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# Biological implications of macrophage infiltration in human tumor angiogenesis

**Abstract** Tumor angiogenesis is believed to be induced by increased production of angiogenic factors and decreased production of angiogenic inhibitors by cancer cells, vascular endothelial cells, and other stromal cell types. Most solid tumor cells are surrounded by stroma comprising interstitial connective tissue, blood vessels, fibroblastic cells, etc. Interaction between the stroma and malignant cells appears to have a critical role in the development of tumor neovasculature. We focused on macrophages, which demonstrate wide heterogeneity in biological function and have an essential role in tumor angiogenesis. Macrophages are terminally differentiated cells which produce a number of potent angiogenic cytokines and growth factors such as vascular endothelial growth factor, tumor necrosis factor-alpha, interleukin-8, and basic fibroblast growth factor. They also modulate events in the extracellular matrix through the secretion of extracellular matrix-degrading enzymes and -modulating enzymes. Thus macrophages could influence various stages of angiogenesis either positively or negatively. We found a close correlation between increased macrophage index, malignancy, and high vascular grade in malignant melanoma, and present a model for the possible involvement of activated macrophages in neovascularization in human malignant melanoma.

**Key words** Macrophages · Tumor necrosis factor alpha · Vascular endothelial growth factor · Interleukin-8

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## Is macrophage infiltration associated with angiogenesis?

Tumor angiogenesis is believed to be induced due to increased production of angiogenic factors and decreased production of angiogenic inhibitors by cancer cells, vascular endothelial cells, and other stromal cell types [7]. Almost all solid tumor cells are usually surrounded by stroma, which comprises interstitial connective tissues, basal lamina, blood vessels, blood cells, and fibroblastic cells. Interaction between the stroma and malignant cells is believed to have a critical role in the development of neovasculature in tumors [3]. Of these stroma constituents, macrophages, which show widespread heterogeneity in biological function, also have an essential role in tumor angiogenesis.

Macrophages are the major terminally differentiated cell type of the mononuclear phagocyte system, and are also one of the key angiogenic effector cells, producing a number of growth stimulators and inhibitors. A number of angiogenic cytokines are known to be produced by macrophages, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-alpha (TGF-α), tumor necrosis factor alpha (TNF-α), platelet-derived endothelial cell growth factor/thymidine phosphorylase (PDECGF/TP), and interleukin-8 (IL-8) [8, 11, 13]. Macrophages are able to modulate events in the extracellular matrix either via the direct secretion of degradative enzymes or via extracellular matrix-modulating cytokines (Table 1). Macrophages are thus expected to influence every stage of angiogenesis.

Several studies have shown a relationship between angiogenesis and prognosis in patients with various tumor types [5, 17]. Leek et al. [12] have further demonstrated that focally increased macrophage numbers are closely related to vascularization and prognosis in patients with breast cancer. These studies suggest a close correlation between monocytes/macrophages and angiogenesis in human tumors. We have investigated whether macrophage infiltration is associated with vascularization in human melanomas [unpublished results].

**Table 1** Extracellular matrix (ECM)-modulating enzymes and cytokines produced by macrophages (*EGF* epidermal growth factor, *HGF/SF* hepatocyte growth factor/scatter factor, *IGF-1* insulin-like growth factor-1, *tPA* tissue-type plasminogen activator, *PAI-1* plasminogen activator inhibitor-1, *PDGF* platelet-derived growth factor, *TAM* tumor-associated macrophage)

| Angiogenic cytokines produced by TAMs | Macrophage-derived ECM modulators | |
|---|---|---|
| | Enzymes and inhibitors | Cytokines |
| VEGF | Collagenase | bFGF |
| bFGF | tPA | TGF-β |
| EGF | uPA | TNF-α |
| IL-8 | PAI-1 | Angiotropin |
| TNF-α | | PDGF |
| IL-1 | | IL-6 |
| TP | | |
| HGF/SF | | |
| IGF-1 | | |

In this study, resected specimens from 37 melanoma patients were analyzed. Histochemical analysis showed that in melanoma in situ only a few microvessels were present and almost no macrophage infiltration was observed; in malignant melanoma, microvessels together with macrophages were heavily stained. Thus we identified a close correlation between infiltrating macrophages and microvessel density. We also observed that mean macrophage counts and mean microvessel counts in malignant melanoma were significantly higher than those in melanoma in situ. Therefore we demonstrated a close correlation between tumor-associated macrophage infiltration and angiogenesis and tumor malignancy in melanoma. Below we present a model for the mechanism of the possible involvement of activated macrophages in neovascularization in human tumors.

