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Angiogenesis and Antiangiogenesis in Multiple Myeloma

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Abstract

Multiple myeloma progression is characterized by a dense interaction between cancer cells and bone marrow microenvironment. The interactions of myeloma cells with various stromal cells and extracellular matrix components are the main regulator of the biological processes that underlie the progression of the disease and of the classic symptomatology correlated. The bone marrow of myeloma patients has recognized autocrine and paracrine loops that regulate multiple signaling pathways and the malignant phenotype of plasma cells. One of the pivotal biological processes which are responsible for myeloma progression is the formation of new vessels from existing ones, known as angiogenesis. It represents a constant hallmark of disease progression and a characteristic feature of the active phase of the disease. Near angiogenesis, other two ancestral processes were active in the bone marrow: vasculogenesis and vasculogenic mimicry. These processes are mediated by the angiogenic cytokines, interleukins, and inflammatory cytokines directly secreted by plasma cells and stromal cells. Neovascularization is also mediated by direct interaction between plasma cells and the various components of bone marrow microenvironment. The observation of the increased bone marrow angiogenesis in multiple myeloma and its correlation with disease activity and overall survival led to consider angiogenesis as a new target in the treatment of multiple myeloma.

Keywords: angiogenesis, antiangiogenesis, bone marrow microenvironment, multiple myeloma, tumor progression, vasculogenesis, vasculogenic mimicry

1. Introduction

In the past decades, myeloma research has been focalized on the malignant cell leading to the identification of various genes (i.e., oncogenes and tumor suppressor genes) and of signaling pathways by which the identified genes themselves control survival and proliferation of cancer cells [1–4]. More recently, newly developed technologies have enabled us to investigate cancer cells at the genomic level. Such gene profiling studies are providing insight into the pathogenesis and risk stratification of plasma cell diseases, and help to predict both prognosis and treatment response [3, 4].

Cancer cells interact with all cells composing the microenvironment and with components of extracellular matrix (ECM) [5, 6]. These interactions play the most important role in the epigenetic control of the malignant phenotype, as in primary sites as in the metastatic ones [6, 7]. Moreover, interactions between host cells in the niche microenvironment and ECM represent an intense area of research [5–9]. The aim of these studies is the better understanding of the pathophysiological events in the tumor process, including malignant cells, surrounding cells, and ECM components [5–9].

Multiple myeloma (MM) is a malignancy of plasma cells that home to and expand in the bone marrow (BM) [9]. MM is characterized by a high genomic heterogeneity but, generally, it shows the same histological features, [8–10]. The interactions between MM plasma cells and BM microenvironment (stromal cells, hematopoietic cells, and ECM) represent near genetic modifications an important factor for disease progression [11–14]. Pathophysiological interactions of myeloma cells with the components of BM microenvironment are pivotal during the progression-associated bone disease and neovascularization [13]. These interactions are mediated by autocrine and paracrine loops that regulate multiple signaling pathways and influence many fundamental biological aspects of the malignant phenotype (i.e., apoptosis, survival, proliferation, invasion, bone damage, and angiogenesis) [12–14].

Neovascularization is the formation of new vessels from existing ones (angiogenesis) or from endothelial precursors (vasculogenesis) and represents one of the principal biological processes controlled by the interactions between plasma cells and BM microenvironment. It is a constant hallmark of disease progression [11–15]. Angiogenesis is controlled by several angiogenic cytokines [14, 15]. The major of these are vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and hepatocyte growth factor (HGF) directly secreted not only by the tumor plasma cells but also by stromal cells [14, 15].

The observation of an increased BM angiogenesis in MM, an overexpression of angiogenic cytokines, and their correlation with disease activity, overall survival and the development of new antiangiogenic compounds, led to consider angiogenesis as a new target in the treatment of MM [11–15].

2. Neovessels formation in multiple myeloma

Neovessels in the BM of patients with active MM appear thin, tortuous, and arborized and are highly permeable showing fenestrae, vesicles, transcellular holes, widened intercellular

junctions, and a discontinuous basement membrane [16]. These alterations are consequent to the rapid neovascularization induced by tumor plasma cells by mean of three different processes: (i) angiogenesis, (ii) vasculogenesis, and (iii) vasculogenic mimicry [17].

2.1. Angiogenesis

In 1994, Vacca and colleagues [16] demonstrated for the first time that BM microvascular density was significantly increased in MM compared to monoclonal gammopathy of undetermined significance (MGUS) and moreover in active (diagnosis, relapse, and leukemic phase) versus non-active (complete/objective response and plateau) MM. The authors first hypothesized that progression from MGUS to MM is accompanied by an increase in BM microvascular density. Subsequent studies by other groups confirmed the observation of increased angiogenesis in active MM compared to healthy individuals or MGUS patients [17–20].

