

# Modulation of angiogenesis by tumor associated macrophages in the tumor microenvironment

## Abstract

Macrophages are important cells in wound healing, providing aids for tissue cell growth, tissue matrix remodeling and angiogenesis. Solid tumor comprises not only tumor cells, but also tissue matrix and many stromal cells such as fibroblasts and macrophages. Tumor associated macrophages (TAMs) promote cancer metastasis through several mechanisms including tumor angiogenesis, tumor growth and cell migration and invasion. Complex paracrine signaling networks exist between TAMs and cancer cells to activate each other. The colony stimulating factor 1/epidermal growth factor paracrine loop is well known of breast cancer cell invasion. TAMs derived protease, such as matrix metalloproteinases, urokinase-type plasminogen activator, and cathepsin B can promote cancer cell metastasis. By interacting with cancer cells, TAMs can be induced to express more cytokines and tissue matrix-degrading enzymes, such as matrix metalloproteinases, plasminogen activators and cathepsin B, that are either direct angiogenic factors or tissue matrix modulators responsible for promoting tumor angiogenesis. With evidence indicating TAMs tumor-promoting roles, many investigations have been undertaken to study the potential of using TAMs as the therapeutic target while some conflicting investigations have demonstrated their role in anti-tumor activity. More detailed mechanisms regarding the interaction between TAMs and tumor still need to be explored, and a thorough understanding of this interaction will provide some helpful conceptual suggestions for future cancer therapy. This mini review will summarize information about the role of TAMs on modulating angiogenesis in the inflammatory micro-environment of solid tumors and discuss the potential targets for future therapeutic approaches.

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**Abbreviations:** TAM, tumor associated macrophage; MCP, monocyte chemotactic protein; GMCSF, granulocyte macrophage colony stimulating factor; HIF, hypoxia-inducible factor; PyMT, polyoma virus middle t oncoprotein; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; TGF- $\alpha$ , transforming growth factor- $\alpha$

## Introduction

The inflammatory infiltrate in most cancers contains several cell types, including lymphoid cells, granulocytes, mast cells, dendritic cells, natural killer cells and macrophages, which are the most highly represented cell type. Macrophages are associated with the development and progression of a variety of tumors, including breast, prostate, glioma, lymphoma, bladder, lung, cervical and melanoma. In breast cancer, macrophages have been reported to comprise up to 50% of the tumor cell mass.<sup>1</sup> An important inflammatory cell type commonly found in the stroma of tumor is the tumor-associated macrophage (TAM2).<sup>2</sup> In normal immune system, macrophages play an important role in wound healing. They provide aids for tissue matrix remodeling, cell growth and angiogenesis.<sup>3,4</sup> Similar to macrophages in wound healing, TAMs play an important role in tumor progression by exerting their multiple functions in tumor cell proliferation, tumor cell invasion, and tumor angiogenesis.<sup>5</sup> When a solid tumor grows beyond 2mm in diameter, simple diffusion of oxygen and nutrients to metabolizing tissues becomes insufficient.

One of the common features of solid tumors is hypoxia is a common feature of solid tumors. Some of these factors, such as monocyte chemotactic protein-1 (MCP-1) and granulocyte-macrophage colony-stimulating factor (GMCSF), are potent chemokines chemotactic toward the monocytes in nearby blood vessels.<sup>6</sup> Monocytes are thus continually recruited into tumors, differentiated into TAMs, and then

accumulate in the hypoxic areas. These cells have been considered to have dual effects on the tumor, i.e. anti-tumor and pro-tumor effects. They exhibit tumoricidal activity toward tumor cells at initial contact, but when they reside in the areas of hypoxia, macrophages respond to hypoxia by up-regulating transcriptional factors like hypoxia-inducible factors 1 and 2 (HIFs 1 and 2), which in turn activate a broad array of genes for promoting tumor cell invasion and tumor angiogenesis.<sup>7</sup> Eventually, TAMs function as the provider of tumorigenic factors as well as regulators for malignant development. Accumulating evidence has suggested that high TAM infiltration is advantageous to tumor spreading in certain cancers, most notably via enhancement of tumor angiogenesis and tumor cell migration and invasion,<sup>8</sup> which could explain why high levels of TAMs correlate with poor prognosis in these cancers.<sup>9</sup>

