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Multiple Myeloma: The Role of Angiogenesis in Disease Progression

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Abstract

Angiogenesis, the formation of new blood vessels from pre-existing ones, plays an important role in the biology of multiple myeloma and has a prognostic value in this disease. Multiple myeloma is a plasma cell malignancy that home to and expand in the bone marrow where actively interacts with stromal cells inducing neovascularization, a constant hallmark of disease progression.

Myeloma-induced angiogenesis involves either the direct production of angiogenic molecules by myeloma cells and the recruitment and activation of bone marrow stromal cells. Indeed, the angiogenic factors released in the bone marrow microenvironment by multiple myeloma plasma cells stimulate stromal cells to secrete their own angiogenic factors and induce the acquisition of a phenotypic and functional adaptation by non-endothelial cells, such as macrophages, which contribute to the completion of the neovessel wall (vasculogenic mimicry).

In this review we summarize recent data which give strong evidence for an increased angiogenic activity in the bone marrow microenvironment and support the hypothesis that angiogenesis is not only important for tumour growth but may also promote plasma cell growth in multiple myeloma.

Keywords: Multiple myeloma; Angiogenesis; Bone marrow microenvironment; Tumor progression; Vascular endothelial growth factor

Introduction

Multiple Myeloma (MM) is a malignancy of immunoglobulin (Ig)-synthesizing plasma cells, that home to and expand in the bone marrow [1]. Although presenting with the same histological features, MM is characterised by a high genomic heterogeneity [2,3]. There is a growing awareness that the interactions between MM plasma cells, stromal cells, hematopoietic cells, and the extracellular matrix (ECM) is as important as the genetic changes in the disease progression. Pathophysiological interactions of myeloma cells within the bone marrow microenvironment are highlighted by the progression-associated bone disease and neovascularization, and are witnessed by autocrine/paracrine circuits that activate multiple signalling pathways and affect the most important aspects of the malignant phenotype, *i.e.*, apoptosis/survival, proliferation, invasion, bone damage and angiogenesis [3,4].

Neovascularization, the formation of new vessels, represents one of the principal aspects of these interactions and a constant hallmark of disease progression [5].

This process is supported by angiogenic factors such as *Vascular Endothelial Growth Factor* (VEGF*), Fibroblast Growth Factor-2* (FGF-2) and *Hepatocyte Growth Factor* (HGF) which are directly secreted by the tumour plasma cells and the stromal cells.

The observation of an increased bone marrow angiogenesis in MM, an overexpression of angiogenic cytokines, and their correlation with disease activity, overall survival and the development of new anti-angiogenic compounds, led to consider angiogenesis as a new target in the treatment of MM.

Angiogenesis in multiple myeloma progression

Angiogenesis is an essential event for progression disease (growth, invasion and metastasis) in solid and haematological tumours, including MM, and has a prognostic value [6]. It is a tightly regulated multistep process [7] which begins with the activation of resting endothelial cells, continues with the degradation of the ECM, proliferation and migration of endothelial cells toward the angiogenic stimulus, and ends with the constitution of new blood vessels enveloped by a basement membrane [8].

Angiogenesis is uncontrolled and unlimited in time, and essential for tumour growth, invasion and metastasis during the transition from the avascular to the vascular phase [9]. Angiogenesis occurs in step with the transition from *Monoclonal Gammopathy of Undetermined Significance* (MGUS) to non active MM (complete response, plateau phase) and to active MM. Vacca et al. [10] showed that angiogenesis was increased in BM sections of patients with active MM disease. They reported an increase in BM microvessel density (MVD), a marker for angiogenesis, in MM patients, especially in advanced disease, compared with MGUS patients, which suggests a vascular phase during active MM and a pre-vascular phase in non active MM and MGUS [11]. Rajkumar [12] demonstrated in a large cohort of 400 patients that a progressive increase in bone marrow-MVD occurs across the whole spectrum of plasma cells disorders, including primary amyloidosis, MGUS, smoldering and active myeloma, which added further evidence to the hypothesis that angiogenesis is related to malignant plasma cells growth [12,13].

The pro-angiogenic bone marrow microenvironment

Recent advances in myeloma biology demonstrated the emerging role of the bone marrow microenvironment in promoting proliferation and survival of the malignant plasma cell clone [14]. Bone marrow angiogenesis is regulated by soluble pro- and anti-angiogenic factors

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which mediate the paracrine interactions between myeloma cells, endothelial cells and bone marrow stromal cells (BMSC) [15].

