INTRODUCTION

The microvessel, with a caliber of 6-9 μm, is the place where the material exchange between blood and tissues is carried out, and changes of its structures and functions are important mechanisms that directly or indirectly result in the occurrence and progression of diseases. For this reason, it is considered as a possible target for disease evaluation reference and treatment. Hematological diseases are originated from abnormal bone marrow hematopoiesis, and abnormal hemopathic marrow microvessel and its clinical significance have been thoroughly and widely discussed in more and more studies. This paper reviewed the advances of these studies and summarized as follows.

MICROVESSEL ANGIOGENESIS AND ITS EFFECT FACTORS

Microvessel angiogenesis-promoting factors

Vascular endothelial growth factor (VEGF) is a central regulatory factor for marrow angiogenesis, which is synergistic with basic fibroblast growth factor (bFGF) in stimulating the migration, proliferation and splitting of vascular endothelial cells, and plays a key role in inducing endotheliocyte differentiation and increasing survival rate. Epoxidase-2 (COX-2), strongly positively expressed by marrow megakaryocytes, can likewise induce the expression of angiogenesis factors including VEGF, transforming growth factor-β (TGF-β) and interleukin (IL)-6, and can inhibit apoptosis and weaken the adhesion ability of cells and extracellular matrix. Hypoxia-inducing factor-1α (HIF-1α) is an up-regulating factor for hypoxia reaction, which can regulate most angiogenesis factors through marrow-derived vascular regulatory factor which is mediated by stroma-derived factor 1-α (SDF1-α). In interleukin family, IL-8, IL-1 and IL-6 can also promote angiogenesis; the application of IL-6 monoclonal antibody can partly inhibit the process of angiogenesis. In addition, TGF (including TGF-α and TGF-β) and TNF-α are also stimulating factors for angiogenesis. Metalloproteases (MMPs) induce the activation and migration of endotheliocytes through degrading basilar membrane glycoproteins and extracellular matrix components. By combining with different ligands and integrin family mediates the migration and adhesion of vascular endothelial cells, which is helpful for the maturation and stability of neovessels, especially MMP2, MMP-9, MT1-MMP.

ABSTRACT

Bone marrow microvessel plays an important role in the onset and progression of hematologic diseases whose genesis is regulated by a variety of regulatory factors. Since abnormal angiogenesis has been found in a number of malignant and non-malignant hematologic diseases, microvessel density can be a valuable prognostic indicator, and also a stratifying factor in some of these diseases. In some cases, inhibiting or stimulating angiogenesis with certain treatments may be very important in improving therapeutic outcomes. However, mechanisms underlying these effects are yet to be further investigated.

Key words: bone marrow microvessel, hematologic diseases, regulatory factor
**Relevant anti microvessel angiogenesis factors**

Pigment epithelium derivation factor (PEDF) is the most potential angiogenesis inhibiting factor, considering its effect of activating endotheliocyte Fas/FasL apoptosis pathway. IL-10, an inflammatory negative regulating factor, can significantly inhibit the release of VEGF by type M1 macrophages. IL-27 can significantly inhibit myeloma angiogenesis, and further inhibit tumor amplification and osteoclasts differentiation, and promote osteoblasts proliferation.\(^2\) In addition, angiostatin, endostatin and thrombospondin-1 are also confirmed to possess the effect of inhibiting angiogenesis, which may be directly related to the inhibition of mitochondrion protein, including the down-regulation of Bel-2.

**CHANGES AND CLINICAL SIGNIFICANCE OF MARROW MICROVESSEL IN COMMON HEMATOLOGIC DISEASES**

**Hematologic malignancies**

**Multiple myeloma**

Multiple myeloma (MM) is the first hematological neoplastic disease that was found to have increased marrow angiogenesis. The formation of neovessels plays an important role in the progression of myeloma, and creates a favorable microenvironment for the growth of neoplastic cells. The tumor plasmyocyte itself can release a large number of angiogenesis promoting factors, including bFGF, hepatocyte growth-promoting factor, etc. and can secrete VEGF with the stimulation of IL-6, forming vicious cycles. A large number of studies have discussed about the clinical significance concerning MM marrow microvessel density (MVD), demonstrating that MVD showed a tendency of gradual increase from monoclonal gammapathy of undetermined significance (MGUS) to primary MM, or to recurrent MM, although there was no significant change in various types of VEGF and bFGF. MVD has been considered as an independent prognosis factor, indicating disease progression or poor prognosis,\(^1\) and it can be recommended as one item of the routine examination.

