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Angiogenesis in multiple myeloma

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ABSTRACT

Multiple myeloma (MM) was the first haematological malignancy in which a prognostic relevance of bone marrow microvessel density (MVD) was shown. Myeloma-induced angiogenesis involves either the direct production of angiogenic molecules by myeloma cells or their induction in bone marrow stromal cells or endothelial cells (EC). Recent data demonstrate an increased angiogenic potential and a paracrine stimulatory effect of bone marrow EC on plasma cells (PC) in MM. Soluble angiogenic factors are elevated in bone marrow (BM) and in peripheral blood samples from myeloma patients. Furthermore, correlation with disease stage and prognosis was shown for serum levels of the angiogenic factors basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF). In this review we summarize recent data which give strong evidence for an increased angiogenic activity in bone marrow microenvironment and support the hypothesis that angiogenesis is not only an epiphenomenon of tumour growth but may also promote PC growth in MM.

1. Background

Angiogenesis refers to the process of new blood vessel formation from a pre-existing vasculature which occurs in either physiological or pathological conditions.[1,2] Angiogenesis develops in a multi-step process comprising perivascular detachment of existing vessels, matrix degradation, migration of EC and formation of a functional vascular plexus which is supported by perivascular apposition of pericytes and basement membrane constituents.[1,3] Tumour angiogenesis develops through the same steps but shows a markedly increased proliferative activity of EC and has significant functional and structural differences in the vascular plexus.[3,4] In solid tumours angiogenesis is well characterized as a critical step for growth, invasion and metastasis.[5–9] The "angiogenic switch", i.e. the transition from an avascular to a vascular phase of tumour growth is caused by an imbalance of pro- and anti-angiogenic factors in the tumour microenvironment.[7,8] Tumour cells promote vessel formation through the expression of angiogenic molecules or their induction in the microenvironment.[3,4,9] Several angiogenic activators and inhibitors have been identified. Among the pro-angiogenic molecules especially VEGF, bFGF and HGF have been identified to drive tumour-related angiogenesis.[10,11] Furthermore, metalloproteinases (MMP) and angiopoietins play an important role in tumour-induced base membrane matrix remodelling, EC migration, stabilization and tube formation.[12,13]

In recent years, growing evidence indicates that BM angiogenesis is also involved in the pathogenesis and progression of certain haematological malignancies.[14–18] Recent advances in myeloma biology indicate that the microenvironment plays a critical role in the regulation of PC growth, drug resistance and myeloma bone disease. The complex interactions between PC and BM microenvironment are described in detail

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in other articles of this special issue. Among the interactions that occur between myeloma cells and the microenvironment, the role of BM angiogenesis has been highlighted as critical in the progression of MM.[19–22] MM was the first haematological malignancy in which a prognostic relevance of angiogenesis was demonstrated.[23,24] The observation of increased BM-MVD, increased angiogenic cytokine expression, their correlation with disease activity and survival and the development of new anti-angiogenic compounds led to consider angiogenesis as a new target in the treatment of MM.[25–27]

2. Bone marrow microvessel density (BM-MVD) in MM

2.1. BM-MVD increases parallel to disease progression

Prognostic relevance of increased MVD was shown in a wide range of solid tumours[28–31] and in haematological malignancies.[14,15,17,23,24,32] Different techniques have been developed for estimating the grade of angiogenesis in various tissues.[33] Tumour microvessels can be visualized by immunohistochemical staining of CD31, CD34, CD105 or factor VIII-related antigen/von Willebrand factor (vWF). Each marker has its specific advantages or limitations.[34] The quantification of MVD can be done either in terms of microvessel count per field or by calculating the percentage of the surface area which is covered by microvessels. Since it was suggested that EC proliferation is particularly active in highly vascularized regions, the so called "hot spots",[35] double-blinded counting of microvessels in hot spots of anti-CD34 or anti-vWF stained trephine biopsies became a common method for immunohistochemical BM-MVD quantification in MM.[23,24,36] In 1994, Vacca and colleagues demonstrated for the first time that BM-MVD was significantly increased in MM compared to monoclonal gammopathy of undetermined significance (MGUS) and moreover in active versus non-active myeloma. The authors first hypothesized that progression from MGUS to myeloma is accompanied by an increase in BM-MVD.[36] Subsequent studies by other groups confirmed the observation of increased angiogenesis in active myeloma compared to healthy individuals or patients with MGUS.[37–40] MM patients with active disease have increased BM-MVD compared to patients with smoldering or stage I myeloma[41] or compared to MM patients in remission after therapy.[42] Rajkumar could demonstrate in a large cohort of 400 patients that a progressive increase in BM-MVD occurs across the whole spectrum of PC disorders, including primary amyloidosis, MGUS, smoldering and active myeloma,[38] which added further evidence to the hypothesis that angiogenesis is related to malignant PC growth.[43]