The increased angiogenic potential of active myeloma was first demonstrated by Vacca et al. who reported that serum-free conditioned media from bone marrow plasma cells of patients with active MM had a significantly higher angiogenic activity *in vitro* (matrigel capillarogenesis assay) and *in vivo* (chick chorioallantoic membrane assay) than samples from patients with non-active MM or MGUS [16]. Bone marrow plasma extracts contained higher levels of FGF-2 in samples from active patients versus those in non-active MM or MGUS. Moreover, the *in vitro* and *in vivo* angiogenic activity of the conditioned media could be reduced by FGF-2 antibodies [16]. A variety of growth factors, including VEGF, *Hepatocyte Growth Factor* (HGF), *Platelet-derived Growth Factor* (PDGF), and *Tumor Necrosis Factor-Alpha* (TNF-alpha), are known to promote angiogenesis [17].

Tumour cells and inflammatory cells, such as mast cells and macrophages, release these factors which stimulate resting endothelial cells by means of cognate specific tyrosine-kinase receptors [18,19]. Angiogenic cytokines may represent a potential indicator of response to therapy in solid and hematologic tumors [20,21]. Receptor/cytokine binding activates endothelial cells and increases cell proliferation, modifies cell adhesion proteins and increases secretion of proteolytic enzymes, cell migration and invasion [22].

VEGF stimulates proliferation and chemotaxis in both endothelial cells and stromal cells [23]. Moreover, VEGF acts as an autocrine inducer of growth and chemotaxis via VEGFR-1 [24]. It increases IL-6 (a major growth and survival factor for MM plasma cells) production by bone marrow stromal cells via VEGFR-2 and thus forming a paracrine loop for tumor growth and angiogenesis [25]. Also, adhesion of plasma cells to bone marrow stromal cells increases VEGF secretion by both cell types enhancing angiogenesis [26]. VEGF production by plasma cells is also regulated by TNF-α of bone marrow stromal cells [27]. TNF-α mediates the upregulation of adhesion molecules of plasma cells and bone marrow stromal cells, and thus enhances heterotypic adhesion and activates IL-6 and VEGF secretion by bone marrow stromal cells [28]. This mechanism mediates MM cell homing and migration, as well as angiogenesis [29,30].

VEGF signalling also contributes to inhibit antiangiogenic signals such as *Semaphorin3A* (SEMA3A) whose autocrine loops usually activates to self-limit the physiologic angiogenesis [31].

FGF-2 is another important angiogenic growth factor, and it represent a potent activator of endothelial proliferation and can thus stimulate angiogenesis, promote stromal fibroblast proliferation and extracellular matrix formation leading to excessive bone marrow fibrosis and can directly affect neoplastic cells by acting on their high affinity to FGFRs [32].

FGF-2 increases IL-6 secretion; conversely IL-6 enhances FGF-2 expression and secretion by MM plasma cells, thus forming a paracrine IL-6/FGF-2 cross-talk between MM plasma cells and bone marrow stromal cells that triggers neovascularisation as well as MM cell growth and survival [33].

Endothelial cells

All the angiogenic stimuli present in the bone marrow microenvironment act on endothelial cells causing a series of modifications that in turn remain stabilized and irreversible. In fact, tumor endothelial cells differ greatly from those of quiescent healthy vessels [34]. They proliferate rapidly in keeping with the enhanced angiogenesis that accompanies tumor progression [35].

Their intercellular adhesion and to the ECM during sprouting (that implies cell proliferation and migration) is greatly reduced since they have different profile and level of cell adhesion molecules [36]. Their survival is markedly dependent on growth factors secreted by the tumor and its microenvironment, and on their expression of specific receptors for these factors [37]. They are abnormal in shape and highly permeable due to the presence of fenestrae, vesicles, transcellular holes, widened intercellular junctions, and a discontinuous basement membrane. They share the lining of new vessels with tumor cells able to mimic vessels [38,39].

The fast growth of endothelial and tumour cells, coupled with their structural and functional abnormalities, make tumour vessels thin, tortuous, and arborized.

As a consequence, tumor blood flow is chaotic and variable and leads to hypoxic and acidic environment that stimulate further angiogenesis [40].