Angiogenesis is the sprouting of new blood vessels from pre-existing ones and is finely regulated [17, 18]. Angiogenesis is essential for tumor growth, invasion, and metastasis starting from the balanced early avascular phase of cancer up to being uncontrolled and unlimited in time during the vascular phase [6, 17, 20]. The angiogenic switch from the avascular to the vascular phase is controlled by the many oncogenes, among which c-myc, c-fos, c-jun, and ets-1 have been recognized [20, 21]. They are activated in tumor plasma cells as a consequence of immunoglobulin translocations and genetic instability [20, 21], and induce the angiogenic phenotype in MM plasma cells [21]. MM plasma cells become CD45-negative and begin to produce VEGF [22]. The same angiogenic switch represents a crucial event for the progression from asymptomatic to symptomatic MM [23]. So, angiogenesis represents an important process in MM progression as well as an important prognostic factor [17, 19, 20].

2.2. Vasculogenesis

Vasculogenesis is responsible for the primary development of the vascular system during embryogenesis and is fundamental for the formation of the yolk sac vasculature, of the heart, and of the dorsal aortae [24]. It derives from the differentiation of endothelial progenitors, namely angioblasts, deriving from mesoderm and aggregate into a primitive capillary plexus [24]. Important evidence suggests that vasculogenesis contributes to neovascularization in the bone marrow of MM patients [25–27]. In fact, putative endothelial progenitor cells have been isolated from peripheral blood and several studies have suggested that angioblasts contribute to the formation of tumor neovessels [25, 26]. It has been demonstrated that when CD34+ VEGFR-2+ cells isolated from peripheral blood of MM patients were cultured on fibronectin-coated plates and exposed to angiogenic cytokines, they acquire a typical spindle-shaped morphology and express endothelial cell markers (CD34, CD31, Flk-1, Tie-2, and E-selectins) [26]. Moreover, in the BM of MM patients, but not of MGUS patients, some endothelial cells of neovessel wall express on their surface the typical endothelial cell markers: factor VIII-related antigen (FVIII-RA), vascular endothelial-cadherin (VE-cadherin), VEGFR-2, and TIE/Tek, as well as the CD133 staminal antigen whose expression was found in the microvascular wall together with FVIII-RA or VE-cadherin in some active MM patients [26].

2.3. Vasculogenic mimicry

The phenomenon called “vasculogenesis mimicry” represent a model of neovascularization in aggressive solid and hematologic tumors, owing to the specific capacity of malignant cells and other non-endothelial cells to form vessel-like networks [27–33]. This phenomenon can be an escape mechanism for antiangiogenic drugs that are now incorporated into standard clinical practice [29]. Also, inflammatory cells (i.e. macrophages and mast cells) participate in this process [30–33] because they can generate endothelial progenitor and can produce functional capillary-like structures *in vitro* when stimulated by VEGF and/or FGF-2 [30–36].

Scavelli et al. demonstrated that when exposed to VEGF and FGF-2, macrophages isolated from BM of myeloma patients develop phenotypic and biologic properties similar to those of endothelial cells, and exhibit numerous cytoplasmic extroversions arranged in tube-like structures [35]. Finally, in BM biopsies of MM, the participation of inflammatory cells in the formation of the capillary network has been directly demonstrated [35, 36].

3. The BM microenvironment

The BM microenvironment plays a pivotal role during MM disease progression by mean neovascularization, bone disease, and activity of inflammatory cells. All the BM microenvironment components surround and support MM plasma cells proliferation, migration, and survival, and are implicated in drug resistance [34, 37].

3.1. Endothelial cells

BM endothelial cells of patients with MM are altered in shape and characterized by different phenotype (in term of expression of cell adhesion molecules, receptors for cytokines and growth factors together with FVIII-RA, and VE-cadherin) from those of normal resting endothelial cells and shows the capacity to proliferate rapidly and spontaneously enhanced angiogenesis [36–39]. In fact, Vacca et al. [38] demonstrated that the phenotype of MM endothelial cells is characterized by expression of surface receptors such as VEGFR-2 and Tie2/Tek (indicators of active angiogenesis), increased expression of the β 3-integrin (that plays a pivotal role in the prevention of apoptosis, adhesion to the ECM, proliferation, migration, and capillarogenesis), expression of endoglin (implicated in the expression of the ligand of the plasma cell CD38 (CD31) enhancing plasma cells interaction with the new-formed blood vessels, favoring plasma cells entry into circulation, and disseminate). The expression of a water transporter, namely aquaporin 1, has been also demonstrated [39]. It enhances vascular permeability, facilitates plasma extravasation, increases interstitial pressure, induces hypoxia, and upregulates hypoxia-inducible factor-1 alpha (HIF-1 α) and VEGF [39]. Some MM endothelial cells express the CD133 indicating their derivation from a subset of CD133+ progenitor cells which contribute to the formation of blood neovessels [26, 40, 41]. MM plasma cells recruit BM and circulating CD133+ progenitor cells into the tumor microenvironment by mean the release of a high quantity of VEGF, FGF-2, and IGF [26]. In the BM microenvironment, CD133+ progenitor cells differentiate into MM endothelial cells and complete the formation of the new vessel wall [26].