**Leukemia**

The marrow microvessel count in children with acute lymphoblastic leukemia (ALL) is significantly higher than that in the normal control group. MVD in children with T-cytotype ALL is higher than that in children with forerunner B cytotype; in forerunner B cytotype, MVD is correlated to karyotype. Patients with t (12, 21) are significantly lower than those with hyperdiploid, and the prognosis is relatively better. An association is present between MVD and high leucocytes in ALL.\(^4\) Recent studies have found that MVD in patients with chronic lymphocytic leukemia is significantly increased, and is related to the existence of CD38 expression and abnormal genetics.\(^3\) In myeloid leukemia, high MVD, manifested as short-term total survival period, is an independent prognostic factor. More and more scholars suggest that MVD should be taken as a routine examination. Contrast enhancement magnetic resonance is gradually used in MVD detection in acute myeloid leukemia (AML), which has been confirmed to have close association with pathologic detection.\(^6\)

**Myelodysplastic syndrome**

Myelodysplastic syndrome (MDS) refers to a group of heterogenous hematopoietic stem cell diseases, gradually defined as neoplastic diseases. Recent studies have found that MVD is also of significance in different risk stratifications of MDS. MVD is significantly higher than RA and RARS types in RAEB-t, RAEB and chronic myelogenous leukemia (CMML, now it is classified as MPN), higher in patients with IPSS 3 scores than in patients with IPSS score 0 or 1.\(^7\) However, MVD is unexpectedly significantly decreased when MDS is secondary to leukemia, and it is simultaneously lower than that in initially primary AML, which may be related to the increased release of angiogenesis inhibiting factors in MDS secondary to leukemia. It should be discriminated when applying angiogenesis-inhibiting agents.

**Myeloproliferative neoplasms**

Typical myeloproliferative neoplasms (MPN) includes polycythemia vera (PV), essential thrombocytopenia (ET) and primary myelofibrosis (PMF). JAK2 V617F mutation can be found in 90-95% of PV and 50% of ET and PMF patients. Studies have found that severity degrees of MVD varied with different types of MPN, manifested as PMF>>PV>ET and that myelofibrosis was an independent risk factor,\(^8\) and JAK2 V617F allele mutation loading dose was also positively correlated to MVD,\(^8\) indicating that JAK2 gene mutation may participate in partial links of angiogenesis.

**Others**

The prognosis of malignant non-Hodgkin’s lymphoma has received more concerns clinically. The presence of VEGF expression significantly affected the 2-year survival rate of peripheral T lymphoma (25.4% vs. 83.3%), resulting in disease progression. Apart from promoting angiogenesis in lymphatic tissues, VEGF was found to participate in the processes of marrow infiltration and microvessel angiogenesis of malignant lymphoma. In addition, systemic mastocytosis (SM) is a neoplastic disease with abnormal hyperplasia of mastocytes, affecting multiple tissues and organs. MVD is significantly higher in SM patients accompanied with marrow infiltration than in simple skin
type, and the infiltration degree is positively correlated with marrow MVD.

NON-MALIGNANT HEMATOLOGICAL DISEASES

Aplastic anemia
Aplastic anemia (AA) belongs to hematopoietic function failure with an increased ratio of non-hematopoietic cells. Proliferation and differentiation of hematopoietic cells need the stimulation by autocrine of bone marrow cells and various factors in peripheral circulation. Considerable recent studies have discussed about the marrow microvessel in AA patients, and have noticed that MVD in patients with this disease has obviously decreased, and significantly decreased in severe AA, compared to normal controls; MVD and VEGF are both significantly increased after treatment with immunosuppressants or bone marrow transplantation. However, the therapeutic efficacy of immunosuppressants is more significant, possibly related to cyclosporine directly stimulating hematopoietic cells or non-hematopoietic cells which secrete VEGF.[10]

Others
Thalassemia and sickle cell disease (SCD) are both genetic hemoglobin molecular function disorders with manifestations of chronic hemolysis, anemia, and long-term anaerobic status of organism. The bone marrow of patients with thalassemia is manifested as hypertrophic hyperplasia and strengthened vascular proliferation. Recent studies indicate that VEGF in peripheral blood is significantly higher in such patients than in healthy controls, and hold that spleen excision or premature use of chelating agents are risk factors for further aggravating angiogenesis, and the specific mechanism is yet to be identified. In SCD patients, manifestations of increased peripheral SDF-1 and pre-angiogenesis factor are present, possibly related to the up regulation of angiogenin-2.

TREATMENT SPECIFIC FOR ANGIOGENESIS

Inhibit angiogenesis
Anti-VEGF monoclonal antibody (Bevacizumab)
Bevacizumab is the first anti-VEGF IgG monoclonal antibody which can block the combination of VEGF with its receptors. Studies found that although single administration of Bevacizumab did not have therapeutic reaction to refractory recurrent leukemia, it could significantly inhibit VEGF expression. For this reason, some scholars suggest that it should be taken as a sequential therapy following the chemotherapy of refractory recurrent AML. In a phase II clinical study, Bevacizumab (10 mg/kg) was administered at Day 8 following MA regimen (cytarabine 2 g/m² on Day 1, mitoxantrone 40 mg/m² on Day 4), resulting in the overall response up to 48% and complete remission 33%. In addition, it could also significantly reduce bone marrow MVD, and the serum VEGF in 93% patients was decreased at two hours following the administration of monoclonal antibody.[13]

Immunomodulators (thalidomide, revlimid)
Thalidomide regulates immunization mainly through inhibiting the synthesis of TNF-α and selectively regulating the distribution of T lymphocyte subsets to increase the ratio of T-help cells. Studies about MM treatment with thalidomide showed that thalidomide had a significant effect of anti-angiogenesis, which could significantly down regulate the expressions of VEGF and IL-6 in serum. Revlimid has a stronger effect (2-3 folds) and the side effects are relatively decreased. Recent studies found that these two drugs also had certain advantages in the treatment of PMF and could achieve clinical improvement in 42.9-57.1% patients.[12] Studies confirmed that immunomodulators played a role in MDS (especially patients with 5q syndrome), AML (the overall response rate was up to 25%) and CLL, partially directly related to their effect of anti-angiogenesis to reduce bone marrow MVD.

