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ARTICLE

Increased Expression of Vascular Endothelial Growth Factor (VEGF) in Bone Marrow of Patients with Myeloproliferative Disorders (MPD)

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Angiogenesis is a multistep process of the development of capillaries from established blood vessels. Angiogenesis probably plays a significant role in the development and progression of hematopoietic malignancies. Higher microvascular density and increased serum levels of proangiogenic factors such as vascular endothelial growth factor (VEGF) or basic fibroblasts growth factor (bFGF) have been reported in acute and chronic leukemias, myeloproliferative and myelodysplastic disorders, multiple myeloma and lymphomas. The microvessel density of bone marrow stroma in myeloproliferative disorders is increased and VEGF is considered as the most potent endothelial cell activator. The purpose of this study was to examine the expression of VEGF in bone marrow of patients with MPD. 60 paraffin-embedded bone marrow core biopsy specimens from newly diagnosed patients with MPD were evaluated. In addition 10 bone marrow core biopsy specimens from adult patients without evidence of malignancy were used as controls. Bone marrow sections were stained immunohistochemically for VEGF (PharMingen, USA). Obtained data show that MPD are associated with an increased expression of VEGF in the bone marrow. This observation support previous studies suggesting that angiogenesis may play a role in the pathophysiology of myeloproliferative disorders. Clinical significance of this phenomenon needs further investigation however thus provides rationale for use of angiogenesis inhibitors in MPD therapy. (Pathology Oncology Research Vol 9, No 3, 170–173)

Keywords: angiogenesis, myeloproliferative disorders, VEGF

Introduction

Angiogenesis is a multistep process of the formation of new capillaries from existing blood vessels. It involves extracellular matrix remodeling, endothelial cell migration and proliferation, capillary differentiation and anastomosis formation. Angiogenesis plays very important role in physiologic vascularisation during normal menstrual cycle, and it occurs in pathophysiological conditions such as wound healing, proliferative retinopathy, rheumatoid arthritis and solid tumors.[4] Angiogenesis is associated with the growth, dissemination and metastasis of solid tumors. There is increasing evidence that neovascularisation may be important in hematological malignancies. Several positive and negative regulatory cytokines have been reported to be involved in the angiogenic process. Vascular endothelial growth factor (VEGF), the most potent direct-acting angiogenic protein known, is a endothelial cell-specific mitogen and angiogenic factor that also increases vascular permeability. VEGF overproduction has been identified as a major factor underlying pathological angiogenesis.[9] High serum concentration of VEGF and increased vascularity in bone marrow were reported in MPD patients.[15]

Myeloproliferative disorders are clonal diseases originating in a pluripotential hematopoietic stem cell. The clonal expansion results in increased and abnormal hematopoiesis and produces a group of interrelated syndromes. MPD are classified according to the predominant phenotypic expression of myeloproliferative clone. The

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Table 1. **Clinical and laboratory characteristics of 60 patients with myeloproliferative disorders and healthy controls**

	Age (years)	*Sex (M/F)*	*Hemoglobin (g/dl)*	*White blood cell count (G/l)*	*Platelet Count (G/l)*
PV	64 (48-70)	8 / 2	16,2 (13,2–18,1)	11,5 (6,7–16,5)	390 (190–630)
MF	59 (37-68)	6 / 8	11,6 (8,2–14,0)	9,8 (4,5–17,2)	182 (110–450)
ET	60 (24–72)	9 / 15	12,8 (9,1–15,2)	7,3 (3,9–12,3)	956 (550–1500)
CML	56 (32-69)	7 / 5	12,5 (7,5–13,9)	21,1 (9,5–170)	370 (150–660)
Normal controls	62 (45-68)	5 / 5	14,0 (12,8–15,5)	6,9 (4,5–8,1)	255 (140–270)

Some PV patients were treated by phlebotomy at the time of laboratory testing

clinical course and management differ among the various disorders. MPD include chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocytosis (ET), and myelofibrosis with myeloid metaplasia (MF).[3,18]

The purpose of this study was to determine the expression of VEGF in bone marrow of patients with MPD.

Material and methods

60 paraffin-embedded bone marrow core biopsy specimens from newly diagnosed patients with MPD were evaluated (polycythemia vera (PV), n=10; myelofibrosis (MF), n=14; essential thrombocytosis (ET), n=24; chronic myelocytic leukemia in chronic phase (CML), n=12. The PV, ET and MF were diagnosed according Polycytaemia Vera Study Group criteria [11,13,18]. The diagnosis of CML was confirmed by the presence of Philadelphia chromosome and/or BCR-ABL oncogene.

In addition 10 bone marrow core biopsy specimens from adult patients without evidence of malignancy were used as controls. In the control patients bone marrow biopsy has been made for evaluation of cytopenias or tumor staging.

Clinical data are shown in the *Table 1*.

Bone marrow sections were stained immunohistochemically for VEGF. Studied samples were fixed in 10% buffered formalin and then embedded in paraffin. The preparations were stained with hematoxylin and eosin and evaluated histopathologically. Deparaffinised sections were incubated with mouse monoclonal antibodies against VEGF (clone G153-694) (Pharmingen, USA) in dilution 2ug antibodies/ml. In the next step streptavidin-biotinylated peroxidase (LSAB2, Dako, Denmark) complex was used and activity of the latter was estimated using DAB (Dako, Denmark). In each case the negative control was included with Primary Negative Control (Dako, Denmark) and then – LSAB2 and DAB.[5,17]

Intensity of immunocytochemical procedure was evaluated using the ImmunoReactive Score (IRS) according Remmele (*Table 2*).[16] The applied scale took into account both intensity of the color reaction and percentage of cells, which exhibited the positive reaction. Final result represented the product of the two parameters and its value ranged between 0 and 12.