MM endothelial cells are functionally different from MGUS endothelial cells, are characterized by an overangiogenic phenotype, and resemble transformed cells because of they downregulate or upregulate some genes like tumor cells [41]. These changes are influenced by the MM microenvironmental and/or plasma cells factors (such as hypoxia, inflammation, expression of multiple cytokines, growth factors, etc.) that render endothelial cells unstable and heterogeneous, with progressive characteristics comparable with a cancer cell. In addition, those factors may have genetic causes and consequences (i.e., increased expression of oncogenes and loss of tumor suppressor genes) [41]. This reciprocal interrelationship and heterogeneity may translate into a site- and stage-specific changes in the regulation of BM-microvessel density and angiogenesis dependence, and ultimately to changes in the proliferation and antiapoptotic potential of MM tumor cells, even in the same patient [17]. Moreover, the overangiogenic activity of MM endothelial cells is linked to a well-defined protein expression [42]. This proteomic signature renders MM endothelial cells very similarly to transformed (such as tumor) cells than normal endothelial cells, confirming the results obtained in the studies at the genomic level [41].

3.2. Fibroblasts

The stromal microenvironment is characterized by a modified extracellular matrix, enhanced angiogenesis, and cells with an activated phenotype, including fibroblasts referred to as 'activated myofibroblasts' or 'cancer-associated fibroblasts' (CAFs) [6, 43–48]. In the poorly vascularized hypoxic or necrotic areas of tumors, they accumulate numerous tumor-associated fibroblasts [43, 44]. They respond to experimental hypoxia by producing high amounts of VEGF-2, FGF-2, tumor necrosis factor alpha (TNF- α), urokinase and matrix metalloproteinases and synthesizing inducible nitric oxide synthase, which increases blood flow and promotes angiogenesis [45]. In breast, prostate, and pancreatic carcinomas, the number of CAFs is associated with an increased malignancy grade, tumor progression, and poor prognosis [46]. CAFs are heterogeneous [45] and display phenotypes similar to those of myofibroblasts derived from quiescent fibroblasts that have undergone activation during tissue remodeling in wound healing, fibrosis [47]. CAFs can arise from resident fibroblasts, BM-derived progenitor cells and cells undergoing the endothelial-mesenchymal transition (EndMT) or mesenchymal transition (MT) [47] in the BM of MM patients, an important interplay between CAFs and plasma cells during MM initiation and progression has been demonstrated [48]. Plasma cells induce and maintain the CAF-activated phenotype, which, in turn, supports tumor progression by promoting extracellular matrix remodeling, cell proliferation, apoptosis resistance, and angiogenesis [48]. Moreover, CAFs play a key role in the bortezomib resistance of MM cells. The protective effect is not related to cell-to-cell interactions but to the ability of bortezomib to trigger bortezomib-resistant CAFs to release in the BM microenvironment several cytokine/growth factors with antiapoptotic effects, such as IGF-1, IL-6 IL-8, and exosomes [48].

3.3. Macrophages

There are several published data on the association between macrophage infiltration, vascularity, and prognosis in cancer [49–52].

In patients with active MM, macrophages contribute to building neovessels through vasculogenic mimicry [35]. Under a synergistic stimulation by VEGF/FGF-2, they undergo a

phenotypic and functional adaptation but retain their own CD14 and CD68 lineage markers which can be evidenced in the neovessel wall [35]. They display oblong and spindle shape with thin cytoplasmic expansions, some of which are either arranged to form a microvessel-like lumen or anastomosed with each other and with those of nearby macrophages to form tubular-like structures [35]. In the BM of patients with active MM, plasma cells secrete VEGF and FGF-2 that bind to VEGFR-1 and FGFR-1, -2 and -3 expressed on monocytes/macrophages surface and induce monocyte migration and infiltration and macrophage to secrete their own VEGF and FGF-2 [17, 35, 49, 52]. These cytokine circuits further promote angiogenesis and vasculogenic mimicry [17].

3.4. Mast cells

Mast cells recruitment in the tumor bed has been associated with enhanced growth and invasion in solid and hematological malignancies [49, 53–56]. In MM, tumor plasma cells secrete stem cell factor (SCF), FGF-2, VEGF-2, and platelet-derived growth factor (PDGF) that recruit mast cells [14, 52]. The granules of mast cells contain several angiogenic factors: (i) tryptase and chymase that favor the formation of capillary structures via a direct action on endothelial cells and activate latent metalloproteinases and plasminogen activator [53]; (ii) heparin that induces endothelial cell proliferation and migration [54]; (iii) histamine, that has a direct angiogenic effect, induces VEGF production in the granulation tissue [54] and contributes to the hyperpermeability of newly formed microvessels, increasing leakage of plasma proteins and hence deposition of fibrin whose degradation products are angiogenic in vivo [55]; and (iv) TGF- β , TNF- α , IL-8, FGF-2, and VEGF, which are all angiogenic factors [52, 53]. Moreover, in the new vessels wall typical tryptase-positive mast cells connected by a junctional system with the endothelial cells can be evidenced. As macrophage, mast cells keep their lineage marker indicating their adaptation to contribute to vasculogenesis mimicry [33]. In patients with MM BM angiogenesis, evaluated as microvessel area, and mast cells counts are highly correlated [53, 56] and both parameters increase simultaneously in the active phase of disease [56].