MM endothelial cells intensely express markers of vivid angiogenesis such as VEGFR-2 and Tie/Tek. This implies synergistic activity of VEGF and Ang-2, produced by plasma cells, in the induction of sprouts from existing vessels [34]. MM endothelial cells sizably express CD133, a marker of the progenitor endothelial cells involved in pre-natal vasculogenesis [41]. It has been proved that some CD133+ hematopoietic stem and progenitor cells contribute to the formation of the vessel wall of newly forming blood vessels together with FVIII-RA+, VEGFR-2+, and VE-cadherin+ MM endothelial cells [42]. MM plasma cells and inflammatory cells secrete high levels of VEGF, FGF-2, and I*nsulin-like Growth Factor* (IGF), which recruit bone marrow and circulating hematopoietic stem and progenitor cells into the tumour microenvironment, where they differentiate into MM endothelial cells and participate to the formation of the new vessel wall. High expression of β3-integrin, which prevents apoptosis of endothelial cells and favours their adhesion to the ECM, proliferation, migration, and capillarogenesis, also implies vivid neovascularisation [43,44]. Overexpression of endoglin by endothelial cells, that enhances the expression of the adhesion molecule CD31, which is the ligand of the

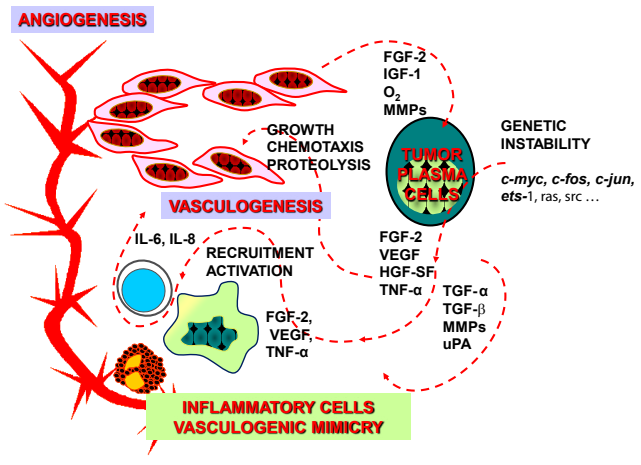

Figure 1: A paracrine loop for tumour angiogenesis.

plasma cell CD38, suggests enhanced opportunities for plasma cells to interact with the new-formed blood vessels enter circulation and disseminate [34].

Frequent interactions between plasma cells and new-formed blood vessels are also mediated by the high expression of E-selectin by endothelial cells [45]. Moreover, MM endothelial cells intensely express a water transporter, namely Aquaporin-1, which enhances vascular permeability, facilitates plasma extravasation, increases interstitial pressure, induces hypoxia, and upregulates *Hypoxia Inducible Factor-1 Alpha* (HIF-1α) and VEGF [46].

A paracrine loop for tumour angiogenesis (figure 1) and growth has been demonstrated in MM patients, mediated by VEGF-A and FGF-2 [47,48]. Plasma cells secrete VEGF-A and this induces endothelial cell proliferation and chemotaxis through VEGFR-2, prevalently expressed on these cells, which display constitutive autophosphorylation of VEGFR-2 and the associated kinase ERK-2 [24,49].

Another important role is played by the paracrine loop existing between MM endothelial cells and plasma cells involving CXC-chemokines and their cognate receptors, which mediate plasma cell proliferation and chemotaxis [50].

Bone marrow endothelial cells express and secrete high amounts of the CXC-chemokines CXCL8/IL-8, CXCL11/interferon-inducible T-cell alpha chemoattractant (I-TAC), CXCL12/stromal cell-derived factor (SDF)-1α, and CCL2/monocyte chemotactic protein (MPC)-1 [50].

Several MM cell lines display a complex expression pattern of chemokine receptors (CXCR, CCR), some of which also mediate the interactions between plasma cells and stromal cells in the bone marrow microenvironment [51].

To summarize, MM endothelial cells show constitutively ultrastructural features of enhanced metabolic activation, an high expression of typical endothelial markers (Tie2/Tek, VEGFR-2, FGFR-2, CD105-endoglin, and VE-cadherin), an high secretion of matrix metalloproteinases-2 and -9, and up-regulation of angiogenic genes (VEGF, FGF-2, Gro-α chemokine, transforming growth factor beta, Tie2/Tek, HIF-1α, ETS-1, and osteopontin) [34].

Vasculogenic Mimicry in Mm

In some aggressive tumours the vessel wall is lined with only cancer cells as a mosaic of cancer cells and endothelial cells. This phenomenon is called *"vasculogenesis mimicry"* [52].

