Levels of angiogenic factors in patients with multiple myeloma correlate with treatment response

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ORIGINAL ARTICLE

Levels of angiogenic factors in patients with multiple myeloma correlate with treatment response

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Abstract Angiogenesis plays a significant role in the pathogenesis of multiple myeloma (MM). We have measured concentrations of angiogenesis activators, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and hepatocyte growth factor (HGF), and inhibitors, including endostatin, thrombospondin-1 (TSP-1), and angiostatin in the peripheral and bone marrow blood of MM patients at diagnosis and after high-dose chemotherapy. We have analyzed 96 patients with secretory MM. Serial measurements of angiogenesis factors/inhibitors were analyzed in the plasma by subgroups based on the best treatment response. Concentrations of angiogenic factors were determined in the peripheral blood and bone marrow plasma. There were significant decreases of VEGF and HGF levels and a significant increase in TSP-1 concentrations in the bone marrow plasma of patients who achieved complete or very good partial response in contrast to those who had partial or no response. VEGF and HGF levels decrease but those of TSP-1 increase after successful treatment for MM, indicating a reduction in the rate of angiogenesis.

Keywords Angiogenesis · Cytokines · High-dose chemotherapy · Multiple myeloma · Therapeutic response

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Introduction

Multiple myeloma (MM) is characterized by proliferation of malignant plasma cells that accumulate in the bone marrow and often produce a monoclonal immunoglobulin [1]. MM was the first hematological malignancy in which increased angiogenesis rate was detected [2, 3]. Angiogenesis activators thought to play a role in MM pathogenesis include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF) [3–5]. They have been shown to stimulate the proliferation of malignant plasma cells as well as nonmalignant stromal cells. Plasma concentrations of these cytokines correlate with the Durie and Salmon stage [6–11], but the results of studies published to date are not conclusive as to their relative importance in pathogenesis and prognosis of MM [12]. There is little information on

the role of angiogenesis inhibitors such as angiostatin, endostatin, thrombospondin-1 (TSP-1), or platelet factor 4 in MM [13]. So far, only one report has been published that showed increased levels of endostatin in a small cohort of MM patients [14]. It has also been reported that angiostatin gives a reduction in HGF levels, inhibits neovascularization, and slows tumor growth in an animal model of plasma cell malignancy [15].

The aim of this study was to measure the concentrations of selected angiogenesis factors/inhibitors including VEGF, bFGF, HGF, endostatin, TSP-1, and angiostatin in the platelet-poor bone marrow plasma (BMP) and platelet-poor peripheral blood plasma (PBP) of MM patients at diagnosis and after high-dose chemotherapy. The working hypothesis was that angiogenesis activators decrease, and conversely, inhibitors increase after successful therapy, reflecting a decreased rate of angiogenesis in responding patients. There is a clear evidence that the resistance to high-dose chemotherapy is also associated with a poor prognosis [16].

Patients and methods

Patients

We have analyzed 96 patients with secretory MM enrolled in the randomized CMG 2002 clinical trial [17]. The study treatment consisted of four cycles of induction chemotherapy with vincristin (0.5 mg i.v., day 1–4), doxorubicin (9.0 mg/m^2, day 1–4), and dexamethasone (40 mg p.o., day 1–4, 10–13, and 20–23; VAD). Stimulation chemotherapy with cyclophosphamide 2.5 g/m^2 followed by granulocyte colony-stimulating factor and myeloablative chemotherapy with melphalan 200 mg/m^2. Baseline characteristics of the patients are shown in Table 1.

Treatment response

Serial measurements of angiogenesis activators/inhibitors were analyzed by subgroups based on the best treatment response. Very good partial response (VGPR) was defined as reduction of monoclonal (M)-component by at least 90% of initial values. In our analysis, this subgroup included patients with complete response, i.e., disappearance of the M-component on electrophoresis and immunofixation. Partial response (PR) was defined as decrease in M-component by 50% to 90% and insufficient response (IR) as decrease in M-component by less than 50% [18].