3.5. Osteoclasts and osteoblasts

MM plasma cells that home and expand in the BM causes an unbalanced bone remodeling that induces osteolytic lesions and causes pain, the main symptom of MM [34]. In MM, plasma cell-dependent alterations of Runx2 and the Wnt pathways induce the differentiation of resident macrophages in osteoclasts and plasma cells themselves can transdifferentiate to functional osteoclasts [57, 58]. Bone disease results from the local production of osteoclast-activating factors (OAF), as well as IL-6, IL-1 α or -1 β , IL-11, TNF- α , TNF- β , and M-CSF [11]. In particular, the receptor activator of nuclear factor ligand (RANKL), the decoy receptor osteoprotegerin (OPG), its receptor (RANKR), and the chemokine macrophage inflammatory protein-1 α (MIP-1 α) trigger differentiation and activation signals in osteoclasts precursors, and thus promoting bone resorption [48]. Adhesion molecules, such as β 1 integrins, mediate the binding of MM plasma cells to stromal cells and VCAM1 induces overexpression of RANKL in both cell types and suppresses OPG production by stromal cells. Furthermore, plasma cells interfere with the regulation of the bone resorption by the secretion of IL-7 and DKK1, a Wnt inhibitor [59].

It has demonstrated a close link between myeloma cells, osteoclasts, and vascular endothelial cells to form a vicious cycle between bone destruction, angiogenesis, and myeloma expansion

in the MM bone marrow and that the inhibition of VEGF produced by plasma and stromal cells and osteopontin produced by osteoclasts, reduce angiogenesis and osteoclastogenic activity by vascular endothelial cells [11, 57].

Some issues demonstrated that CD38 is expressed by effectors and inhibitory cells, and by both osteoblasts and demonstrating the role of CD38 in bone remodeling, in mice and rabbit models [60] as in human [61]. Horenstein AL. et al. [62] recently shown that the ectoenzymatic network CD73/CD203a is active even in MM bone niche in the alternative production of ADO, which levels correlate with disease aggressiveness and ISS staging of MM patients [61]. Moreover, the role of CD38 in human OC differentiation and as well as the reduction of the area of osteoclast bone resorption in vitro by the anti-CD38 monoclonal antibody daratumumab have been also demonstrated [63]. Overall these findings suggest the possibility of a role of CD38 during osteoclast formation supporting the potential activity of daratumumab on MM bone disease and on the protection of MM plasma cells by stromal cells of the bone niche [60–63].

3.6. Hematopoietic stem cells

Hematopoietic stem cells (HSCs) reside in the BM in the endosteum niche and in the vascular niche, where they self-renew and differentiate into mature blood cells [64, 65]. This is a finely controlled-process by mean numerous signals from the bone marrow components [64, 65]. In MM, the BM niches (endosteum and vascular) components play a pivotal role in the regulation of vasculogenesis and angiogenesis [11, 14, 26, 64], and alterations of the signals in niche microenvironment modulate myeloma progression and spread [26, 64].

In the BM of patients with MM, the expression of the CD133 staminal antigen in some cells of the neovessel wall has been demonstrated [26, 38]. Moreover, a subset of CD34+/CD133+ cells mobilized in the peripheral blood for collection during transplant procedure express VEGFR-2 and are able to differentiate in mature endothelial cells in appropriate culture conditions [28].

3.7. Endothelial progenitor cells

Various studies have demonstrated that endothelial progenitor cells (EPCs) can be isolated from patients with MM [40, 66–69] and contribute to the formation of new blood vessels [40]. Moreover, circulating EPCs expressing CD146+, CD105+, and CD34+ are increased in MM patients compared to healthy controls [66, 67].

Rigolin et al. [69] have hypothesized a possible origin of EPCs and plasma cells from a common progenitor namely hemangioblast in MM patients. In their work, they demonstrated that EPCs, isolated from MM patients presents the 13q14 deletion and the great part of them are positive for the CD133 [69]. Finally, some evidence indicates a prognostic significance of the circulating EPCs also after treatment with new drugs [66, 68].

3.8. Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are the major component of BM stroma [11, 57, 70–72]. These cells, of unclear origin in MM [71], are potentially able to differentiate into multiple histotypes (i.e. fibroblasts, adipocytes, chondrocytes, and osteoblasts) and in the BM form specialized niches namely “vascular niche” and “osteoblast niche” [57, 70, 71]. MSCs support tumor cell

growth, metastasis, survival, bone marrow colonization, and evasion of the immune system [72]. MSCs can migrate toward primary tumors and metastatic sites, implying that these cells might modulate tumor growth and metastasis. In the BM of patients with MM, functional abnormalities of MSCs and complex interaction with MM plasma cells have been demonstrated indicating that they play a critical role in MM development and disease outcome [70, 71]. In fact, MSCs can induce bortezomib-resistance in MM plasma cell by increasing Bcl2 expression and enhance NF- κ B activity via cell-cell contact [73, 74]. Moreover, MSCs are able to modulate engraftment of HSC, to suppress T- and B-lymphocyte activation and proliferation, and to affect dendritic cell maturation [71]. Finally, since MSCs represents the osteoblasts progenitors, in the BM of MM patients, MSCs play a critical role in the pathophysiology of myeloma bone disease [75]. They present reduced osteogenic potential and promoting osteoclasts formation and activity by increasing RANKL to OPG expression, augmenting secretion of activin A, uncoupling ephrinB2-EphB4 signaling, and augmenting Wnt5a production [75].