2.2. MVD is an independent prognostic factor for survival in MM

MM was the first haematological malignancy in which a significant correlation of angiogenesis with prognosis and survival could be identified.[23,24] In 2000, two independent studies by Rajkumar and by our group demonstrated that increased BM-MVD in MM patients is associated with an unfavourable prognosis.[23,24] Rajkumar could first demonstrate that overall survival was significantly different among myeloma patients with high, intermediate or low low-grade angiogenesis, using immunohistochemical anti-vWF staining of BM biopsies.[23] In immunohistochemical studies on CD34 stained BM sections, we could also show that patients with high MVD (>48 vessels/mm^2) at the time of diagnosis, have significantly shorter overall survival versus those with low MVD.[24] Moreover Munshi and Wilson could show a prognostic significance of pre-treatment MVD for progression-free survival and for median duration of complete response in newly diagnosed MM patients who were treated in the total therapy protocol.[44] Prunieri compared the prognostic significance of BM microvessel staining, using anti-CD34 antibodies or anti-CD105, which was reported to bind preferentially to EC of newly formed vessels.[45] They found a prognostic significance of BM-MVD in the anti-CD34 stained samples but not by using anti-CD105 immunohistochemistry, suggesting that microvessel staining with anti-CD34 provides more accurate prognostic information in MM.[39] The prognostic significance of pre-treatment MVD for progression-free and overall survival was also confirmed in patients undergoing high-dose chemotherapy (HDT).[46]

2.3. MVD correlates with established parameters of disease activity

In the initial report on increased BM angiogenesis in MM, the MVD was found to correlate to PC proliferative rate, evaluated as PC labelling index (PCLI).[36] Furthermore we could find a correlation of MVD with BMPC infiltration rate,[47] suggesting a causal relationship between PC growth and marrow angiogenesis. Rajkumar reported that MVD correlated with PCLI but did not find a correlation with BMPC percentage in their initial study.[23] In a large cohort of patients with PC disorders the same group could later demonstrate a significant correlation of MVD with BMPC percentage.[38] Studies on circulating PC in myeloma have demonstrated that an increased BM-MVD correlates with the presence of circulating PC, which was independent from the BMPC infiltration rate, and suggested that angiogenesis may promote PC proliferation and migration into the peripheral blood.[48] An association of MVD with PC proliferative activity was also demonstrated by histomorphological and immunohistochemical studies, which showed a correlation of BM-MVD with Ki67 expression in PC and with cytological grade according to Bartl score.[41] Further evidence that increased BM-MVD is associated with disease activity and poor prognosis came from studies which correlated MVD with established prognostic parameters. Increased MVD significantly correlated with the presence of deletion 13q14.[37] Moreover, pretreatment BM-MVD was correlated with parameters of tumour burden and disease activity such as β2-microglobulin (β2-MG) as shown by our group[39,47] or lactate dehydrogenase (LDH)[39,40] in mixed cohorts of symptomatic and asymptomatic myeloma patients. In a further study, including patients who received HDT and had a lower median age than in the other studies,[40,47] a correlation of pre-treatment MVD with β2-MG, but not with LDH or cytogenetics could be demonstrated.[46]

In three studies a multivariate analysis was performed, including previously identified prognostic factors β2-MG and C-reactive protein (CRP),[49] age, Bartl grade or deletion 13q14. In the Cox regression analysis, MVD remained significant as a prognostic variable for overall survival.[23,39,44]