In healthy subjects, cells of monocyte lineage (other mesodermal-derived cells) can generate endothelial cell progenitors or act as pluripotent stem cells [41,53,54]. They can develop an endothelial cell phenotype, especially when stimulated by VEGF and/or bFGF, and produce a functional capillary-like mesh permeable by blood cells, hence recapitulating embryo vasculogenesis [54-57].

Bone marrow monocytes and macrophages of MM patients can be induced to assume a number of endothelial cell properties and form capillary-like structures *in vitro* through vasculogenesis. Moreover, macrophages contribute to build neovessels in MM through vasculogenic mimicry, and in MGUS they are prone to a vascular switch that marches in step with the progression toward MM [58].

In fact, MM bone marrow macrophages exposed to VEGF and bFGF develop a number of phenotypic properties similar to those of paired bone marrow endothelial cells, and form capillary-like structures morphologically mimicking those produced by MM endothelial cells. At the ultrastructural level, MM macrophages exhibit numerous cytoplasmic extroversions arranged in tube-like structures [58].

All these features are lacking or minimal in macrophages of patients with MGUS or with benign anaemia which, however, will become phenotypically and functionally similar to those of MM under angiogenic stimulation [58].

Bone marrow biopsies of MM, but not of MGUS, harbour 'mosaic' vessels since these are formed by MM endothelial cells, endothelial cell-like macrophages and macrophages themselves [58].

Antiangiogenic strategies

The actual therapeutic strategies of MM consist of conventional chemotherapy in combination with biological-based therapies in various settings, targeting not only the MM plasma cells but also its microenvironment and new therapeutic targets are currently available [59].

The proteasome inhibitor bortezomib (Velcade, formerly PS-341), a boronic acid dipeptide, is a potent, highly selective, and reversible proteasome inhibitor that targets 26S proteasome complex and inhibits its function [60]. The 26S proteasome is an ATP-dependent multicatalytic protease mediating intracellular protein degradation [61].

Proteasomal degradation of misfolded or damaged proteins proceeds by recognition of polyubiquitinated proteins by the 19S regulatory subunit of the 26S protease and subsequently hydrolysis to small polypeptides [61].

Besides eliminating damaged/misfolded proteins, the proteasome also regulates key cellular processes, including modulation of transcription factors, such as NF-κB, cell cycle progression, inflammation, immune surveillance, growth arrest, and apoptosis [62].

Bortezomib has inhibitory effects on the NF-κB activity in MM cells. NF-κB is a major transcriptional factor which mediates expression of many protein including cytokines, chemokines, cell adhesion molecules, as well as those involved in anti-apoptosis and cellular growth control [62]. Its activity is regulated by association with IκB family proteins [63]. Varius stimuli, including cytokines, such as TNFα and IL-1β, trigger phosphorylation of IκB protein by IκB kinase [64]. Phosphorylated IκB is subsequently polyubiquitinated by specific enzymes and degradated by the 26S proteasome, which allows p50/p65 NF-κB nuclear translocation and binding to consensus mtifits in the promoter region of target genes [62,64].

Expression of adhesion molecules, such as ICAM-1 and VCAM-1, on both MM cells and bone marrow stromal cells are also regulated by NF-κB [65,66]. Thus inhibition of NF-κB by bortezomib downregulates these adhesion molecules, thereby enhancing susceptibility of MM cells to therapeutic agents in the context of the bone marrow milieu [62]. Another important aspect is that induction of IL-6 transcription and secretion by bone marrow stromal cells is mediated via NF-κB activation which, in turn, increases secretion of other cytokines, such as VEGF, from MM plasma cells [67].

Furthermore, MM cell adherence to bone marrow stromal cells triggers IL-6 secretion via NF-κB activation, associated with an increased MM cell growth and leads to a reduction of VEGF secretion. Bortezomib significantly blocks the MM cell adhesion induced by IL-6 secretion from bone marrow stromal cells [62].

Bortezomib is also directly cytotoxic, triggering stress response and apoptotic signalling via multiple pathways [62].

As the result of inhibition of proteasome activity, it causes the accumulation of misfolded polyubiquinated proteins, resulting in endoplasmic reticulum stress which triggers caspase-4 and downstream signalling [68]. Bortezomib also induces ROS which play a critical role in the initiation of the apoptotic cascades by disruption of membrane potential and the release of cytochrome c from mitochondria, followed by caspase-9 activation [69]. Proteasome inhibitors have a potent activity against mitotic endothelial cells, so they target aberrant blood vessel development associated with tumor growth, in fact, bortezomib inhibits the proliferation of MM endothelial cells associated with downregulation of VEGF, IL-6, IGF-I, Ang-1 and Ang-2 [62]. Moreover, bortezomib inhibits DNA repair activity by cleavage of DNA dependent protein kinase catalytic subunit (DNA-PKcs), thereby restoring sensitivity to DNA-damaging chemotherapeutic agents, such as doxorubicin and melphalan [70].