Statistical analysis was done using the U Mann-Whitney test. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

The patients with MPD consisted of 30 women and 30 men. The control group of patients consisted of 5 women and 5 men. The mean age of MPD patients was 64 years (range 48-70). In the control group the mean age was 62 (range 45-68). The mean percentage of VEGF positive cells in MPD group and in the control were 47% (range 5-70%) and 5% (range 0-9%) respectively. The mean intensity of reaction in MPD bone marrows was 2 (range 1-3) while in the control was 1 (range 0-1). The differences between VEGF expression measured by IRS scale

Table 2. **Evaluation of the reaction results using the IRS scale**

% positive cells		*Intensity of the reaction*	
0	no positive cells	0	no positive reaction
1	<10% positive cells	1	faint colour reaction
2	10-50% positive cells	2	moderate colour reaction
3	51-80% positive cells	3	intense colour reaction
4	> 81% positive cells		

Table 3. **VEGF expression in the MPD patients and the control**

	MPD patients	*Control*	*P*
Mean %VEGF (+) cells (range)	50% (10–75)	5% (0 –9)	<0,001
Mean intensity of reaction (range)	2 (1-3)	1 (0-1)	<0,001
Mean IRS (range)	6 (2-9)	1 (0-1)	<0,001

Table 4. **VEGF expression in MPD patient's subgroups according to diagnosis**

	ET	*PV*	*MF*	*CML*
Mean % (+) cells (range)	50% (10-75)	46% (15-70)	51% (10-75)	52% (20-75)
Mean intensity of reaction (range)	2 (1-3)	2 (1-3)	3 (2-3)	3 (1-3)
VEGF – mean IRS (range)	5 (2-9)	4 (2-6)	7 (4-9)	6 (2-9)

in MPD and control marrows were highly significant (6 and 1 respectively; $p < 0,001$). Among PV, MF, ET and CML groups VEGF expression did not differ significantly. The results of VEGF expression are summarized in the *Table 3 and 4*.

The myeloproliferative disorders are collectively characterized by stem cell origin of the clonal process. A chronic myeloid process is initially investigated for the presence of t(9;22) (Philadelphia chromosome) and BCR-ABL oncogene. On the basis of the presence of the BCR-ABL gene diagnosis of CML is established. PV, ET and MF constitute the classical group of BCR-ABL negative chronic myeloproliferative disorders. PV is characterized by clonal increase of red blood cell mass, ET by thrombocytosis, MF by bone marrow fibrosis and CML by leucocytosis. Most of these features are not diagnostically specific, and secondary causes increased hematopoiesis and bone marrow fibrosis must be excluded.[3,7,18]

Angiogenesis probably plays a significant role in the development and progression of hematopoietic malignancies. Higher microvascular density and increased serum levels of proangiogenic factors such as VEGF or bFGF have been reported in acute and chronic leukemias, myeloproliferative and myelodysplastic disorders, multiple myeloma and lymphomas.[1,10,14,20,21] VEGF plays an important role in angiogenesis by acting as a potent inducer of vascular permeability as well as serving as specific endothelial cell mitogen.[9] The importance of proangiogenic factors such as VEGF, although clearly established in solid tumors, has not been fully understood in hematopoietic neoplasm. Prominent bone marrow neovascularisation occurs in MPD.[8] Circulating serum levels of proangiogenic cytokines (i. e. VEGF) are increased in MPD but the frequent thrombocytosis that accompanies these disorders could limit the interpretation of these data since platelets and megakaryocytes may be considered a major source of VEGF.[15] Musolino et al. found a significant correlation between the VEGF levels and the platelet count in the ET patients. VEGF level, in this study, was associated with increased risk of thrombotic complications.[12] Among MPD myelofibrosis is the disease with the most pronounced angiogenesis (increased microvessel density).[8] In our study expression of VEGF in bone marrow in MF patients was not significantly higher than in other myeloproliferative disorders. Therefore many other cytokines such as basic fibroblast growth factor, transforming growth factor and platelet-derived growth factor may take part in MF pathogenesis. These cytokines, beside other activities, are strong inducers of angiogenesis.[15]

There are many techniques of the assessment of angiogenesis in hematological malignancies. One of them is bone marrow microvessel density evaluated by staining and quantitating vascular endothelial cell. However falsely elevated MVD may result from immunostaining of platelets, megakaryocytes and some plasma cells with CD31, immature myeloid cells with CD34, erythroid and stroma cells with Ulex europeus or nonspecific staining with polyclonal factor VIII-related antigen.[19]

We demonstrate increased VEGF expression in bone marrow of MPD patients. Statistical analysis showed that VEGF expression in bone marrow of MPD patients was significantly higher than the control. This data indicate that VEGF may play role in the growth of MPD. It may occurs through either a paracrine or an autocrine mechanism. In MPD VEGF may be expressed and secreted by tumor cells as well as bone marrow stromal cells.[6] Increased stromal proliferation in MPD is a reactive process mediated by cytokines released by clonal megakaryocytes. The similar profile of cytokines receptors of endothelial and stromal cells in bone marrow supports the functional role of corresponding ligands in angiogenesis and fibrinogenesis in MPD.[7] Prominent bone marrow angiogenesis provides rationale for use of angiogenesis inhibitors (i. e. Thalidomid) in MPD therapy.[2,19]

Conclusions

These data, although based on relatively small number of patients, show that myeloproliferative disorders are associated with an increased expression of VEGF the bone marrow. Our findings suggest that targeting VEGF may be a potential therapeutic strategy in MPD.

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