## Possible positive effect of TNF-α on macrophage infiltration in angiogenesis in melanoma

TNF-α is a cytokine synthesized mainly by cells of the monocyte-macrophage lineage [2]. Other sources are mast cells, T lymphocytes, natural killer cells, neutrophils, and some tumor cells [9]. Many effects of TNF-α on endothelial cells have been reported to date, although some of these are controversial due to the different concentrations of TNF-α used and the exposure time, as well as to the different endothelial cells used [4].

Pleiotropic factors such as TNF-α and IL-1, representative macrophage-derived cytokines, and plasminogen activators are believed to be involved in macrophage-associated angiogenesis. In particular, TNF-α upregulates expression of the potent angiogenic factors IL-8, bFGF, and VEGF through activation of transcription factors in vascular endothelial cells and other cell types including melanoma cell lines [15]. TNF-α-induced tubular morphogenesis in vascular endothelial cells is inhibited by the administration of anti-IL-8, anti-VEGF, and anti-bFGF antibodies, and coadministration of all three antibodies almost completely abrogates tube formation [19]. Moreover, treatment with Sp1, NF-κB, and c-Jun antisense oligonucleotides inhibits TNF-α-induced tubular morphogenesis by microvascular endothelial cells [19]. Administration of a NF-κB antisense oligonucleotide almost completely inhibits TNF-α-induced IL-8 production and partially abrogates TNF-α-induced VEGF production [19]; Sp1 antisense oligonucleotides partially inhibit TNF-α-induced production of VEGF, and a c-Jun antisense oligonucleotide partially inhibits TNF-α-induced bFGF production [19]. Finally, administration of anti-IL-8 or anti-VEGF antibodies also blocks TNF-α-induced neovascularization in rabbit corneas in vivo [19]. Thus angiogenesis by TNF-α appears to be modulated by various angiogenic factors, both in vitro and in vivo, and this pathway is controlled through paracrine and/or autocrine mechanisms (Fig. 1). Moreover, oxygen stress induced by activating macrophages might also induce angiogenesis [16].

## Are mast cells angiogenic?

The involvement of other leukocytes such as mast cells, basophils, and T and B lymphocytes in addition to macrophages is not well understood. To determine whether mast cells might be associated with angiogenic states in angiogenic diseases, we examined whether IL-4 has any role in angiogenic activity.

IL-4 is a 20-kDa glycoprotein secreted by activated T lymphocytes, basophils, and mast cells. It is a potent lymphocyte growth factor and a major regulator of IgE production and has a direct effect not only on leukocytes but also on vascular endothelial cells [14]. IL-4 enhances expression of vascular cellular adhesion molecule-1 in vascular endothelial cells [1], resulting in increased adhesiveness to lymphocytes and eosinophils. IL-4 also induces urokinase-type plasminogen activator expres-

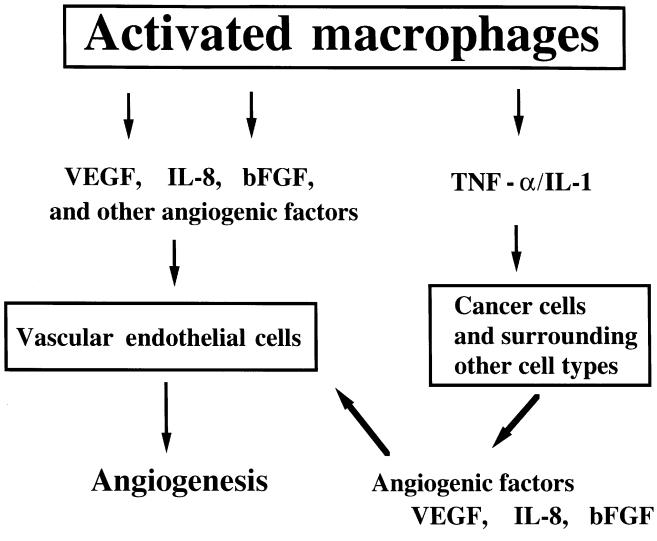


**Fig. 1** Proposed model for the control of angiogenesis

sion [18] and morphological changes and proliferation of human vascular endothelial cells [10].

Treatment of human or bovine vascular endothelial cells with IL-4 resulted in the formation of tube-like structures in collagen gel, and also angiogenesis in the cornea of rats, at rates comparable to the effect of bFGF [6]. Thus IL-4 might be involved in angiogenesis under pathological conditions which are associated with the appearance of mast cells or T cells.

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