Correlation of Mast Cell Density With Angiogenic Cytokines in Patients With Active Multiple Myeloma

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ABSTRACT

Purpose: The aim of the study is to estimate whether bone marrow mast cell density (MCD) in multiple myeloma (MM) correlates with circulating levels of various angiogenic factors.

Methods: In 70 patients with newly diagnosed active MM, we measured MCD using immunohistochemical stain for tryptase and serum levels of matrix metalloproteinase 9 (MMP-9), angiopoietin 2 (ANGIOP-2), and angiogenin (ANG) with ELISA.

Findings: Levels of MCD, ANGIOP-2, and ANG were significantly higher in MM patients compared with the control group. The MMP-9 level was higher in MM patients compared with the control group but without statistical significance. All values were increasing in parallel with clinical stages. Furthermore, MCD correlated positively with MMP-9, ANGIOP-2, and ANG.

Implications: MCs participate in the angiogenic processes of MM, with complex implicated mechanisms. This interplay between MCs and the other participants favors angiogenesis and MM growth. (*Clin Ther*. 2016;38:297–301)

Key words: angiogenesis, cytokines, mast cells, multiple myeloma.

INTRODUCTION

The genesis of new blood vessels has been an important mechanism in multiple myeloma (MM) progression. It is a complex procedure with a plethora of participants. The transition from an avascular to a vascular phase of tumor growth is caused by an imbalance of proangiogenetic and antiangiogenetic factors in the tumor microenvironment and is considered as presuppositionfor tumor growth and expansion. Among participants, inflammatory cells modify the tumor microenvironment, favoring all aspects of tumor progression.[1,2]

Tumor cells promote vessel formation through the expression of angiogenic molecules. In this framework, several cells and molecules with multifaceted properties participate in this procedure. There are angiogenic activators and inhibitors, as well host cells, whose interplay favors angiogenic switch and vascular expansion. Among proangiogenic molecules, with direct mitogenic action on endothelial cells, vascular endothelial growth factor (VEGF), basic fibroblast growth factor, hepatocyte growth factor, and angiogenin (ANG) drive tumor-related angiogenesis.[3,4] Furthermore, metalloproteinases (MMPs) and angiopoietins (ANGIOPs) regulate the process of angiogenesis because they play an important role in tumor-induced base membrane matrix remodeling, endothelial cell migration, stabilization, and tube formation.[5,6] On the other hand, among host cells, inflammatory cells, such as mast cells (MCs), are recruited and activated by tumor cells via paracrine mechanisms, acting synergistically with tumor cells by secreting a plethora of molecules participating in these processes.[3,4,7,8] MCs are among the major cells that participate in a plethora of inflammatory and malignant diseases. Mastocytosis is a primary MC disease where somatic mutations of c-kit are detected.[9]

For the case of MM, BM angiogenesis and MC counts have been highly correlated in patients with

Accepted for publication November 30, 2015.
http://dx.doi.org/10.1016/j.clinthera.2015.11.022
0149-2918/$ - see front matter

monoclonal gammopathy of undetermined significance, nonactive MM, and active MM.[10] By these means, there has been an extensive study regarding the participation of MCs in the MM angiogenic process. The aim of the present study is to estimate MC density (MCD) in the BM of patients with active MM and to correlate it with angiogenic factors, such as ANG, ANGIOP-2, and MMP-9.

MATERIAL AND METHODS

Patients

Seventy patients with newly diagnosed active MM (36 men and 34 women; median age, 64 years; range, 46-87 years) participated in the study. Patients with renal or lever impairment, history of active or previous other malignant tumors or other BM diseases, uncontrolled infectious diseases, use of immunomodulatory drugs, or incapability to consent were excluded from the study. According to the International Staging System (ISS), 15 patients were in stage I, 31 in stage II, and 24 in stage III of the disease. The types of monoclonal proteins were as follows: IgG in 37, IgA in 23, and light chain disease in 10. None of the patients had received any myeloma-related treatment before examination. Eighteen age- and sex-matched healthy individuals were used as controls. The study was performed in accordance with the ethical standards outlined in the Declaration of Helsinki. Informed consent for the study was obtained from all participants before their inclusion in the study.

Methods

Circulating levels of the above molecules were measured on serum samples, which were stored at −70°C, after collection, and assayed at the end of the study, to avoid interassay variability. Serum levels of MMP-9, ANGIOP-2, and ANG were measured by the solid-phase sandwich ELISA, using monoclonal anti–MMP-9, ANGIOP-2, and ANG antibodies (Quantikine; R&D Systems, Minneapolis, Minnesota), according to manufacturers' instructions.

BM biopsy was performed in all patients as a routine diagnostic examination. BM MCD was estimated using monoclonal antibody to tryptase in the MCs (Ab-2, clone AA1; ThermoFisher Scientific, Waltham, Massachusetts), as has been previously described.[11] In each specimen, BM MCs were quantified in 3 areas (of neoplastic infiltration for the patients) containing the highest number of MCs (hot spots) in 10 high-power fields (×400) for each hot spot separately. MCD was the mean percentage of positive staining. MCs calculated in the 3 hot spots for each specimen and expressed as MCs per 0.0625 mm^2.

Statistical Analysis

The descriptive measures of central tendency and dispersion and the continuous variables are presented as mean (SD). Differences in continuous variables between the 2 groups were examined with the independent sample t test and the nonparametric Mann-Whitney test. One-way ANOVA and the nonparametric Kruskal-Wallis test were applied for comparisons of >2 groups. Correlations between variables were examined using Spearman's ρ. SPSS statistical software, version 21.0 (SPSS Inc, Chicago, Illinois), was used for statistical analysis. A level of .05 was set as significant.

RESULTS

MCs were observed in areas both within paratrabecular regions and diffusely in cellular myeloma marrow in isolated forms. Their morphologic features did not resemble spindle-shaped systemic mastocytosis. Mean (SD) serum values of each analyzed parameters, as well BM MCD, in both patients and the healthy population are given in Table I. Table I reveals that all of them except MMP-9 were significantly higher in MM patients ($P < 0.001$ for all cases). Table II gives their values in ISS stages. All values were increasing in parallel with disease stage ($P < 0.001$ for all cases). Positive correlations between BM MCD with angiogenic factors MMP-9 ($r = 0.412$), ANGIOP-2 ($r = 0.682$), and ANG ($r = 0.694$) ($P < 0.0001$ for all cases) were noted (Figure 1 for ANGIOP-2 and MMP-9).

DISCUSSION

The cellular and molecular basis of disease progression in MM is favored by mechanisms that involve the BM microenvironment. Angiogenesis represents a major feature and is supported by a plethora of cellular and extracellular elements of BM.[1–4,10] It is uncontrolled, unlimited in time, and essential for tumor growth, invasion, and metastasis during the transition from the avascular to the vascular phase.[1] BM MCD has been already correlated with microvascular density in patients with inactive and active MM, being increased in parallel with disease