Table 1 Baseline characteristics of patients

Number		96
Mean age (range; years)		55.1 (28–72)
Males/females		51/45
Durie–Salmon stage	IA	9
	IB	1
	IIA	13
	IIIA	56
	IIIB	14
	Not known	2

Sample processing

After giving written informed consent to the study, patients had bone marrow aspiration from either the sternum or iliac crest. Peripheral venous blood sample was taken on the same day. All samples were immediately mixed with EDTA and centrifuged at 3,800 rpm for 15 min at room temperature. BMP was always prepared from the first milliliter of the aspirated bone marrow.

Cytokine measurements

Measurements were done using ELISA kits according to the manufacturers' instructions. The following ELISA kit were used: human VEGF (R&D systems, Minneapolis, USA), human bFGF (R&D systems, Minneapolis, USA), human HGF (R&D systems, Minneapolis, USA), human TSP-1 (Chemicon, Millipore, Billerica, USA), human endostatin (Chemicon, Millipore, Billerica, USA), and human angiostatin (Ray Biotech, Norcross GA, USA). For each patient, levels of the angiogenesis activators/inhibitors were measured at diagnosis and at the time of maximum treatment response, i.e., 1 to 6 months after high-dose chemotherapy.

Statistical analysis

Repeated-measures analysis of variance method was used to compare the changes in angiogenesis activators/inhibitors before treatment and after high-dose chemotherapy in subgroups with different treatment responses. The level of statistical significance was set at $p=0.05$.

Results

Of the 96 patients, 72 (75%) had evaluable paired PBP samples, and 81 (84%) had evaluable paired BMP samples taken at diagnosis and 1 to 6 months after high-dose chemotherapy. Hemolysis was the most common reason for sample invalidation. Results of HGF, VEGF, and TSP-1 measurements are summarized in Table 2. The decrease of VEGF levels in PBP of complete

Table 2 HGF, VEGF, and TSP-1 levels in bone marrow plasma in multiple myeloma patients at diagnosis (timepoint A) and after high-dose chemotherapy (timepoint B)

Sample	Bone marrow plasma					
Response	VGPR n=38		PR n=28		IR n=13	
Timepoint	A	B	A	B	A	B
VEGF median (range) pg/ml	58 (40–707) p=0.005	29 (19–102)	40 (31–114) p=0.281	47 (13–169)	120 (63–247) p=0.082	83 (17–91)
HGF median (range) pg/ml	886 (828–2,111) p=0.041	782 (707–1,641)	1,165 (1,140–2,656) p=0.244	987 (903–2,432)	1,760 (1,182–4,210) p=0.315	2,090 (1,199–4,865)
TSP-1 median (range) pg/ml	351 (337–917) p=0.048	511 (479–1,124)	303 (279–705) p=0.065	466 (354–1,210)	246 (167–798) p=0.893	329 (244–1,345)

VGPR very good partial response, *PR* partial response, *IR* insufficient response, *VEGF* vascular endothelium growth factor, *HGF* hepatocyte growth factor, *TSP-1* thrombospondin-1

response (CR)+VGPR patients was statistically significant (p=0.016). There was also a statistically significant reduction of both HGF and VEGF concentrations in BMP after treatment in the CR+VGPR subgroup (p=0.041 and p=0.005, respectively; Table 3). In contrast, there was no significant change in the levels of HGF or VEGF in patients who had PR or IR.

The patients who achieved CR or VGPR had a significant increase in the levels of TSP-1 in BMP after treatment (p=0.048; Table 3). There was no change in TSP-1 concentration in the subgroups with PR or IR. Changes in TSP-1 concentration in PBP were not statistically significant for any subgroup (Table 2).

No statistically significant differences between bFGF, endostatin, or angiostatin concentrations at diagnosis and after treatment in any of the patients' subgroups for PBP or BMP were observed (data not shown).

Discussion

Measurement of angiogenesis factors in the peripheral blood or in the bone marrow is a relatively simple and reproducible way for monitoring angiogenesis in hematological malignancies, including MM [4, 6, 10, 19, 20]. We have undertaken serial measurements of selected angiogenesis activators and inhibitors in MM patients. In accordance with published data, HGF and VEGF were the two angiogenesis activators that significantly responded to successful treatment [8, 19]. There are contradictory reports

Table 3 HGF, VEGF, and TSP-1 levels in peripheral blood plasma in multiple myeloma patients at diagnosis (timepoint A) and after high-dose chemotherapy (timepoint B)

