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Original article

Increased angiogenesis in primary myelofibrosis: latent transforming growth factor-β as a possible angiogenic factor

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ABSTRACT

Objective: The aim of this work was to demonstrate a possible relationship between anti-latency-associated peptide human latent transforming growth factor beta 1 (latent TGF-β1) expression in megakaryocytes and microvascular density in bone marrow biopsies from patients with essential thrombocythemia and primary myelofibrosis.

Methods: Microvascular density was evaluated by immunohistochemical analysis and the expression of latent TGF-β1 in samples (100 megakaryocytes per bone marrow sample) from 18 essential thrombocythemia and 38 primary myelofibrosis (19 prefibrotic and 19 fibrotic) patients. Six bone marrow donor biopsies were used as controls. Fibrosis in the bone marrow biopsies was evaluated according to the European Consensus.

Results: The average fibrosis grade differed between essential thrombocythemia and primary myelofibrosis groups when compared to the control group. Latent TGF-β1 expression differed significantly between the fibrotic primary myelofibrosis (PMF) group and the control group (p-value < 0.01). A high degree of neo-angiogenesis (demonstrated by analysis of CD34 expression) was detected in patients with myelofibrosis. There were correlations between latent TGF-β1 expression and microvascular density ($r = 0.45$; p-value < 0.0009) and between degree of microvascular density and fibrosis grade ($r = 0.80$; p-value < 0.0001). Remarkable differences for neo-angiogenesis were not observed between patients with essential thrombocythemia and controls.

Conclusion: Angiogenesis participates in the pathogenesis of primary myelofibrosis, in both the prefibrotic and fibrotic stages, while latent TGF-β is differentially expressed only in the prefibrotic stage.

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Introduction

Chronic myeloproliferative neoplasms (MPNs) were categorized for the first time in 1951 by William Dameshek, who noticed phenotypic similarities and characteristics common to chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).[1]

PMF is a clonal stem cell disorder that is characterized by bone marrow myeloproliferation associated with a stromal reaction, including fibrosis, osteosclerosis and angiogenesis.[2]

Of the different types of MPN, PMF is associated with the worst prognosis; the primary causes of death are thrombohemorrhagic events, bone marrow failure and leukemic transformation. The median survival ranges from two to over 15 years and varies according to the stage: prefibrotic or fibrotic.[3]

According to the World Health Organization (WHO), the major differential criterion between ET and PMF is fibrosis, which is either minimal or absent in ET patients and present to various degrees in PMF patients.[4]

The classification of bone marrow fibrosis by the European Consensus involves an assessment of the stroma and bone characteristics on a scale that ranges from 0 to 3. Grade zero corresponds to few linear reticulin, Grade 1 to a few fiber intersections in focal areas around vessels, Grade 2 to a diffuse increase in reticulin, many intersections and some foci of collagen or osteosclerosis and Grade 3 to conspicuous and diffuse increases in reticulin fibers, many fiber intersections, collagenization and osteosclerosis.[5]

Special stains are necessary to identify and classify bone marrow fibrils. Mallory and Masson stains are used for collagen I while silver (Gomori) is used for reticulin fibers.[6–9]

Another histological feature observed in PMF is increased angiogenesis. Angiogenesis is the generation of new blood vessels from the pre-existing vasculature.[2]

In PMF, angiogenesis seems to be associated with stromal fibrosis triggered by the release of several cytokines produced by megakaryocytes, including transforming growth factor-beta (TGF-β).[10]

TGF-β is a multifunctional cytokine with three isoforms: TGF-β1, TGF-β2 and TGF-β3, with TGF-β1 being the most abundant.[11] TFG-β is synthesized as an inactive form (latent TGF-β) by various cells (epithelial, endothelial, hematopoietic, neuronal cells and connective tissue).[12] Platelets and megakaryocytes are an abundant source of TGF-β1 in humans.[11]

For TGF-β to signal through its receptors, it must be converted into the active state; once activated, this cytokine acts on target cells, thereby producing different effects depending on the cell type and on the degree of differentiation of the cell.[13–15]

Of the several functions of TGF-β, the stimulation of extracellular matrix production and angiogenesis should be highlighted.

Fibroblasts display TGF-β receptors, which are stimulated to produce structural proteins, primarily collagen.[12,14] TGF-β also inhibits the synthesis of extracellular matrix proteases and is therefore involved in the pathogenesis of diseases associated with the over deposition of connective tissue.[10]

Endothelial cells (ECs) signal via two different type I receptors, also known as activin receptor-like kinases (ALK), with opposite effects. While the activation of ALK5 by TGF-β results in the inhibition of migration and proliferation, TGF-β-induced ALK1 activation results in the increased migration and proliferation of ECs. Both ALK1 and ALK5 are functional TGF-β type I receptors in ECs. The ratio of TGF-β signals via ALK1 versus ALK5 determines whether TGF-β exerts pro- or anti-angiogenic effects.[16]

TGF-β is also an indirect angiogenic factor promoting angiogenesis via the downstream induction of other cytokines, such as vascular endothelial growth factor (VEGF).[17] VEGF is a potent, direct-acting regulator of angiogenesis that is detectable in various types of malignancies.[18,19]

Angiogenesis is essential in the pathogenesis of PMF, while it is less pronounced in PV and ET.[18] The increase in microvascular density (MVD) in PMF correlates with cellularity and megakaryocytes clusters. Clonal proliferation of megakaryocytes in PMF is accompanied by an abnormal release of cytokines, including angiogenic factors, resulting in an excessive stromal reaction and an increase in bone marrow vascularity.[20]

Because megakaryocytes and platelets are a major source of TGF-β1, which is able to enhance collagen synthesis and vessel proliferation, it is thought to be one of the key cytokines involved in the development of primary myelofibrosis.[9]

The aim of this work was to demonstrate a possible relationship between latent TGF-β1 expression in megakaryocytes and MVD as assessed by immunohistochemical analysis of bone marrow biopsies from ET and PMF patients. As fibrosis is used to differentiate between ET and PMF,[3] possible associations between MVD, fibrosis and latent TGF-β1 expression were evaluated.

Methods

The study was conducted using bone marrow biopsy samples collected from 56 patients with either ET or PMF. Samples from six bone marrow donors were used as normal controls. Patients previously evaluated for the relationship between TGF-β1 and bone marrow fibrosis[21] were the participants in this study. All patients were examined in the Hematology and Pathology Departments of Hospital São Paulo, Universidade Federal de São Paulo between 1992 and 2010. Participants were included in the study after informed consent was obtained according to the guidelines of the Institutional Ethics Committee.

The clinical, cytological and histological aspects of all cases were reviewed and classified according to WHO criteria[3] before being selected for the study. The cases were selected based on the diagnosis by biopsy and before the patient had started any type of therapy.

Subjects were divided into four groups: 18 cases of ET; 38 cases of PMF, which included 19 cases at each phase (prefibrotic PMF and fibrotic PMF); and six controls. The controls were six bone marrow biopsies from patients who were disease-free and were on the bone marrow donor registry. Two cases of reactive thrombocytosis were used as controls for the immunohistochemistry test.