3. Treatment related changes of MVD in MM

Although the prognostic relevance of MVD at the time of diagnosis is well established, the clinical relevance of MVD quantification under therapy is less clear. In a longitudinal study comparing pre- versus post-treatment BM-MVD, we could demonstrate that BM-MVD decreases significantly in myeloma patients achieving a remission after CT or HDT, but not in patients who had no response to therapy.[50] In our study we could show that progression-free survival in patients who achieved a reduction in MVD was significantly longer than in those without a decrease in MVD.[50] A decrease of MVD in patients who achieved at least a partial response after CT or HDT was also shown by Kumar, but this did not reach statistical significance.[51]

In a recent study, a significant decrease in MVD could be demonstrated in patients who were responsive to thalidomide while there was no change in non-responders.[52] Post-treatment MVD counts in responders to chemotherapy were comparable to that of untreated patients in stage I[50] but remained higher than in normal controls,[51] suggesting that there was an ongoing angiogenic stimulus by residual myeloma cells. Although a decrease in MVD after chemotherapy could be shown in some studies,[50,51] there are some limitations in using MVD for accurate monitoring of therapy effects.[53] MVD is the cumulative result of certain angiogenic mechanisms in the BM microenvironment and as such must not be a surrogate of the angiogenic activity at the time of biopsy. Furthermore chemotherapy-induced apoptosis of EC or MVD-reduction may not occur parallel to reduction of tumour cells.[53] The clinical relevance of post-therapy MVD quantification has to be further evaluated in the context of more specific anti-angiogenic drug therapies.[26,27]

4. Angiogenic interactions in the bone marrow microenvironment

4.1. *The pro-angiogenic environment in the bone marrow of myeloma patients*

Recent advances in myeloma biology demonstrate the emerging role of the BM microenvironment in promoting proliferation and survival of the malignant PC clone in MM.[54] BM angiogenesis is regulated by soluble pro- and anti-angiogenic factors which mediate the paracrine interactions between myeloma cells, EC and BM stromal cells (BMSC).[22] The increased angiogenic potential of active myeloma was first demonstrated by Vacca who reported that serum-free conditioned media from BMPC of patients with active MM had a significantly higher *in vitro* (matrigel capillarogenesis assay) and *in vivo* (chick chorioallantoic membrane assay) angiogenic activity than samples from patients with non-active MM or MGUS.[55] BM plasma extracts contained higher levels of bFGF 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 bFGF-antibodies.[55] Kumar could also demonstrate an increased angiogenic activity of BM plasma samples of patients with active MM but did not find a difference in the expression levels of VEGF or bFGF in PC obtained from MGUS, smoldering myeloma (SMM) or newly diagnosed active myeloma.[56] The difference in the *in vitro* angiogenic activity was explained by the observation that MGUS samples markedly inhibited angiogenesis compared to SMM or MM.[56] Although it has been well established that myeloma cells drive angiogenesis by the secretion of bFGF, VEGF and certain other angiogenic factors (as described below), there is also evidence for a loss of an anti-angiogenic activity of BM plasma with disease progression.[56]

4.2. *Myeloma cells express angiogenic factors and promote the bone marrow angiogenic switch*

The angiogenic switch in MM is driven by various angiogenic cytokines, which are secreted in the BM microenvironment and can directly stimulate BMEC for proliferation and vessel formation. Among the angiogenic factors, VEGF has been identified to play a key role in sustaining angiogenesis in solid tumours,[10] in several haematologic malignancies[11] and especially in MM.[57,58] VEGF stimulates endothelial cell growth, mobilization of endothelial precursors, vascular development and proliferation of stromal cells (SC). Several studies have demonstrated that myeloma cells directly produce VEGF.[57–59] Moreover, in a paracrine loop, PC derived VEGF stimulates IL-6 and VEGF secretion in SC, whereas stromal cell-derived IL-6 promotes proliferation, survival and VEGF production in PC.[60] In addition to the direct angiogenic stimulation by the VEGF production of MM cells, recent data suggest also a loss of VEGF inhibitory potential by the downregulation of the soluble VEGF antagonist sVEGFR-1 (soluble VEGF receptor) in MM patients compared to healthy subjects.[61]

Basic FGF, another well characterized pro-angiogenic molecule which induces migration, proliferation, and differentiation of EC,[62] is also secreted directly by myeloma cells[42] and triggers paracrine myeloma – stromal cell interactions in an IL-6/bFGF paracrine loop.[63] Syndecan-1 (CD138), a low affinity receptor of bFGF, which is a regulator of myeloma cell growth and survival,[64] is also highly expressed by myeloma cells.[65,66] A simultaneous expression of the angiogenic cytokine HGF[67] and its receptor c-Met was also demonstrated in myeloma cell lines.[68] The expression of bFGF, HGF and their receptors on myeloma cells suggests that these factors are also involved in autocrine loops, which stimulate MM cells.

