**Review**

Host microenvironment in breast cancer development

**Inflammatory and immune cells in tumour angiogenesis and arteriogenesis**

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**Abstract**

Breast cancer progression is associated with and dependent upon robust neovascularization. It is becoming clear that tumour-associated ‘normal’ cells, such as immune/inflammatory cells, endothelial cells and stromal cells, conspire with cancer cells in promoting this process. In particular, infiltrating immune/inflammatory cells secrete a diverse repertoire of growth factors and proteases that enable them to enhance tumour growth by stimulating angiogenesis and, as we suggest here, by promoting ‘tumour arteriogenesis’ – enlargement of feeding vessels supplying the expanding tumour capillary bed. Macrophages and their chemoattractants (e.g. macrophage chemoattractant protein-1) are critical for the arteriogenic process in ischaemia, and probably also in breast neoplasia. A better understanding of these various cellular and molecular constituents of breast cancer neovascularization may be useful in designing more effective therapies.

**Keywords:** angiogenesis, arteriogenesis, cancer, immunity, inflammation

**Introduction**

Infiltration of lymphocytes, macrophages, mast cells and neutrophils is a hallmark of inflammatory, defense and tissue repair reactions, which are often present in tumours [1,2]. Various types of tumour-infiltrating lymphocytes, including cytotoxic T cells, natural killer cells and lymphokine activated killer cells, are viewed as potential effectors of antitumour immunity and may oppose tumour expansion [3]. Tumour-associated macrophages (TAMs) constitute a major component of the leucocytic infiltrate [4], and activated macrophages have been shown to possess both direct and indirect tumouricidal activity [5,6]. However, evidence increasingly suggests that these cells may in fact symbiotically promote rather than inhibit tumour growth and development.

Macrophages, lymphocytes and mast cells have all been implicated in another host-dependent process, namely that of angiogenesis [7–9]. Clinical studies have linked the extent of immune/inflammatory cell infiltration with increased blood vessel density and poor prognosis in various types of cancer, suggesting that these cells may contribute to tumour progression in large part by stimulating tumour neovascularization [10,11]. Several studies in mice support these observations, and demonstrate a critical role for macrophage and mast cell infiltration in promoting angiogenesis during the earliest stages of neoplastic progression [9,12,13].

**Molecular regulators of inflammatory cell infiltration into tumours**

The infiltration of host immune cells into tumours is regulated by cues from the tumour microenvironment, in combination with tumour-derived chemokines, which together influence the adhesion, extravasation and migration of leucocytes. Breast carcinomas are known to contain a high proportion of infiltrating leucocytes, particularly TAMs. Macrophages are a heterogeneous population of cells that...
belong to the mononuclear phagocyte system and are derived from blood-borne monocytes that migrate into tissues, where they undergo final differentiation. Tumour hypoxia is an important stimulus for extravasation of monocytes [14], which migrate into the tumour tissue along gradients of chemoattractants, and these TAMs become immobilized in ischaemic, necrotic areas of tumours, where they can remain for an extended time [15–18].

Many studies have linked increased TAM density to poor prognosis in breast cancer [15,19–21], and in fact certain genetic alterations that increase the malignancy of the tumour may concomitantly increase the degree of macrophage infiltration. A strong association has been reported between HER-2, c-myc and int-2 oncogene amplification in breast tumour samples and the density of lymphocyte infiltration of the tumour [22]. In inflammatory breast cancer, expression of constitutively activated RhōC oncoprotein is associated with concomitant upregulation of both angiogenic (vascular endothelial growth factor [VEGF]) and inflammatory (IL-6) cytokines, leading to the formation of a specific type of inflammatory/angiogenic stroma in this particularly aggressive form of disease [23].

Some of the tumour molecular alterations that increase macrophage infiltration and macrophage-mediated angiogenesis include increased expression of monocyte chemoattractant protein (MCP)-1 and VEGF, both of which are highly expressed in breast tumour cells. MCP-1, a member of the C–C chemokine family, is involved in monocyte and T-lymphocyte migration, and is secreted by many human and murine tumour cells in addition to activated stromal cells [24,25]. MCP-1 expression in tumour cells is significantly correlated with the extent of TAM infiltration [26,27], and in particular both MCP-1 and VEGF expression have been positively correlated with TAM infiltration, angiogenesis and poor survival in breast cancer [28–30].

VEGF is a potent angiogenic growth factor that is overexpressed in the majority of human cancers [31]. VEGF produced by tumours promotes the proliferation, survival and migration of endothelial cells by binding to its receptors, namely VEGF receptor (VEGFR)-1 and VEGFR-2, which are expressed on the endothelial cell surface. However, in addition to these direct effects on endothelial cells, VEGF also stimulates monocyte migration through VEGFR-1 [32], which is expressed on monocytes and macrophages, as well as on endothelial cells [33,34]. A positive correlation between VEGF expression and degree of macrophage infiltration has been observed in invasive breast carcinoma [28,35] and other malignancies [36,37]. Placental growth factor and VEGF-C – two VEGF-related tumour-derived growth factors – can also stimulate monocyte chemotaxis [38,39]. Placental growth factor may also act as a survival factor for both endothelial cells and macrophages [40].