## Clinical association of TAM with tumor angiogenesis in human cancers

It is well established that the majority of malignant tumors contain numerous macrophages as a major component of the host leukocytic infiltrate.<sup>10</sup> These cells were originally thought to be part of the host immune system used to defend the tumor cells; however, many clinical investigations have indicated the correlation between high TAM level and worse prognosis in human cancers. Several studies have elucidated studies of macrophage infiltration in various human cancers and its relationships with tumor angiogenesis. Several human cancers, such as breast,<sup>11</sup> prostate,<sup>12</sup> uterine cervical,<sup>13</sup> uterine endometrial,<sup>14</sup> liver<sup>15</sup> have been reported to display significant correlation of macrophage infiltrating level with tumor angiogenesis. Macrophage infiltration is also well associated with poor prognoses in these cancers.<sup>9</sup> In addition to the clinical correlation between increased TAM level and higher vascular density, the pro-angiogenic function

of TAMs has been demonstrated by experimental mouse models.<sup>5</sup> In polyoma virus middle T oncoprotein (PyMT)-induced mouse mammary tumors, macrophages were recruited to the lesions where angiogenesis subsequently occurred before transition into malignant sites. Depletion of macrophages in PyMT-induced tumors resulted in a ~50% decrease of vascular density and thus the delay of malignant transition. In contrast, enhanced recruitment of macrophages by over expression of colony-stimulating factor- 1 (CSF-1) resulted in accelerated vascularization and progression to malignancy.<sup>16</sup>

Bingle et al.<sup>17</sup> studied the effect of macrophages on tumor angiogenesis by using a mouse dorsal skin fold chamber model, which allowed direct in situ visualization and monitoring of implanted tumor spheroids and the surrounding blood vessels *in vivo*. Their results indicated that the presence of macrophages in tumor spheroids resulted in at least a 3-fold increase of VEGF release and significantly greater angiogenesis.

### Mechanistic studies for macrophage-associated angiogenesis

The increasing body of evidence has indicated that tumor angiogenesis was induced not only by the tumor cells but also the TAMs.<sup>18-21</sup> *In vitro* studies have also shown that TAMs are capable of producing a broad spectrum of angiogenesis-modulating factors including growth factors, cytokines and tissue matrix-degrading enzymes.<sup>22-24</sup> Growth factors: A number of growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and transforming growth factor- $\alpha$  (TGF- $\alpha$ ), are known to be produced by TAMs. They are not only growth factors for tumor cells, but also potent mitogens to promote endothelial cell proliferation.<sup>25</sup> Among them, VEGF is well-documented angiogenic factor that can promote endothelial cell growth, maturation and survival in a range of experimental systems.<sup>26</sup> VEGF is strongly expressed in glioblastoma, the most frequent brain malignancy, which is characteristically highly vascularised.<sup>27</sup> Up-regulation of VEGF has also been observed in human breast malignancy, and correlated with microvessel density in invasive breast carcinoma.<sup>28</sup> VEGF also functions as a monocyte chemotactic factor to attract TAMs to the hypoxic areas of tumor. In addition, VEGF can cause persistent extravasation of fibrin and fibronectin and continuous generation of tissue matrix.<sup>1</sup>

### Cytokines

Several inflammatory cytokines have been known to participate in macrophage associated angiogenesis during the wound healing process.<sup>22,23</sup> Interleukin-8 (IL-8) is mitogenic to endothelial cells and stimulates angiogenesis in animals.<sup>29</sup> The conditioned media of macrophages have been observed to achieve the same effects, and the effects can be markedly abolished by anti-IL-8 antibody, suggesting that IL-8 produced by macrophages plays an important role in macrophage-associated angiogenesis.<sup>30</sup>