3.9. Adipocytes

The cancer-associated adipocytes (also namely peritumoral, intratumoral, or tumor-infiltrating adipocytes) influence tumor biology also by promoting angiogenesis [76–81]. A great number of signaling factors contributing to angiogenesis in both adipose tissue and tumors: VEGF, Ang-1 and -2, leptin, adiponectin, TNF- α , FGF, TGF- β , HGF, IL-6, and IL-8 [77–79, 81]. The VEGF/VEGFR system is the main mediator of angiogenic activity in adipose tissue [77]. In particular, adipocytes produce VEGF, Ang-2, and HGF [77, 81]. In MM, the hypoxic environment of BM favors the production of angiogenic factors by adipocytes, particularly VEGF, and decreases adipogenic differentiation increasing adipose-derived stem cell proliferation and migration [82, 83], supporting aberrant microvessel growth and neovascularization, and MM plasma cell proliferation [82, 83]. Paracrine and autocrine signaling of VEGFA between BM adipocytes and MM cells have been also demonstrating [77, 80, 81].

3.10. Soluble factors and transduction pathways

The progression from in situ to invasive and metastatic solid tumors are accompanied and enhanced by the switch from the perivascular to the vascular phase [84, 85]. The same process has been demonstrated in MM in which active disease represent the ‘vascular phase’ of plasma cell tumors, and non-active disease (remission or plateau phase), smoldering MM and MGUS their ‘perivascular phase’ [22, 26, 43].

VEGF is the main angiogenic cytokine secreted in the BM of patients with MM [86–88]. VEGF carries out its activity through the MEK-1/ERK pathway by the interaction with his receptors (VEGFR1–3) [86]. In the BM of patients with MM paracrine loops between endothelial cells and plasma cells [89] and autocrine loops on the same endothelial cells have been demonstrated [90]. Moreover, plasma cell-derived VEGF stimulates IL-6 and VEGF secretion in BM stromal cells, whereas stromal cells-derived IL-6 promotes proliferation, survival, and VEGF production in plasma cells, activating a loop between both growth factors [91].

Levels of FGF isoforms are significantly higher in the serum and plasma cell lysates of patients with active MM compared with non-active MM and MGUS patients [92–94]. Moreover, FGF-2 inhibition suppresses the angiogenic potential of plasma cells from patients with active MM

in vitro and in vivo [92, 93]. Finally, FGF-2 triggers paracrine MM-stromal cell interactions in an IL-6/FGF-2 paracrine loop [92, 95] and syndecan-1 (CD138), a low-affinity receptor of FGF-2, is also expressed by MM cells [96]. The high expression/activation of the FGF2 signaling in active MM also overcomes the inhibitory effect of the Pentraxin 3 (PTX3) [97, 98], a soluble pattern recognition receptor that binds with high affinity and selectivity to FGF2 inhibiting its pro-angiogenic activity, autocrine loops usually activated to self-limit physiologic angiogenesis in a normal subject or MGUS patient [97].

HGF has been identified in human MM cell lines and in freshly isolated plasma cells from patients with MM [99, 100]. Serum levels of this factor are higher in newly diagnosed MM patients and decline after induction therapy in the responding patients. Ferrucci et al. demonstrated the co-expression of HGF and c-MET in MM endothelial cells, suggesting autocrine stimulation [99]. Moreover, BM stromal cells produce HGF, paracrine stimulation of MM cells within the BM microenvironment can also take place [99, 100]. The inhibition of this pathway causes reduction of spontaneous and plasma cell-induced angiogenesis in MM endothelial cells in vitro and in vivo [99–101].

The Ang-1/Ang-2 expression in MM patient serum and BM samples correlates with the BM microvascular density [102–106]. It has been demonstrated that Ang-1, as well as Ang-2 expression, is upregulated in MM cell lines and in plasma cells obtained from MM patients [103, 105] and that the angiopoietin receptor Tie-2 is upregulated in the BM endothelial cells in the presence of MM cells [104]. Moreover, anti-Tie-2 antibodies blocked the in vitro angiogenic activity of MM cells [104]. Higher levels of Ang-1 and Ang-2 have been detected in MM patients as compared to controls [102] and their ratio may represent an independent prognostic factor in these patients [106].

Osteopontin (OPN) contributes to angiogenesis in MM [107–109]. Its expression correlates with BM microvascular density, and OPN-immunodepleted conditioned media from myeloma cells fail to induce a pro-angiogenic effect [107, 108] and an anti-OPN antibody block myeloma-induced angiogenesis [107]. Moreover, OPN may represent a useful serum marker of bone disease and BM angiogenic extent in myeloma patients [109].

