when co-injected with human MM cells into severe combined immunodeficiency mouse (SCID) mice, green fluorescent protein-marked human monocytes were found to be incorporated into tumor blood vessels and expressed human vascular endothelial cell proteins and genes that were blocked by anti-pleiotrophin antibodies.

BM angiogenesis and mast cell counts are highly correlated in patients with non-active and active MM and in those with MGUS, and both parameters increase simultaneously in active MM [30]. At the ultrastructural level, vessels from BM biopsies are lined by mast cells showing numerous and irregularly shaped electron dense granules [31]. Moreover, thick endothelial cells, containing endocytotic vescicles, but lacking granules, were connected by a junctional system with the mast cells lining the vessel wall [31]. These ultrastructural findings have been confirmed by confocal laser microscopy using double anti-tryptase (to mark mast cells) and anti-FVIII-RA (to mark endothelial cells) antibodies. Vessels from MM biopsies displayed regions stained by FVIII-RA alternating with regions stained by both tryptase and FVIII-RA. In the MGUS biopsies, the vessels were uniformly stained by the anti-FVIII-RA antibody only, while tryptase-positive mast cells were only recognizable perivascularly [31]. Overall, these data suggest that mast cells also contribute to MM neovascularization.

The role of fibroblasts

Critical elements in the microenvironment include tumor-associated fibroblasts, which provide an essential communication