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is also one of the major cytokines involved in macrophage-associated angiogenesis. TNF- $\alpha$  was postulated to be used by macrophages to kill cancer cells and cause tumor necrosis. When over expressed in endothelial cells, TNF- $\alpha$  was growth-inhibitory and even cytotoxic to endothelial cells.<sup>31,32</sup> In contrast, exogenous application of TNF- $\alpha$  promoted formation of new blood vessels in several *in vivo* models.<sup>33,34</sup> Leibovich et al.<sup>35</sup> further showed that anti-TNF- $\alpha$  antibodies completely neutralized the angiogenic activity in the conditioned media of activated macrophages, supporting the role of TNF- $\alpha$  as an angiogenic molecule

from macrophages. Moreover, macrophages are an important source of interleukin-6 (IL-6). IL-6 is involved in macrophage-mediated wound healing and, although evidence revealing its angiogenic activity is still incomplete, a recent study has demonstrated its role in tumor angiogenesis for colon cancer cells.<sup>36</sup>

### Multiple factors involved in tams-associated tumor angiogenesis

For solid tumors, tumor angiogenesis is an essential process for continuous tumor growth and spreading. Endothelial cell's biomechanical properties are regulated by invasive cancer cells.<sup>37</sup> Tumor angiogenesis can be regulated by a complex interaction between cancer cells and TAMs that produces angiogenic factors and tissue matrix modulating factors. Angiogenic factors exert direct effects on endothelial cells and tissue matrix is also involved in regulating angiogenesis.<sup>38</sup> It is a supporting matrix for vessels but a barrier for protruding or migrating endothelial cells. Tissue matrix is also a reservoir of angiogenic factors. Many angiogenic factors, such as VEGF, bFGF and TGF- $\beta$ , have an affinity for heparin and thus are sequestered by tissue matrix by binding to heparin like glycosaminoglycans. Tissue matrix modulating factors are not only responsible for tissue matrix remodeling; they are also capable of regulating the release of matrix-binding angiogenic factors following the degradation of tissue matrix. It has long been established that endothelial cells can be induced to display the morphology of capillary tubes and form a network structure on Matrigel, which can be an *in vitro* marker of angiogenesis.<sup>39</sup> VEGF is a typical potent inducer in this assay system. Moreover, it has been reported that interaction between cancer cells and macrophages can produce sufficient factors for promoting endothelial cell network formation. Furthermore, this effect of cancer cells/macrophages interaction on endothelial cells can be inhibited by incubation with either anti-IL-8 receptor antagonizing antibody or IL-6 or TNF- $\alpha$  soluble receptor, suggesting that IL-8, TNF- $\alpha$  and IL-6 indeed participate in TAMs related angiogenesis.<sup>40</sup>

### Tissue matrix-degrading proteinases

Macrophages are a rich source of tissue matrix-degrading enzymes and inhibitors. They are capable of producing MMPs (e.g. MMP-2, MMP-7, MMP-9, and MMP-12), serine proteinases (such as plasminogen activators uPA and tPA), cysteine proteinase (e.g. cathepsin B), and plasminogen activator inhibitors to modulate the destruction and re-construction of tissue matrix]. Once the tissue matrix is proteolytically degraded, these factors are released and become angiogenic. Furthermore, some proteolytic products of tissue matrix, e.g. fibrin of plasmin and fragments of hyaluronic acid, can also facilitate angiogenesis.<sup>41,42</sup>

