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Macrophages in multiple myeloma

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ABSTRACT

Tumor associated macrophages (TAMs) are a rich source of pro-angiogenic cytokines and growth factors, and a relationship between the TAMs content, the rate of tumor growth and the extent of vascularization has been shown in several tumors. In this article, we have summarized the literature and our data concerning the involvement of TAMs in angiogenesis occurring in multiple myeloma. Finally, therapeutic aspects concerning the potential role of molecules which inhibit macrophage recruitment in the tumor side are also discussed.

1. Tumor-associated macrophages

Firstly in 1863, Rudolf Virchow described the infiltration of leukocytes into malignant tissues and suggested a link between cancer and chronic inflammation [1]. Macrophages were initially described by Elie Metchnikoff who won the Nobel Prize in 1905 for their identification and the phagocytosis theory.

Tumor associated macrophages (TAMs) derive from circulating monocytes and are recruited at the tumor site by several chemo attractants, including colony stimulating factor-1 (CSF-1), the CC chemokines (CCL-2, CCL-3, CCL-4, CCL-5 and CCL-8), and vascular endothelial cell growth factor (VEGF) secreted by both tumor and stromal elements [2]. Overexpression of CSF-1 enhances the TAMs recruitment and accelerates tumor progression in the mammary epithelium of MMTV-PyMT mice [3]. After inhibition of the expression of CSF-1 or its receptor with short interfering RNA (siRNA) in a mice model, macrophages infiltration and vascularity are decreased compared to controls [4]. Macrophages co-cultured with tumor cells secrete several factors which facilitate tumor cell proliferation [5] and extensive TAMs infiltration correlates with poor prognosis in breast, prostate, cervix, and bladder cancer [6].

The term "macrophage activation" is used to describe the process of recruitment of these cells, which exhibit many differences from resident tissue macrophages. Activated macrophages are categorized into two types: M1 (classically activated) and M2 (alternatively activated) [2]. M1 macrophages are induced by interferon gamma (IFN-γ), microbial stimuli, cytokines [tumor necrosis factor alpha (TNF-α) and interleukin-12 (IL-12)], reactive nitrogen and oxygen intermediates (RNI, ROI) [7]. M1 secrete high levels of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, IL-12 and IL-23, and exhibit high levels of superoxide anions, oxygen and nitrogen radicals [7]. The M2 form of macrophage activation is induced by IL-4 and IL-13 [8].

The molecular mechanisms that promote M1 or M2 subsets within the tumor microenvironment are not completely understood. TAMs are mainly constituted by the M2 elements, which promote angiogenesis, repairing and remodeling and suppressing adaptive immunity [9]. In regressing tumors, TAMs resemble the M1 type, which exhibits anti-tumor activity, while in advanced tumors, TAMs are oriented toward the M2 type that favors tumor growth and progression [10]. In the tumor mass, TAMs can express pro-tumoral or anti-tumoral activities in function of their activation state.

2. Macrophages and tumor angiogenesis

TAMs are a rich source of pro-angiogenic cytokines and growth factors, such as VEGF, TNF-α, IL-8 and fibroblast growth factor-2 (FGF-2). In addition, TAMs express a broad array of angiogenesis-modulating enzymes, including matrix metalloproteinases (MMP)-2, -7, -9, -12, and cycloxygenase-2 (COX-2) [11].

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TAMs accumulate in hypoxia regions of tumors and their adaption to this condition is achieved by the increased expression of hypoxia-inducible and pro-angiogenic genes, including VEGF, FGF-2 and CXCL-8, as well as glycolytic enzymes [12].

A relationship between the TAMs counts, the rate of tumor growth and the density of vascularization has been demonstrated in several tumors, including breast carcinoma malignant uveal melanoma, glioma, squamous-cell carcinoma of the esophagus, bladder carcinoma and prostate carcinoma [13].

3. Angiogenesis in multiple myeloma

Angiogenesis is a constant hallmark of multiple myeloma (MM) progression and has prognostic potential [14]. It is induced by plasma cells *via* angiogenic factors with the transition from monoclonal gammopathies of undetermined significance (MGUS) to MM, and probably with loss of angiostatic activity on the part of MGUS. The pathophysiology of MM-associated angiogenesis is complex and involves both direct production of angiogenic cytokines by plasma cells and their induction within the microenvironment cells.

Bone marrow stroma cells (BMSCs) increase the concentration of angiogenic factors and matrix degrading enzymes in the bone marrow microenvironment by direct secretion or following stimulation by myeloma cells or by endothelial cells through paracrine interactions [15,16]. BMSCs, osteoclasts, osteoblasts and endothelial cells secrete several factors, including VEGF, FGF-2, TNF-α, IL-6, B-cell activating factor, stromal cell-derived factor 1α (SDF1-α, also known as CXCL-12), and various Notch family members, which are further up-regulated by tumor cell adhesion to extracellular matrix proteins and/or BMSCs. Finally, circulating endothelial cells and endothelial precursor cells (EPCs) contribute to the neovascularization, and the presence of EPCs suggests that vasculogenesis may also contribute to the full MM vascular tree [14].