Although bFGF, VEGF or HGF expression of PC plays a crucial role in BM angiogenesis, other factors may additionally be involved. This can be hypothesized by data showing that the *in vitro* and *in vivo* angiogenic effects of myeloma BM samples are not totally abolished by neutralizing bFGF[55] or VEGF antibodies[69] and by the observation that there was no difference in PC bFGF or VEGF expression between MGUS, smoldering and active myeloma.[56]

Recently, angiopoietin-1 (Ang-1) was identified as an additional angiogenic factor in MM.[70] Angiopoietins and their receptor Tie-2 are important in developmental and postnatal

angiogenesis as well as in tumour angiogenesis.[71] Whereas bFGF, VEGF or HGF initiate endothelial cell proliferation, angiopoietins acting via the Tie-2 receptor are essential regulators of subsequent vascular remodelling.[72] Ang-2 antagonizes Ang-1 binding to the Tie-2 receptor which leads to destabilization of the quiescent vasculature and to sprouting of new vessels, a process which furthermore sensitizes the EC to VEGF.[71] Giuliani found that Ang-1 but not Ang-2 expression is upregulated in MM cell lines or in PC obtained from MM patients and that the Ang-1 expression in the patient samples correlated with BM-MVD. Furthermore, the authors could demonstrate that the angiopoietin receptor Tie-2 is upregulated in bone marrow endothelial cells (BMEC) in the presence of MM cells and that anti-Tie2 antibodies blocked the *in vitro* angiogenic effect of myeloma cells.[70] In contrast, Uneda could not find Ang-1 but Ang-2 expression by RT-PCR or immunohistochemistry and could identify Ang-2 as a prognostic factor in myeloma patients.[73] The seemingly contradictory findings by Giuliani and colleagues and Uneda and co-workers may be interpreted by differences in the sample preparations and must be resolved by further investigations, because of the small number of patients in both studies.

Osteopontin (OPN), which has a role in tissue remodelling such as bone resorption, wound healing and angiogenesis,[74] was recently found to contribute to angiogenesis in myeloma[75] and to myeloma bone disease. [76] A recent study by Colla showed that OPN is expressed by CD138+ MM cells and can be detected in BM plasma of MM patient samples. Moreover for the first time, a role of OPN in myeloma-induced angiogenesis could be demonstrated by the correlation of OPN expression with BM-MVD.[77] In an *in vitro* angiogenesis assay the OPN production by myeloma cells was critical for their pro- angiogenic effects.[77]

In addition to those factors which have direct effects on EC, myeloma cells also release metalloproteinases (MMP), especially MMP-2, MMP-9 or urokinase-type plasminogen activator (uPA)[55,78] which play an important role in matrix degradation, base membrane permeabilization and invasion of EC and pericytes for vessel formation.[79] During matrix degradation, matrix bound growth factors, further angiogenic molecules such as bFGF, VEGF or OPN and anti-angiogenic factors are released.[80] MMP-2 secretion is increased in patients with active myeloma versus non-active MM or MGUS.[55] Furthermore, MMP secretion of MM cells is triggered by BMSC or BMEC.[81]

In summary, MM cells themselves can stimulate angiogenesis by the release of pro-angiogenic factors and matrix degrading enzymes or by paracrine induction of these factors in the BM environment. Thus in active MM, PC shifts the balance between pro- and anti-angiogenic factors in favour of angiogenesis.