### TAMs as the target for anti-cancer studies

Tumor angiogenesis is one of the major mechanisms by which TAMs exert their tumor-promoting activity, and therefore agents that can antagonize the angiogenic factors secreted by TAMs would be obviously effective in the inhibition of TAMs-induced angiogenesis.<sup>43</sup> Furthermore, several studies have been undertaken to investigate the possibilities of suppressing secretion of angiogenic factors by TAMs and blocking macrophage infiltration into the tumor mass as anti-cancer strategies. For example, a low dose of interferon- $\gamma$  has been shown to inhibit secretion of TNF- $\alpha$  by macrophages.<sup>44</sup> The compound chloroquine inhibits TNF- $\alpha$  gene transcription in macrophages.<sup>45</sup> Chemokine inhibitors affect tumor growth by decreasing TAM infiltration.<sup>46</sup> Moreover, transplanted tumors transfected with

Interleukin-10 show markedly less macrophage infiltration than their non-transfected counterparts.<sup>47</sup> The anti-angiogenic agent linomide, by suppressing functions of macrophages, exhibits its efficacy to significantly reduce tumor volume in a murine prostate cancer model.<sup>48</sup> Legumain, a member of the asparaginyl endopeptidase family, is over expressed by TAMs and thus serves as a target molecule. A legumain-based DNA vaccine was shown to drastically reduce TAM density in tumor and resulted in a marked decrease in angiogenic factors produced by TAMs.<sup>49</sup> With the seemingly important role of TAMs in not only tumor angiogenesis but also tumor cell migration/invasion, the TAMs themselves become an appealing target for cancer therapeutic approaches. One such approach is photodynamic therapy, which combines a non-toxic photosensitizer with harmless visible light of the specific wavelength to excite photosensitizer to a high-energy triplet state that will subsequently generate reactive oxygen species to kill target cells. Studies have reported specific targeting of photosensitizer to macrophages via conjugation of a photosensitizer to a ligand of the scavenger receptor class A, and the illumination was confined to the tumor. It was assumed that only TAMs would be killed, and circulating monocytes and macrophages in other tissues might still have beneficial antitumor effects such as extinguishing micro metastasis.<sup>50,51</sup> Because TAMs migrate and accumulate in hypoxic areas of tumor, they may serve as vectors to carry genes or drugs into these sites to be activated by hypoxia.<sup>52</sup> The potential of developing such a delivery strategy is to overcome the problem that most drugs or gene therapy vectors are difficult to reach the tumor sites with low or even no vascular density. Antibody-dependent cell phagocytosis (ADCP) represents another promising approach and methods to enhance efficacy of this therapeutic modality have been described.<sup>53</sup> Another approach described by Carta et al.<sup>54</sup> where they transduced macrophages with a viral vector containing IFN- $\gamma$  gene that was activated only in low oxygen conditions. Finally, advances in antibody engineering may represent another avenue. Recent results highlighting the plasticity of TAMs, which are capable of promoting tumor progression and invasion while still retaining tumoricidal function in the presence of tumor-targeting mAbs, represents another promising approach to TAM mediated activity within the tumor microenvironment.<sup>55,56</sup>

## Summary

TAMs are derived from peripheral blood monocytes recruited into the tumor and have multiple effects on tumor progression. They exhibit tumoricidal activity toward one fraction of cancer cells. Upon affected by both tumor cells and microenvironment, the TAMs can release a vast diversity of growth factors, cytokines, proteolytic enzymes, and other inflammatory modulators. With these factors, TAMs can induce tissue matrix remodeling and angiogenesis, and simultaneously enhance tumor cell migration and invasion, ultimately resulting in tumor metastasis. Clinically, the TAM level is significantly correlated with poor prognosis in several human cancers. As accumulating evidence indicates TAM's to tumor promoting roles, many investigations have been undertaken to study the potential of using TAMs as the therapeutic target. Targeting of macrophages in tumors is a promising therapeutic strategy. The depletion of TAMs or their 're-education' as anti-tumor effectors is under clinical investigation and may ultimately contribute to the success of conventional anti-cancer treatments.

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## Conflicts of Interest

There is no conflict of interest.

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