Bortezomib also down-regulates caveolin-1 tyrosine phosphorylation, which is required for VEGF-mediated MM cell migration, and also blocks the caveolin-1 phosphorylation induced by VEGF (transcriptional target of NF-κB) in endothelial cells, thereby inhibiting ERK-dependent cell proliferation. It inhibits the transcription of important adhesion molecules such as ICAM-1, VCAM1 and E-selectin [71].

All these biological activity are responsible of the higher and rapid therapeutic efficacy of this drug as well as of its potential persistence of antiangiogenic activity after the term of patients treatment [72,73].

Thalidomide has a direct tumoricidal activity, an antiangiogenic effect by modulation of various angiogenic genes [74].

It modulates TNF-α signalling through direct and/or indirect effects on the tumour microenvironment, reduces FGF-2, VEGF and IL-6 secretion in bone marrow stromal cells and by MM cells [75-77]. It also stimulates the activation and expansion of T cells and augments NK-cell –mediated cytotoxicity through its direct effect on T cells with a consequent increase in IL-2 and interferon gamma (IFN-γ) secretion, and interferes with NF-κB activity by blocking its ability to bind to DNA or suppresses IκB kinase activity, thus abrogating normal inflammatory cytokine production [78,79]. Thalidomide also disrupts the host marrow-MM cell interaction by selective modulation of the density of cell surface adhesion molecules [80].

Treatment with thalidomide is associated with sedation, fatigue, constipation, rash, deep-vein thrombosis, and peripheral neuropathy [81].

Lenalidomide, a derivative of thalidomide, is less toxic and more potent than the parent drug [82]. In patients with relapsed or refractory MM, lenalidomide can overcome resistance not only to conventional chemotherapy but also to thalidomide [83,84].

The bisphosphonates are other compounds that, although originally used to reduce bone loss in MM due to an anti-osteoclast activity, have also been shown to have a direct effect on MM cells.

In fact, zoledronic acid has a direct cytotoxic activity on tumor cells and suppresses angiogenesis, inhibits FGF-2- and VEGF-dependent proliferation of endothelial cells and inhibits VEGFR-2 in an autocrine loop [83,84]. Neridronate exerts its antiangiogenic activity through both a direct effect on endothelial cell proliferative activity and inhibitory effect on the responsivity of the endothelial cells to the proliferative stimuli mediated by angiogenic cytokines [85].

Conclusion

The bone marrow microenvironment plays a crucial role in the pathophysiology of MM. It is involved in the crosstalk between plasma cells and bone marrow stromal cells, which increases the survival, proliferation and migration of tumor cells themselves, and represents the substrate for angiogenesis which favour to disease progression. Due to interaction with active microenvironment, MM plasma cells also acquire drug resistance giving less opportunity to therapy response.

Many research studies have tried to better understand the biological mechanisms and the genetic basis of all the interactions between MM cells and bone marrow stromal cells. VEGF, FGF-2, IL-6, macrophages, mast cells, and many others cells and molecules, play the most important role in this process.

All these observations are indicative that: *i)* angiogenic stimuli provided by VEGF, FGF-2 and IGF release in the bone marrow microenvironment of MM patients by myeloma plasma cells are sufficient to recruit haematopoietic stem cells and macrophages to the tumor bed and induce their differentiation into endothelial cells contributing to the tumor vasculature; *ii)* haematopoietic stem cells and macrophages may be a source of endothelial cells in the bone marrow of MM patients during disease progression; *iii)* vasculogenic mimicry, contributing together with angiogenesis in MM, is induced by cytokines secreted by myeloma plasma cells.

Several studies have focused their investigation on novel drugs targeting the MM plasma cells and the microenvironment cells. Good results have been already done but MM still remain an incurable malignancy, indicating that the role of bone marrow microenvironment is important in MM progression, but its role is still not completely clear.

The goal for MM therapy remain the simultaneous block of plasma cell proliferation and survival, plasma cells/bone marrow stromal cells interaction, and bone marrow stromal cells activity by the combination of biological target drugs.

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