4.3. *Role of bone marrow stromal cells (BMSC) and endothelial cells (BMEC) in angiogenesis*

BMSC play a crucial role in sustaining PC proliferation, survival, drug-resistance, osteoclast activation, inhibition of osteogenesis and BM angiogenesis in MM.[54] An overview on the important role of BMSC – MM cell interactions is given by Mitsiades in this special issue. BMSC increase the concentration of angiogenic factors and matrix degrading enzymes in the BM environment by direct secretion or by stimulation of myeloma cells or EC via paracrine interactions.[60,63] Additionally, direct BMSC-MM cell adherence triggers the expression of VEGF and IL-6[82,83] and thus enhances PC proliferation and modulation of EC.[84]

BMEC in MM represent a heterogeneous cell population forming tortuous, uneven vessels with irregular branching and shunts. An increased proliferative activity of EC in haematological malignancies could be shown by immunohistochemical studies with CD34 and Ki67 co-staining.[85] Furthermore, it could be shown that EC proliferative activity *in vitro* is increased in MM versus MGUS or healthy controls.[86] Circulating endothelial progenitor cells with *in vitro* angiogenic activity were found in a higher amount in myeloma patients compared to healthy controls and were correlated with M-protein and β2-MG.[87] Differences in BMEC derived from MM patients compared to quiescent human umbilical vein endothelial cells (HUVEC) have been elaborated by Vacca in 2003. In this study it could be shown that MM-BMEC are characterized by an enhanced expression of specific angiogenic receptors such as VEGFR-2, bFGFR-2, Tie-2 or CD105, by increased *in vitro* capillarogenic activity and by the expression of growth and invasive factors such as VEGF, bFGF, Ang-2 or MMP.[88,89] The finding, that MM-BMEC produce soluble PC stimulating factors, indicates that angiogenesis promotes PC growth, invasion and dissemination as it was previously hypothesized by the correlation of MVD with PC proliferative activity and migration.[36,41,48] Further evidence that EC promote myeloma cell growth comes from a recent publication demonstrating that MM-BMEC secretes CXC-chemokines,[90] which are involved in chemotaxis of tumour cells. Since several CXC receptors were identified on myeloma cells,[91–93] EC derived chemokines may mediate PC proliferation and chemotaxis in MM. The direct adherence of BMEC to MM cells is also considered to be a key step in the pathophysiology of myeloma. In several studies with the 5TMM mouse models it could be shown that EC modulate MM cell homing and adherence through certain chemokines and adhesion molecules (e.g. CD44 variants).[85,94]

A new aspect on the role of EC has been added by a recent study in B-cell lymphomas, in that identical genetic aberrations could be found both in tumour cells and in EC, suggesting a close relationship between the genetic events in those two cell types.[95] Thus, tumour EC could be derived from common malignant precursors or develop by endothelial cell - tumour cell fusion, a possible explanation for the increased proliferative activity and expression of growth factors by tumour-related EC.[95] Since it has been demonstrated that BMEC in myeloma show significant phenotypic differences in morphology, angiogenic potential and in expression levels of several growth factors, the frequency of myeloma specific genetic aberrations in MM-BMEC is of special interest and remains to be investigated.

5. Clinical significance of angiogenic cytokine levels in MM

5.1. *Elevated levels of angiogenic cytokines in MM*

Angiogenic cytokines which are produced in the tumour or BM microenvironment can be detected in peripheral blood

samples of cancer patients by commercially available immunoassays. In several types of solid tumours and in haematological malignancies the prognostic role of angiogenic cytokines was shown.[96,97] First evidence for a clinical relevance of angiogenic cytokine levels in MM came from Borset, and Seidel and colleagues who found that serum levels of HGF are elevated in myeloma patients compared to healthy individuals.[98,99] These findings were later confirmed by several other groups.[40,100,101] In 2001 we could demonstrate that serum levels of circulating bFGF are elevated in MM patients and increase parallel to the stage of the disease.[102] To overcome possible inaccuracies by the release of VEGF from platelets during coagulation,[103] Sato measured VEGF in plasma samples and could show a significant elevation in MM patients versus controls, but no correlation with disease activity.[104] In further studies it could be demonstrated that progression of MM to advanced disease stages is accompanied by an increase in plasma or serum VEGF,[100,105] bFGF,[104–106] HGF[40,100,105] and Syndecan-1.[106,107] The important question whether peripheral blood levels of angiogenic cytokines in myeloma reflect the cytokine concentrations in the BM compartment, has been addressed by two studies.