The other important factor responsible for increased macrophage infiltration in breast cancer is the macrophage colony-stimulating factor (CSF)-1; this is a haematopoietic growth factor that regulates the proliferation, survival and differentiation of monocytes and macrophages, which express the CSF-1 receptor [41]. Although it is secreted by many types of cells, increased expression of CSF-1 occurs in breast tumours, where it has been associated with high TAM infiltration and poor prognosis [42–44]. The critical importance of CSF-1 production, not only for macrophage recruitment but also for tumour vascularization and progression, has also been demonstrated in transgenic mouse models of breast cancer [45,46].

**Contribution of inflammatory cells to tumour angiogenesis and lymphangiogenesis**

Although tumour cells themselves promote the recruitment and expansion of their own blood supply, tumour-associated immune/inflammatory cells can modify and contribute to this process by supplying a repertoire of growth factors, cytokines and proteases comparable to that secreted by tumour cells themselves (summarized in Table 1). Inflammatory cells can produce a myriad of cytokines and growth factors, many of which are proangiogenic, and directly stimulate the migration and proliferation of endothelial cells. For example, macrophages, mast cells and neutrophils all secrete VEGF, IL-8 and transforming growth factor-α. Some of these molecules, for example VEGF, act not only on endothelial cells but also stimulate migration of further inflammatory cells into the tumour, potentially forming self-perpetuating positive feedback loops. Factors that increase vascular permeability are also proangiogenic because they promote deposition of fibrin, providing a matrix favourable for endothelial cell and leucocyte migration. For example, macrophage-derived VEGF, substance P, platelet-activating factor and prostaglandins induce vessel hyperpermeability [47]. Histamine, which is stored and released by mast cells, has wide-ranging biological effects that include proangiogenic activity [48].

Inflammatory cells also secrete a variety of proteases that degrade and remodel the extracellular matrix [47]. For example, macrophages, mast cells, neutrophils and lymphocytes all secrete metalloprotease (MMP)-9 – a MMP that has emerged as an important modulator of angiogenesis and tumour development [49]. Some of these proteases (e.g. urokinase-type plasminogen activator and heparanase) release proangiogenic growth factors (e.g. basic fibroblast growth factor) that are sequestered by heparan sulphate proteoglycans in the extracellular matrix. However, it should be kept in mind that MMPs, including MMP-9, may also have an antiangiogenic effect (at later stages) by processing the α3 chain of type IV collagen to the angiogenesis inhibitor tumstatin [50]. Furthermore, at least one macrophage-derived protease, namely...
MMP-12 (metalloelastase), has been shown to generate angiostatin, an endogenous angiogenesis inhibitor, from its precursor – plasminogen [51]. MMP-7 and MMP-9 have also been shown to have angiostatin-converting activity [52].

It should be noted that not all of the growth factors and cytokines released by inflammatory cells are proangiogenic. For example, macrophages secrete thrombospondin-1, interferon-α and interferon-γ, which are antiangiogenic. Many cytokines (e.g. transforming growth factor-β, IL-1β, IL-6, tumour necrosis factor-α) are known to have pleiotropic effects, stimulating angiogenesis under certain conditions and inhibiting it under others [47]. It is not known how the net proportion of the various proangiogenic or antiangiogenic activities of macrophages and other inflammatory cells is regulated. However, this balance between angiogenesis promoting, inhibiting and modulating influences at given times and locations in the tumour microenvironment clearly has a potential to determine the overall course and dynamics of blood vessel formation.

Finally, immune/inflammatory cells may also promote tumour lymphangiogenesis by secreting the lymphangiogenic growth factors VEGF-C and VEGF-D [53,54]. The formation of peritumoural lymphatic vessels, which in one study correlated with the extent of TAM recruitment, represents an important conduit for the metastasis of tumours to regional lymph nodes, with major implications for patient prognosis [54,55].

‘Tumour arteriogenesis’: the possible role of inflammatory cells

Although various processes that affect tumour microcirculation (both blood and lymphatic) have attracted considerable attention and are being extensively characterized at the molecular level, there has been essentially no emphasis on the events that must implicitly occur in the vascular tree upstream of the site of active angiogenesis. The recruitment of large numbers of capillary microvessels during tumour growth (as a result of angiogenesis) considerably increases the intratumoral capillary volume – a circumstance that would be expected to require the concurrent expansion of upstream arterioles and downstream venules (i.e. tumour ‘feeding vessels’). Indeed, such dilatations of feeding vessels have been observed using angiography in cancer patients [56]. This peritumoral remodelling and expansion of pre-existing arteries and arterioles probably involves processes similar to collateral vessel formation or ‘arteriogenesis’, which occurs during ischaemic limb or heart disease. We postulate that an analogous process of ‘tumour arteriogenesis’ must accompany angiogenic expansion of the microvasculature, and hence such a process could similarly be viewed as a rate-limiting and targetable event during tumour expansion.