Matrix Metalloproteinase-2 and -9 (MMP-2 and MMP-9) secretion is increased in patients with active MM versus non-active MM or MGUS [92, 110, 111] and usually, the MMP-2 expression is stronger [92, 110]. MM cell lines and freshly isolated BM plasma cells of MM patients produce MMP-9 [112], and MMP secretion of MM cells is triggered by BM stromal or endothelial cells [92, 112].

PDGF-Receptor Beta (PDGF-Rbeta) is expressed in plasma cells of MM patients [111, 113], and PDGF-BB/PDGF-Rbeta kinase axis promotes MM tumor growth by activating ERK-1/2 and AKT [113, 114]. Dasatinib, an orally bioactive TK-inhibitor significantly delays MM tumor growth acting as an inhibitor of PDGF-Rbeta kinase activation [113].

Airoldi et al. [115] demonstrated that IL-12 receptor B2 (IL-12Rbeta2) is downregulated in MM plasma cells and IL-12 reduces their pro-angiogenic activity by downregulation of a wide panel of angiogenic factors, including FGF-2, VEGF, Ang-2, and IL-6 and upregulation of some inhibitors of angiogenesis, including CXCL-4, interferon alpha and gamma (IFN- α and IFN- γ), and tissue inhibitor of metalloproteinase-2 (TIMP-2).

IL-27 exert strong antitumor activities against MM cells from patients by binding with its specific IL-27 receptor [116, 117] inhibiting the angiogenic potential of MM plasma cells. In animals injected with the U266 MM cell line, the expression of the genes encoding the chemokines CCL-2, CXCL-3, CXCL-5, and CXCL-6 is significantly downregulated by IL-27 treatment [116, 117].

Another important paracrine loop between MM endothelial cells and plasma cells involves CXC-chemokines and their cognate receptors have been evidenced in the BM of MM patients [118, 119]. In fact, BM 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)-1 α , and CCL2/monocyte chemotactic protein (MPC)-1 [118] that mediate the interactions between plasma cells and stromal cells interacting with the respective chemokine receptors (CXCR and CCR) [118, 120].

HIF-1 α has been demonstrated to be stabilized in MM plasma cells, in hypoxic as in normoxic conditions [82, 83, 119, 121–123]. The constitutive stabilization of HIF-1 α in myeloma cells is associated with the oncogenic c-Myc activity, suggesting that a common signaling pathway is active in MM plasma cells [122]. Among target genes controlled by HIF-1 α , the genes coding for the pro-angiogenic cytokines VEGF, IL-8, and OPN have been evidenced, and HIF-1 α silencing significantly suppresses the pro-angiogenic properties of MM cells reducing their secretion [87]. Moreover, MM endothelial cells from relapsed/refractory MM patients, but not those of newly diagnosed or non-active MM patients, showed a stabilization and activation of the HIF-1 α protein in normoxic conditions [124]. This stabilization is induced by ROS and correlated with the expression of HIF-1 α pro-angiogenic targets [124]. The inhibition of HIF-1 α in MM plasma cells [123] as well as in endothelial cells [124] impaired the MM plasma cells/stromal cells communication, the angiogenesis-related functions, and revert bortezomib- and lenalidomide-resistance [123, 124]. It may also have prognostic significance because patients with MM endothelial cells expressing the stabilized HIF-1 α protein had shorter overall survival [124].

The mammalian target of rapamycin (mTOR) is an intracellular serine/threonine kinase that mediates intracellular metabolism, cell survival, and actin rearrangement. mTOR is made of two independent complexes, mTORC1, involved in protein synthesis and autophagy inhibition, and mTORC2, involved in progression promotion, survival, actin reorganization, and drug resistance [125–127]. In MM endothelial, a significantly higher activation of mTORC2 have been demonstrated. Its inhibition induces a reduction of the angiogenic abilities of MM endothelial cells, suggesting a major role of mTORC2 in the “angiogenic switch” and indicates that mTORC2 might be a new antiangiogenic target in MM [127].

In MM endothelial, cell-to-cell contact-dependent homotypic activation of Notch pathway has been shown [128, 129]. MM plasma cells cocultured with MM endothelial cells trigger Jagged1/2-mediated Notch activation enhancing endothelial angiogenic activity. Moreover, halting Notch axis reduces angiogenesis *in vitro* and *in vivo* suggesting Notch pathway as a novel therapeutic target in MM [129].

The ephrins (Efn) and their receptors (Eph), a large family of receptor tyrosine kinases, are involved in several biological processes including cancer growth, progression, and angiogenesis [130–133]. Caivano et al. [134] recently demonstrated that EphA3 is highly overexpressed in MM endothelial cells and its expression correlates with disease progression. They have also

defined the biological role of EphA3 in MM angiogenesis and their preliminary data indicate that EphA3 could represent an angiogenic target in patients with MM [134].