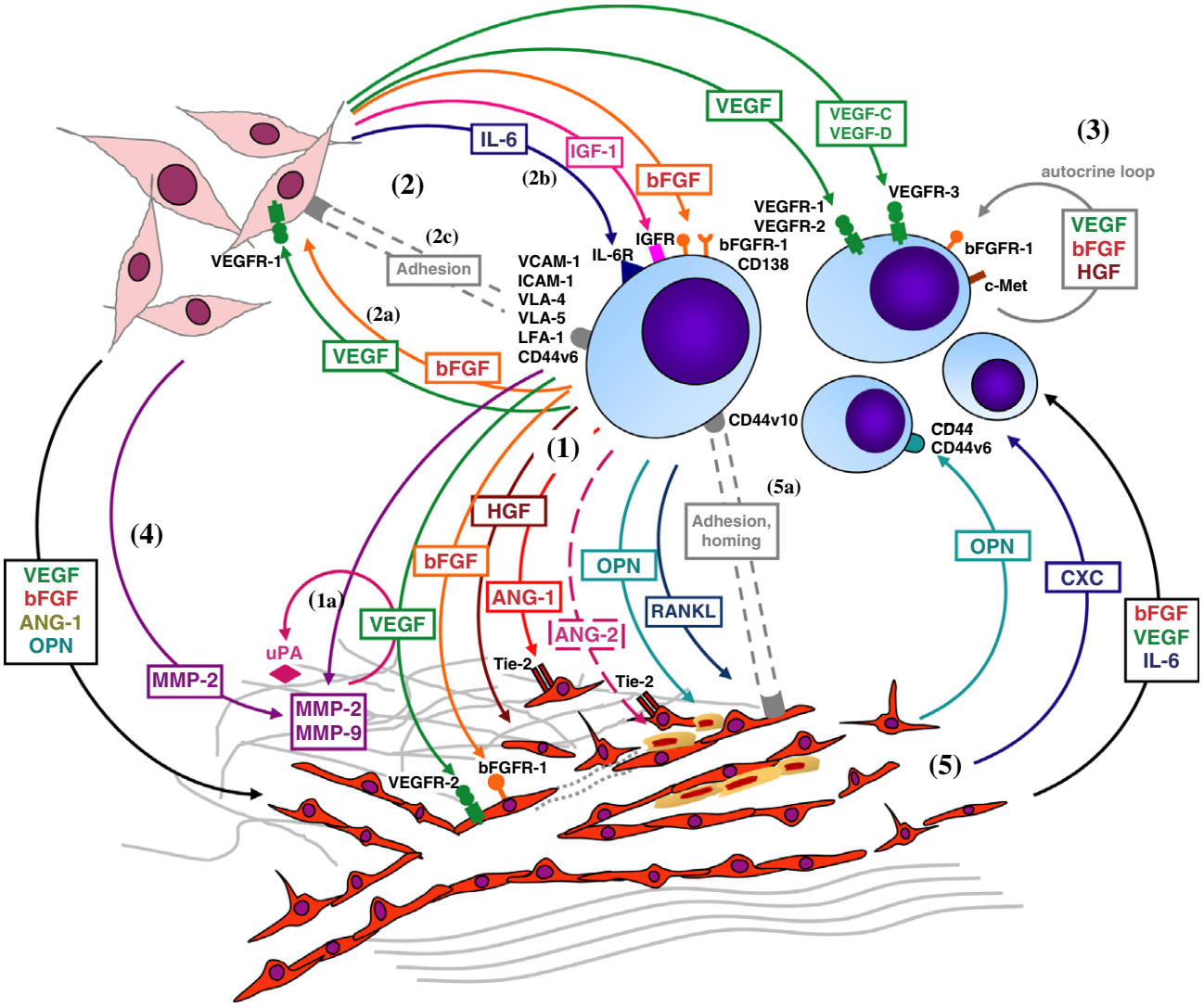

Fig. 1 – Interactions in the BM microenvironment mediated by angiogenic factors in MM. Myeloma cells produce angiogenic factors VEGF, bFGF, HGF, Ang-1, Ang-2, OPN and metalloproteinases MMP-2 or MMP-9 which leads to EC proliferation, matrix degradation and tube formation (1). Subsequently, further matrix degrading factors such as MMP or uPA and angiogenic peptides are released or activated by matrix degradation (1a). PC derived VEGF and bFGF stimulate BMSC (2a) to release cytokines such as VEGF, bFGF or IL-6 (2b) which in turn promote myeloma cell growth in a paracrine loop (2). Cytokine release, survival support and drug resistance is further triggered by direct adhesion of MM cells to BMSC via certain differentially expressed adhesion molecules (2c). Autocrine loops by the angiogenic factors VEGF, bFGF and HGF and their receptors on PC may stimulate MM cell growth (3). In addition to myeloma cells, activated BMSC also promote angiogenesis by the release of angiogenic factors and matrix degrading enzymes (4). Endothelial cells produce angiogenic factors such as VEGF, bFGF, OPN and MMP, but also IL-6 and CXC chemokines (5) and thereby also contribute to promote growth, survival and homing (5a) of PC in the BM.

Di Raimondo could demonstrate that plasma levels of VEGF, bFGF and HGF were higher in the BM and correlated significantly with the concentration in corresponding peripheral blood samples.[105] Recently, Andersen could also demonstrate a higher concentration in the BM and a correlation with peripheral blood levels of bFGF, HGF and syndecan-1,[101] thus suggesting that BM is the major source of circulating angiogenic cytokines in myeloma. Among the different cell types which contribute to the angiogenic cytokine release in the BM