4. Multiple myeloma-associated macrophages

Multiple myeloma is characterized by the accumulation of monoclonal plasma cells in the bone marrow, suggesting the importance of the bone marrow microenvironment in supporting the MM cell growth and survival. The MM microenvironment is formed by clonal plasma cells, extracellular matrix proteins, and BMSCs, and reciprocal positive and negative interactions between plasma cells and BMSCs are mediated by any array of cytokines, receptors and adhesion molecules. Interactions between these cell components determine the proliferation, migration and survival of plasma cells as well as their acquisition of drug resistance and the development of a progressive behavior [15].

In MM, macrophages are an important component of the stromal cells, and in active MM (at diagnosis, at relapse, on refractory phase), plasma cells secrete VEGF and FGF-2 that induce inflammatory cells to secrete their own VEGF, FGF-2, and hepatocyte growth factor (HGF), able to recruit and activate the MM-associated macrophages; these pathways are irrelevant in nonactive MM (at complete/partial remission) [17]. Moreover, bone marrow macrophages protect MM cells from spontaneous and melphalan-induced apoptosis [18].

Chen et al. [19] demonstrated that monocytes induce vascular endothelial cell gene expression and develop tube-like structures when they are cultured with the bone marrow from patients with MM that express an angiogenic factor, namely pleiotrophin; the tubulogenesis was specifically blocked by anti-pleiotrophin antibodies. Moreover, when co-injected with human MM cells into SCID mice, green fluorescent protein-marked human monocytes were found to be incorporated into tumor blood vessels and expressed human vascular endothelial cell protein markers and genes that were blocked by anti-pleiotrophin antibodies.

In patients with active MM, fluorescence activated cell sorting (FACS) analysis on isolated bone marrow mononuclear cells revealed higher percentages of CD68+ macrophages than in patients with nonactive disease or those with MGUS [20]. Bone marrow macrophages in patients with active MM were functionally, phenotypically, and morphologically different from those of patients with nonactive disease and MGUS. Indeed, macrophages of these patients were similar to paired endothelial cells and contributed to angiogenesis through a vasculogenic mimicry, in parallel with progression of plasma cell tumors [20]. When macrophages from active MM patients were exposed *in vitro* to VEGF and FGF-2, they transformed into cells functionally and phenotypically similar to MM endothelial cells because they were able to generate capillary-like networks [20]. Moreover, bone marrow biopsies of active MM patients examined on confocal laser microscopy displayed macrophages with both endothelial cell-like (*i.e.*, CD68/FVIII-RA double positive) and apparently typical (*i.e.*, CD68 positive/FVIII-RA negative) features located in the microvessel wall. Figures of this type were rare in nonactive MM patients and absent in MGUS (Fig. 1) [20].

The MM macrophages give increased levels of VEGF-A and VEGF-C mRNAs [21]. It has been assumed that mesenchymal stromal cells (MSCs) are the major source of the VEGF isoforms [22],

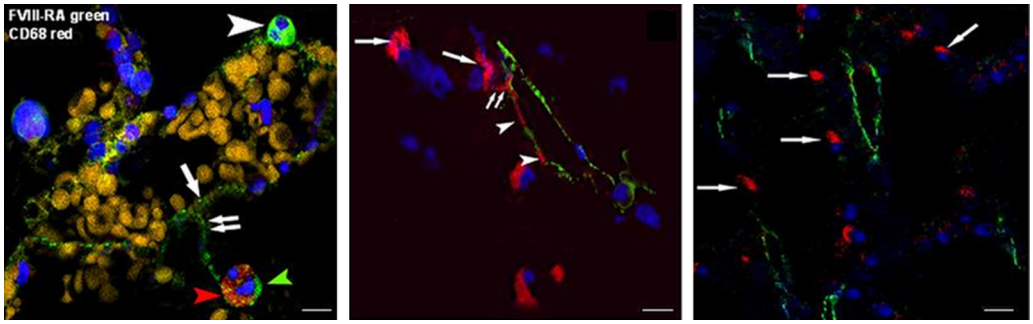

Fig. 1. On the left, microvessel lined by flattened FVIII-RA positive multiple myeloma endothelial cells (arrow), an FVIII-RA positive macrophage (arrowhead) showing protrusions connected to multiple myeloma endothelial cells, and another macrophage containing double-labeled CD68 (red arrowhead) and FVIII-RA (green arrowhead) granules in the cytoplasm and connected to multiple myeloma endothelial cells by an FVIII-RA positive cytoplasmic protrusion (double arrow). Erythrocytes (orange) are well recognizable inside the lumen. Another microvessel formed by FVIII-RA positive (green) multiple myeloma endothelial cells and CD68 positive (red, arrowheads) tracts that belong to the cytoplasmic protrusions (double arrow) of macrophages, some of which are arrowed (middle panel). On the right, the MGUS microvessels are formed by only FVIII-RA positive endothelial cells: macrophages (arrows) are randomly scattered in the tissue and independent of them. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Source: Reproduced from Ref. [20].