Focal adhesion kinase (FAK) is a tyrosine kinase that localizes at focal adhesion sites of endothelial cell to the ECM [135–137]. It mediates signaling starting from integrin, is upregulated in many cancer types, controlling tumor aggressiveness, and metastasis [135], and is implicated in endothelial cell survival, proliferation, and migration [136, 137]. Integrin/FAK-mediated signaling cooperate with other growth factor receptor signaling (i.e. FGFR signaling) to promote angiogenesis in MM [138].

Various growth factor receptors induced an increase in DNA synthesis in MM endothelial cells by mean PI3K/Akt-MEK/ERK pathway inducing angiogenesis [17, 86, 138]. The role of this pathway in promoting angiogenesis is mainly related to the phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), S6-kinase (S6K), and MAP kinase interacting kinase mediated by ERK [139, 140]. This process leads to an increased rate of mRNA translation into HIF-1 α protein in an oxygen-independent way [139, 140]. ERK is also able to activate the transcription of HIF-1 α by the co-activator CBP/p300 that increases HIF-1 α /p300 complex formation [139, 140].

MicroRNAs are small endogenous non-coding RNAs (21–25 nucleotides) involved in regulating normal physiological processes as well as cancer pathogenesis [141–143]. Particularly, some miRNA have been implicated in tumor angiogenesis individuate as potential therapeutic targets/therapy [143–145]. Evidences suggests that MM cells promote angiogenic activity via HIF-1 α , a key transcription factor of hypoxia, leading to the overproduction of angiogenic cytokines [91, 104]. Moreover, communication between plasma cells, stromal cells, and endothelial cells is mediated also by mean the exosomes, small endosome-derived vesicles, containing a wide range of functional proteins, mRNA, and miRNA [146]. In BM of MM, miR-135b has been involved as the principal pro-angiogenic miRNA by targeting factor-inhibiting HIF-1 [147], whereas miR-199a-5p, which directly targets HIF1- α , miR-15a, and miR-16, and VEGF, have been demonstrated to be strong inhibitors of MM-induced angiogenesis [148, 149]. Overall, published data indicate that circulating microRNAs in exosomes and microvesicles can be useful biomarkers of angiogenesis, and synthetic miRNAs may be potential new antiangiogenic therapeutics tools in MM [150].

4. Antiangiogenesis in multiple myeloma

The combination of biological drugs in the actual therapeutic strategies of MM have improved the outcome of MM patients because of their activity on microenvironment [17, 151–153].

4.1. Proteasome inhibitors

Bortezomib, a potent, highly selective, and reversible proteasome inhibitor targeting the 26S proteasome complex [154, 155] act on key cellular processes, such as cell cycle progression, inflammation, immune surveillance, growth arrest, and apoptosis [154]. Bortezomib acts by mean the modulation of NF- κ B transcription factor, which mediates the expression and secretion of cytokines, chemokines, cell adhesion molecules involved also in anti-apoptosis and cellular

growth control [154–156]. After phosphorylation by I κ B kinase, I κ B is polyubiquitinated and degraded by the 26S proteasome, which allows p50/p65 NF- κ B nuclear translocation and binding to consensus motifs in the promoter region of target genes [155, 156]. NF- κ B regulated also the expression of adhesion molecules, such as ICAM-1 and VCAM-1, on both MM cells and BM stromal cells [156], so, its inhibition downregulates these adhesion molecules favoring the susceptibility of MM plasma cells to therapeutic agents [156]. Moreover, NF- κ B activation controls the production of IL-6 by BM stromal cells that increase production and secretion of VEGF-2 and FGF-2 from MM plasma cells [91]. By blocking NF- κ B, bortezomib inhibits MM cell adherence to the BM stromal cells reducing MM cell growth and VEGF-2 and FGF-2 secretion [17, 91, 154, 155].

Bortezomib is directly cytotoxic on MM plasma cells by blocking proteasome activity that causes the accumulation of misfolded polyubiquitinated proteins and causes ROS production [155, 156]. The accumulation of misfolded proteins in the endoplasmic reticulum triggers caspase-4 activation, and ROS accumulation causes disruption of membrane potential and the release of cytochrome c from mitochondria, and then the caspase-9 activation. These cytoplasmic alterations consequently, initiate the apoptotic cascades causing apoptosis of the cell [155, 156]. Finally, bortezomib downregulates VEGF, IL-6, IGF-I, Ang-1, and Ang-2 production and secretion by MM plasma cells and BM stromal cells, targeting aberrant blood vessel development through a potent inhibition of proliferation of activated endothelial cells [17, 154].

Ixazomib (MLN2238) is a second-generation proteasome inhibitor with a similar activity of bortezomib on the inhibition of NF- κ B [157, 158]. It has been demonstrated that ixazomib affects BM stromal cells triggered MM cell growth and BM stromal cells-induced endothelial cell proliferation suggesting that ixazomib not only directly targets MM plasma cells but also overcomes the cytoprotective effects of the MM host BM microenvironment [158]. In fact, ixazomib is able to impact angiogenesis in vivo decreasing the expression of angiogenic markers in mice as well as in vitro reducing the capillary formation by HUVEC in the Matrigel™ system [159].