microenvironment (Fig. 1), myeloma cells play a key role in sustaining the pro-angiogenic environment. For several angiogenic cytokines, a correlation of angiogenic cytokine levels with parameters of tumour cell mass, such as BMPC count, M-protein or serum β2-MG has been shown. A synopsis of studies on angiogenic cytokine levels in MM is given in Table 1. Although there are some contrary findings regarding

Table 1 – Synopsis of studies on circulating angiogenic cytokine levels in MM

Reference	No. of pts. *n* =	Angiogenic factor	MM patients vs. controls	Correlation disease activity/ stage	Correlation tumor burden	BM vs. PB concentration	Prognostic significance	Change with treatment response
Borset[98]	13	HGF	↑*	–	–	–	–	–
Seidel[99]	398	HGF	↑*	–	BMPC : n.s. M-Prot :* β2-MG:*	– –	OS:* (in β2-MG > 6.0)	↓* (CT)
Seidel[107]	174	Syn-1	↑*	↑*	BMPC :* M-Prot :* β2-MG:*	– –	OS:*	–
Di Raimondo[105]	33	pl-VEGF	–	↑*	–	↑*	–	–
		bFGF	–	↑*	–	↑*	–	–
		HGF	–	↑*	–	↑*	–	–
Sezer[102]	47	s-VEGF	↔	n.s.	β2-MG: n.s.	–	–	↓* (CT+HDT)
		bFGF	↑*	↑*	β2-MG:*	–	–	↓* (CT+HDT)
		HGF	(↑)	(↑)	β2-MG: n.s.	–	–	↓* (CT+HDT)
Neben[111]	51	VEGF	–	–	–	*	–	↔ (Thal)
		bFGF	–	–	–	*	–	↔ (Thal)
		HGF	–	–	–	*	–	↓* (Thal)
Seidel[108]	128	HGF	↑*	–	–	–	OS: n.s.	↓* (HDT)
Sato[104]	45	pl-VEGF	↑*	n.s.	BMPC: n.s. β2-MG: n.s.	–	–	–
		bFGF	↑*	↑*	BMPC:* β2-MG:*	–	–	–
Iwasaki[100]	52	s-VEGF	↑*	↑*	–	–	OS:*	↓* (CT)
		HGF	↑*	↑*	–	–	OS:*	↔ (CT)
Dmoszynska[110]	30	VEGF	–	–	–	–	–	↓* (Thal)
		bFGF	–	–	–	–	–	↓* (Thal)
Alexandrakis[40]	47	HGF	↑*	↑*	BMPC :* β2-MG:*	–	–	–
Kyrtsonis[106]	27	bFGF	–	↑*	BMPC : n.s. β2-MG: n.s.	–	OS:*	↔ (CT)
		Syn-1	–	↑*	BMPC :* β2-MG:*	–	OS:*	↓* (CT) ↓* (Bisph.)
Andersen[101]	67	bFGF	↑*	–	–	↑*	OS: n.s.	
		HGF	↑*	–	–	↑*	OS:*	–
		Syn-1	↑*	–	–	↑*	OS:*	–
Hatjiharissi[112]	25	VEGF	↑*	–	–	–	–	↔ (Thal)
		bFGF	↑*	–	–	–	–	↔ (Thal)