### Table 1

| Cell type       | Activity     | Molecule          | Reference |
|-----------------|--------------|-------------------|-----------|
| Macrophages     | Proangiogenic| VEGF              | [62]      |
|                 |              | TGF-α             | [63,64]   |
|                 |              | IL-8              | [65]      |
|                 |              | bFGF              | [66]      |
|                 |              | TP                | [30,67]   |
|                 |              | PDGF              | [68]      |
|                 |              | Substance P       | [69]      |
|                 |              | Prostaglandins    | [70]      |
| Antiangiogenic  |              | TSP-1             | [71]      |
|                 |              | IFN-α             | [72]      |
|                 |              | IFN-γ             | [73]      |
| Protease        | t-PA         |                   | [74]      |
|                 | u-PA         |                   | [75,76]   |
|                 | MMP-1, -2, -3, -7, -9 and -12 | | [10,12,51] |
| Mast cells      | Proangiogenic| VEGF              | [77,78]   |
|                 |              | bFGF              | [79]      |
|                 |              | TGF-β             | [80]      |
|                 |              | TNF-α             | [81]      |
|                 |              | IL-8              | [81]      |
|                 |              | Histamine         | [48]      |
| Protease        | Chymase      |                   | [9,81,82] |
|                 | Tryptase     |                   | [9,81]    |
|                 | MMP-9        |                   | [12]      |
|                 | Heparanase   |                   | [83]      |
| Neutrophils     | Proangiogenic| VEGF              | [84]      |
|                 |              | IL-8              | [84]      |
| Antiangiogenic  | IL-12        |                   | [84]      |
|                 | IP-10        |                   | [85]      |
|                 | MIG           |                   | [85]      |
| Protease        | MMP-9        |                   | [86]      |
|                 | u-PA         |                   | [86]      |
|                 | Elastase     |                   | [12,87]   |

bFGF, basic fibroblast growth factor; IFN, interferon; IP, interferon gamma inducible protein; MMP, matrix metalloprotease; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumour necrosis factor; TP, thymidine phosphorylase; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.

Monocytes are critical for the initiation of arteriogenesis because they adhere to and invade endothelium activated by the increased shear stress that results from large pressure differences between perfused areas [57]. The involvement of monocytes in arteriogenesis was discovered by Schaper et al. in 1976 [58], shortly before their role in angiogenesis in 1977 [7]. MCP-1 is once again implicated in this process because it not only attracts monocytes but also promotes their adhesion by inducing
them to upregulate Mac-1, the receptor for intercellular adhesion molecule-1 (ICAM-1) that is expressed in activated endothelium [59]. Monocytes that adhere and then invade the arteriolar wall subsequently promote collateral artery growth by producing cytokines such as tumour necrosis factor-α and basic fibroblast growth factor [60]. Studies of collateral growth in rabbits have shown that, although prevention of monocyte adhesion (e.g. using antibodies to ICAM-1) delays arteriogenesis, infusion of MCP-1 or survival factors for monocytes (e.g. granulocyte-macrophage CSF) accelerate the process [57]. Although VEGF has also been shown to stimulate collateral growth, it now appears as though this positive effect of VEGF on arteriogenesis may be due principally to its effect on activating monocytes, stimulating their adhesion to the endothelium and their transmigration through it [61].

Although the large ‘feeding vessels’ that supply tumour vascular beds represent a potentially useful target for anticancer therapies, the exact mechanisms by which they are formed and recruited remains unknown. Again, the participation of monocytes/macrophages in this process has never been examined; however, given their importance in collateral formation, it is clear that they have the capability to play a considerable role. These issues are being actively pursued in our laboratory.

**Conclusion**

The recruitment of monocytes, macrophages and other inflammatory cells to a tumour appears to be a common denominator for the major processes involved in tumour development and progression (Fig. 1). Inflammatory cells contribute to tumour angiogenesis by supplying proangiogenic growth factors, cytokines and proteases. They also contribute factors that promote the formation and enlargement of intratumoural or peritumoural lymphatic vessels, eventually allowing a tumour to metastasize to distant organs. Finally, they may also play a critical role in arteriogenesis by promoting the growth of the larger vessels that supply the expanding capillary bed, feeding the rapidly growing tumour mass.

Is the recruitment of inflammatory cells a good target for cancer therapy? It is important to keep in mind that macrophages and other inflammatory cells, despite their proangiogenic and protumour effects, may also participate in antitumour immunosurveillance. The answer to this question will probably depend on the type of tumour, on the stage at which inflammatory cells provide the greatest contribution during tumour progression, and on the nature of their influence (tumour-promoting or inhibitory). However, it could be speculated that simultaneously targeting the proarteriogenic effects of macrophages and the proangiogenic functions of endothelial cells may lead to synergistic antitumour effects, and exploration of this possibility is therefore warranted.

**Competing interests**

None declared.

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