Sample	Peripheral blood plasma					
Response	VGPR n=33		PR n=28		IR n=11	
Timepoint	A	B	A	B	A	B
VEGF median (range) pg/ml	71 (61–188) p=0.016	22 (20–91)	77 (64–161) p=0.079	34 (21–96)	83 (35–313) p=0.315	103 (13–575)
HGF median (range) pg/ml	472 (417–823) p=0.014	398 (354–475)	623 (493–991) p=0.07	526 (303–1,230)	1,230 (495–3,430) p=0.121	1,036 (321–32,228)
TSP-1 median (range) pg/ml	464 (385–826) p=0.955	599 (425–868)	378 (328–642) p=0.893	323 (297–651)	568 (411–860) p=0.192	470 (379–523)

VGPR very good partial response, *PR* partial response, *IR* insufficient response, *VEGF* vascular endothelium growth factor, *HGF* hepatocyte growth factor, *TSP-1* thrombospondin-1

on the correlation between VEGF level and prognosis. Mileskhin et al. reported that high levels of VEGF were associated with response rate in patients treated with thalidomide [21]. On the other hand, Cibeira and collaborators have not confirmed any correlation between response rate and VEGF level [22]. We are the first to report that the angiogenesis inhibitor TSP-1 increases in patients who achieve CR or VGPR after first-line treatment for MM [23]. In MM, VEGF is produced by malignant plasma cells and stimulates the proliferation of endothelial cells. At the same time, endothelial cells in the tumor microenvironment also produce VEGF that acts in an autocrine and paracrine way on both endothelial and MM cells. Thus, there is a reciprocal stimulation between endothelial cells and malignant plasmocytes [24].

Concentrations of HGF are increased in MM patients as compared to healthy controls [8]. HGF promotes the adhesion of malignant plasma cells to fibronectin, a process which leads to the acquisition of resistance to cytostatic agents by MM cells and their enhanced survival [25]. HGF inhibits the proliferation of osteoblasts, and recent reports underline its importance in MM bone disease. HGF is also critical for increased bone marrow angiogenesis which is a key mechanism in the pathogenesis of MM [3, 4]. According to the results of our study as well as other reports, HGF is the single most useful angiogenesis activator for angiogenesis monitoring because its levels decrease in the peripheral blood as well as in bone marrow after successful treatment.

TSP-1 is an extracellular matrix protein produced by endothelial cells, fibroblasts, macrophages, monocytes, and some tumor cells [26]. There is an evidence for its inhibitory effect on endothelial cells through the induction of apoptosis, blocking of chemotaxis, and inhibition of new vessel formation [27]. Some preclinical data suggest that TSP-1 may have antineoplastic activity [13]. Our results showing an increase in the concentration of TSP-1 in the bone marrow of MM patients with good response to treatment may indicate that TSP-1 has a role in the pathogenesis of MM. In our hands, there was a wide variation in the levels of TSP-1 in the peripheral blood between individual patients probably caused by the release of the cytokine from activated platelets and endothelial cells in the peripheral vasculature. It has been proposed that the type of endothelium which is present in the tumor microenvironment produces less TSP-1 that the endothelium of normal capillaries, thus leading to disinhibition of tumor-associated angiogenesis [28].

It is more reliable to measure the concentrations of angiogenesis factors in the plasma rather than in the serum because platelets contain significant concentrations of these cytokines, releasing them upon coagulum formation during the preparation of serum [6]. Our previous data show the importance of analyzing the first milliliter of aspirated bone marrow because peripheral blood admixture may result in artifactual readings [29]. It is possible that discordant results published recently on the correlation between prognosis and angiogenesis-related cytokine levels are due to this methodological issue. We have also found that pretreatment concentrations of HGF and TSP-1 are predictive factors for treatment response. Patients with low angiogenesis rate as determined by HGF and TSP-1 concentrations were more likely to achieve complete or very good partial response after high-dose chemotherapy and probably had better prognosis than others [23].

In conclusion, the decreases in VEGF and HGF concentrations and the increase in TSP-1 concentration in patients with CR or VGPR indicate that the rate of angiogenesis is decreased after successful treatment for MM. We have shown in a large patient cohort that VEGF and HGF are the key angiogenesis activators, and TSP-1 is the most important angiogenesis inhibitor in MM.

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