Tyrosine kinase inhibitors
Tyrosine kinase inhibitors block angiogenesis directly through inhibiting VEGF receptor mediated signal pathway (especially c-kit and Flt3). A total of 42 AML patients with refractory diseases or unable to tolerate high-intensity chemotherapy were included in a SU5416 phase II clinical study. After one-cycle treatment, 7 cases achieved partial remission, and their bone marrow VEGF mRNA expression and bone marrow MVD were significantly decreased.[13] Some cases reported that continuous remission was achieved following the administration of SU5416 to patients with recurrent AML for 12 weeks. As an inhibitor for Raf kinase and VEGF receptor-2, sorafenib was confirmed to play an antivascular role in MM patients, which may be related to its interference on Akt phosphorylation and its inhibiting effect on the expression of downstream mTOR in synergism with rapamycin. Sorafenib was also applied to patients with refractory and recurrent AML in recent studies with complete remission rate of up to 10% (5/50). Flt-3 gene tandem duplication mutation was present in such patients, indicating that this drug was effective for patients with the presence of Flt-3 mutation. Other applications of PTK787/ZK 222584 in MDS and PMF were also reported, and good effects were achieved. However, there was still no established conclusion about direct association of PTK787/ZK 222584 with angiogenesis inhabitation.
Activated T cells and COX-2. Serum VEGF and bone marrow. Some reported that the increased VEGF may be related to the fact that bortezomib in the VMP regimen plays a role in directly inhibiting bone marrow derived endothelial hyperplasy in MM patients, hence blocking the secretion of VEGF and IL-6 and the transcription process of angiogenin and further anti-angiogenesis, and reduce tumor load.

Others
The prognosis is extremely poor when invasive NHL is recurrent, unresponsive to second line chemotherapy and unable to conduct transplantation. In a phase II clinical study, celecoxib (400 mg, b.i.d), a COX-2 selective inhibitor, was concurrently used with oral administration of cyclophosphamide (50 mg, o.d) and methotrexate (2.5 mg) to treat refractory and recurrent large B cell lymphoma, with the results of partial response in 31.7% of 41 patients, non-progression of disease in 48.8% patients and 12-month of disease-free survival. Thus, it can be taken as an alternative regimen in later stage. In another study, large doses of celecoxib (400 mg, b.i.d) and thalidomide (800 mg, o.d) were concurrently administered to treat patients with refractory and recurrent MM. The overall response rate could be up to 42%, and the patients who received a total amount of celecoxib over 40 g during the first 8 weeks had better response rate (62% vs. 30%). However, it should be selected carefully in view of the high toxicity of large-dose celecoxib. In addition, some studies found that the therapeutic effect of arsenious acid in treating acute promyelocytic leukemia was also related to its effect of anti-microvessel angiogenesis in bone marrow.

Regulate and promote angiogenesis
Immunosuppressants (Cyclosporine, CsA)
The effective rate of single use of cyclosporine A (CsA) as first-line drug for AA is about 40%. Although the impact of CsA on serum VEGF in patients was yet not concluded, some reported that the increased VEGF may be related to the up-regulated expression of angiogenin II. CsA can inhibit angiogenesis by inhibiting the nuclear factor of activated T cells and COX-2. Serum VEGF and bone marrow MVD in AA patients were significantly increased following with treatment with immunosuppressants (ATG or combined with CsA). The role of CsA in promoting angiogenesis could not be excluded, but the specific mechanism is yet to be further identified.

Granulocyte colony-stimulating factor
Granulocyte colony-stimulating factor (G-CSF) has the effects of stimulating the proliferation and differentiation of granulocyte blast cells and strengthening the function of mature granulocyte. G-CSF can promote the activation and recruitment of CD11b+Gr1+ myeloid cells. CD11b+Gr1+ myeloid cells have been confirmed to have the effects of promoting angiogenesis and inhibiting T cell function, which can facilitate tumor mediated immune tolerance and reduce the sensitivity of diseases to anti-VEGF treatment, and therefore promote angiogenesis. It, however, should be used with caution in hematologic malignancies. Significant increase of serum VEGF can be also found in normal donors of hematopoietic stem cell transplantation following the mobilization of G-CSF, and simultaneously, Tie-2 (a kind of negative regulation factor of angiogenesis) expression is decreased. Currently, G-CSF has been used as one of conventional medicines for AA patients accompanied with agranulocytosis in clinical practice; considering the above-mentioned effects, there seems to be more important significance of G-CSF application, which is worth to be further discussed.

REFERENCES


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