↑: increase, ↓: decrease, ↔: no difference, *: $P < 0.05$ in appropriate statistic test, n.s.: no statistical significance, (↑): trend but not statistically significant, OS: overall survival; PFS : progression-free survival, CT: conventional dose chemotherapy, HDT: high dose chemotherapy, Thal: Thalidomide, pl-VEGF: plasma VEGF, s-VEGF: serum VEGF, BMPC: bone marrow plasma cell infiltration, M-Prot: M-protein, β2-MG: beta2-microglobulin.

the correlation of angiogenic factors with some parameters of tumour burden, it can be hypothesized that myeloma cells are the main source of angiogenic cytokines and conversely angiogenic cytokine levels may reflect myeloma cell mass rather than BM angiogenesis.

5.2. *Cytokine levels and treatment response*

Several studies have investigated cytokine levels under therapy in MM. In the study by Seidel on HGF in myeloma, there was a subgroup of 29 patients with response to conventional-dose chemotherapy (CT) who had a significant decrease in post-treatment HGF serum levels after therapy.[99] We could demonstrate that serum levels of VEGF, bFGF and HGF decrease with response to CT or HDT.[102] The decrease of serum HGF levels after CT and HDT was later confirmed by a further study[108,109] whereas in one study no differences in pre- versus post-treatment HGF levels could be found.[100]

The relevance of angiogenic cytokine measurements under thalidomide treatment remains to be clarified. In one study, a significant decrease of VEGF and bFGF in 18 patients responding to thalidomide treatment was reported.[110] In contrast, in two further studies there was no decrease of VEGF or bFGF after thalidomide,[111,112] while there was a decrease in HGF levels after six months.[111] The observation that both thalidomide treatment and chemotherapy can cause a reduction of angiogenic cytokine levels in patients achieving a remission is most probably related to a reduction in myeloma burden rather than to specific effects of these drugs on angiogenic cytokine production.

5.3. *Prognostic value of angiogenic cytokines*

HGF was first reported to be an adverse prognostic factor in a subset of myeloma patients with elevated β2-MG values, but not in the whole group of 398 myeloma patients in stages I to III.[99] In younger patients who received HDT and had a significant decrease in post-treatment serum HGF after response to treatment, there was no prognostic significance of pretreatment HGF for overall survival.[108] In contrast, a further study by Iwasaki found a prognostic significance of serum HGF and VEGF in a smaller number of patients who received CT.[100] The prognostic role of HGF was further confirmed by Andersen who also identified HGF as an independent prognostic factor for overall survival.[101] Furthermore, a prognostic significance could be shown for bFGF[106] and syndecan-1,[101,106,107] while in the study by Andersen, no prognostic significance for bFGF could be calculated.[101]

Since the prognostic significance of circulating angiogenic cytokines is not fully elucidated, we recently performed a univariate and multivariate analysis to investigate the prognostic value of plasma VEGF, serum bFGF and HGF levels for overall survival in 100 untreated MM patients. All of those three factors were independent prognostic factors for overall survival. Additionally, in multivariate analysis including classical parameters of tumour burden and disease activity, VEGF, bFGF and HGF remained independent prognostic variables in a model adjusted to age, international scoring system (ISS) and β2-MG [Jakob, unpublished results].

6. Conclusion

Although it has been well established that disease progression in MM is accompanied by increased BM angiogenesis, there is still controversy as to whether angiogenesis is an epiphenomenon or a driving force in the progression of MM. Since the initial studies on the diagnostic and prognostic relevance of MVD or circulating angiogenic cytokines gave the first evidence for a crucial role of angiogenesis, recent advances in myeloma biology give a more detailed insight into the complex interactions within the BM microenvironment. Especially the recent studies on the characteristics of myeloma-derived BMEC and their interactions with PC or BMSC demonstrate that the EC sustain growth and progression of myeloma, not only by giving a vascular supply, but rather by providing proliferation and survival stimuli for malignant PC. This gives a new rationale for the specific inhibition of angiogenic signalling, which not only inhibits PC-induced angiogenesis, but may also interrupts the endothelial cell-derived growth factor support for myeloma cells.

Conflict of interest statement

None declared.

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