The antiangiogenic activity of another proteasome inhibitor, carfilzomib, has not been clearly demonstrated but it seems to have inhibitory activity on tumor-stromal interactions and angiogenesis [137, 160]. Moreover, VEGF pathway polymorphisms have been associated with clinical outcomes in MM patients [161], and have been reported that polymorphisms of VEGF pathway are associated with response to the combination of carfilzomib and lenalidomide [162].

4.2. Immunomodulators (IMiDs)

Thalidomide, a first generation immunomodulatory drug (IMiD), has a direct tumoricidal activity, an antiangiogenic effect and modulates TNF- α signaling through direct and/or indirect effects on the tumor microenvironment [15, 163–167], reduces FGF-2, VEGF, and IL-6 secretion in BM stromal cells and by MM cells [163]. It also interferes with NF- κ B activity by blocking its ability to bind to DNA abrogating inflammatory/angiogenic cytokine production [165, 166], and disrupts the direct interactions between MM plasma cells and BM stromal cells by modulation of cell surface adhesion molecules [167].

Two new IMiDs, including lenalidomide and pomalidomide, demonstrating up to 50,000 times more potent inhibition of TNF- α than thalidomide, has been developed [168–170]. They

inhibit VEGF and FGF-2 secretion from both myeloma and BM stromal cells and block endothelial cell migration and proliferation in vivo and in vitro [169]. Lenalidomide, a first derivative of thalidomide, is less toxic and more potent than the parent drug, and in patients with relapsed or refractory MM, lenalidomide can overcome resistance not only to conventional chemotherapy but also to thalidomide [169]. De Luisi et al. [170] demonstrated that lenalidomide inhibits angiogenesis and migration of MM endothelial cells and that lenalidomide-treated MM endothelial cells show changes in VEGF/VEGFR-2 signaling pathway, and in several proteins controlling EC motility, cytoskeleton remodeling, and energy metabolism pathways. Both thalidomide and lenalidomide downregulate VEGF. Pomalidomide is a third generation IMiD with increased activity in vitro compared with thalidomide and lenalidomide [171, 172], which exerts anti-MM effects through multiple mechanisms, including induction of apoptosis via caspase-8, reduction of proliferation, inhibition of NF- κ B activation, reduction of stromal cell stimulatory cytokine secretion, and angiogenesis inhibition [172].

4.3. Bisphosphonates

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 antiangiogenic activity [173–175]. 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 [173]. It has also been demonstrated that the addition of zoledronic acid to antimyeloma therapy, bortezomib-, lenalidomide-, or thalidomide-based, is associated with a benefit in term of skeletal-related event rate as well as in term of the progression-free survival rate of myeloma patients [174]. Neridronate exerts its antiangiogenic activity through both a direct effect on endothelial cell proliferative activity and inhibitory effect on the responsiveness of the endothelial cells to the proliferative stimuli mediated by angiogenic cytokines [175].

4.4. Monoclonal antibodies and other drugs

The most successful therapeutic approach to target VEGF in cancer is the use of a humanized monoclonal antibody against VEGF, bevacizumab [176]. Several clinical trials in MM tested the effects of bevacizumab used in conjunction with other agents including lenalidomide, dexamethasone, or bortezomib with discouraging results [177].

In addition to bevacizumab, other VEGFRs targeting compounds (including aflibercept-VEGF-trap), tyrosine kinase inhibitors (cabozantinib, dasatinib, pazopanib, sorafenib, sunitinib, and semaxanib), PI3K/Akt-MEK/ERK pathway inhibitors, FAK inhibitors, interleukin inhibitors (atiprimod), farnesyltransferase inhibitors, other monoclonal antibodies (anti-CD40), and marine cartilage extract (neovastat) have shown antiangiogenic activity but no significant results or only preliminary preclinical data have been reported with the use of this drugs in MM [177–181].

5. Conclusions

Despite the good results obtained in the last decades, MM remains an incurable malignancy, indicating that our knowledge on the mechanisms responsible for disease progression and

drug resistance is still not completely clear. The goal obtained with the introduction of the new target drugs for MM therapy is the improvement of the outcome of MM patients in term of progression-free and overall survival. The simultaneous block of plasma cell proliferation and survival, plasma cells/BM stromal cells interaction, and BM stromal cells activity by the novel agents help us to get these results. In fact, the BM microenvironment plays a crucial role in the pathophysiology of MM. An active crosstalk between MM plasma cells and stromal cells in the BM of myeloma patients is constantly working. It represents a hallmark of active MM favoring survival, proliferation, and migration of plasma cells, and modulates neovessel formation by mean angiogenesis favoring the disease progression. The crosstalk between MM plasma cells and BM microenvironment is not only responsible for drug resistance of plasma cells but also of endothelial cells and other cells composing the microenvironment. The better understanding of the biological mechanisms controlling the interactions between MM cells and BM stromal cells remain fundamental for our knowledge about disease progression and for developing novel drugs targeting these processes.

Conflict of interest

Authors have no conflict of interest to declare.

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