

REVIEW

Mast cell contribution to angiogenesis related to tumour progression

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Summary The current wisdom is that tumours are endowed with an angiogenic capability and that their growth, invasion and metastasis are angiogenesis dependent. It is now well documented that neoplastic cells are influenced by their microenvironment and vice versa. The specific organ microenvironment determines the extent of cancer cell proliferation, angiogenesis, invasion and survival. Tumour cells are surrounded by an infiltrate of inflammatory cells, namely lymphocytes, neutrophils, macrophages and mast cells (MCs), which communicate via a complex network of intercellular signalling pathways, mediated by surface adhesion molecules, cytokines and their receptors. This review article summarizes: (i) the MC mediators involved in angiogenesis; (ii) the experimental evidence concerning the role played by MCs in angiogenesis; (iii) the list of solid and haematological tumours in which a close relationship between angiogenesis, tumour progression and MCs has been demonstrated; (iv) the circumstances in which MCs are a critical source of angiogenic factors *in vivo*, and in such cases, the signals that regulate their production and secretion that need to be determined as a prelude to the elaboration of new therapeutic strategies associated with MC presence and activation.

Angiogenesis and tumour progression

Angiogenesis, the process through which new blood vessels arise from pre-existing ones, is involved during embryonal development and later, in adult life, in several physiological conditions, such as corpus luteum formation, and pathological conditions, such as tumours and chronic inflammation, in which angiogenesis itself contributes to the progression of disease [1]. There are at least two types of angiogenesis: (a) the so-called 'sprouting' angiogenesis, which is characterized by the proliferation and migration of endothelial cells into avascular sites [2]; (b) the 'non-sprouting' angiogenesis or intussusceptive microvascular growth, which occurs by splitting of the existing vasculature by transluminal pillars or transendothelial bridges [3].

Tumours are endowed with an angiogenic capability and that their growth, invasion and metastasis are angiogenesis dependent [4]. After the initial transformation and growth of cells, vascularization must occur if a tumour mass is to exceed 1 mm³ in diameter. The synthesis and secretion of several pro-angiogenic factors by tumour and host cells plays a key role in establishing a vascular network from the surrounding stroma.

Tumour angiogenesis is essentially mediated by angiogenic molecules produced by tumour cells, such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), IL-8, placental growth factor, transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), angiopoietins and others. These are exported from

tumour cells, mobilized from the extracellular matrix or released from host cells recruited to the tumour.

Tumour microenvironment

It is now well documented that neoplastic cells are influenced by their microenvironment and vice versa. The specific organ microenvironment determines the extent of cancer cell proliferation, angiogenesis, invasion and survival [5, 6]. These data indicate that a permissive stromal environment is important in supporting tumour progression in combination with genetic alterations.

Tumour cells are surrounded by an infiltrate of inflammatory cells, namely lymphocytes, neutrophils, macrophages and mast cells (MCs), which communicate via a complex network of intercellular signalling pathways, mediated by surface adhesion molecules, cytokines and their receptors. The inflammatory cell infiltrate, particularly macrophages, may contribute to tumour angiogenesis, and there are many reports of associations between macrophage infiltration, vascularity and prognosis. Tumour-associated macrophages accumulate in poorly vascularized hypoxic or necrotic areas [7] and respond to experimental hypoxia by increasing the release of VEGF and FGF-2 and a broad range of other factors, such as TNF- α , FGF-2, VEGF, urokinase and matrix metalloproteinases (MMP) [8]. Moreover, activated macrophages synthesize and release inducible nitric oxide synthase, which increases blood flow and promotes angiogenesis [9]. Lastly, the angiogenic factors secreted by macrophages stimulate MC migration [10].

Chemokines also have a role; some CXC chemokines, such as IL-8 are pro-angiogenic [11]. Chemokines may exert their regulatory activity on angiogenesis directly or as

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a consequence of leucocyte infiltration and/or the induction of growth factor expression.

The most aggressive human cancers, namely malignant melanoma, breast carcinoma and colorectal adenocarcinoma, are associated with a dramatic host response composed of various inflammatory cells, especially MCs at the tumour periphery. MC infiltrate hyperplasias, dysplasias and invasive fronts of carcinomas (but not the core of solid tumours), where they degranulate in close apposition to capillaries and epithelial basement membranes.

Mast cell-dependent mediators of angiogenesis

There are three kinds of MC-dependent mediators: granule-associated mediators, lipid-derived mediators and cytokines (Fig. 1).

Heparin, a granule-associated mediator, stimulates endothelial cell proliferation and migration *in vitro* [12, 13]. *In vivo*, however, it stimulates [14–16], inhibits [16–18] or has no effect [19, 20]. These differences seem to be related to its molecular size and degree of sulphation. Heparin acts as a soluble form of the low-affinity FGF-2 receptor [21], which displaces FGF-2 in the biologically active form, and allows its rapid interaction with endothelial cells [22].

Histamine has an angiogenic effect through both H_1 and H_2 receptors [23]. It may also increase the permeability of newly formed microvessels during tumour angiogenesis, and hence increase the leakage of plasma proteins and deposition of fibrin. Degradation products of fibrin are angiogenic *in vivo* [24].

It is to note that microvascular leakiness in tumours is also a consequence of a defective organization of microvascular endothelium. In fact, tumour endothelial cells do not form a normal monolayer and thus do not have a normal barrier function [25]. The cells are disorganized, irregularly shaped, overlap one another, have luminal projections and give rise to abluminal sprouts.

MC synthesize and store large amounts of MMP-2, MMP-9 and serine-proteinases of two subclasses: tryptase and chymase [26–28]. Given the ability of MMP-2 and MMP-9

to degrade type IV, V, VII and X collagens, as well as fibronectin [29], namely the major components of the interstitial stroma and subendothelial basement membrane, the findings suggest that MC may contribute to the progression from *in situ* to invasive and metastatic solid tumours, characterized by an enhanced angiogenesis and secretion of proteolytic enzymes [29].

Tryptase is the predominant protease present in MC granules in the lung, skin and gastrointestinal tract, while chymase is present in 85% of the MCs of the skin and intestinal submucosa, but not in the intestinal mucosa or lung. Both enzymes are involved in angiogenesis after their release from activated MC granules. Their proteolytic activities degrade extracellular matrix components or release matrix-associated growth factors [30], and they also act indirectly by activating latent MMP [31] and plasminogen activators [32]. Blair et al. [33] have demonstrated the angiogenic potential of tryptase *in vitro* and its important role in neovascularization. Tryptase added to microvascular endothelial cells cultured on Matrigel caused a pronounced increase in capillary growth, and this was suppressed by specific tryptase inhibitors. Moreover, tryptase directly induced endothelial cell proliferation in a dose-dependent fashion.

Muramatsu et al. [34, 35] used the hamster sponge-implant model to show that angiogenesis is induced by angiotensin II and inhibited by chymase inhibitors, suggesting that MC-derived chymase is an important mediator of MC-dependent angiogenesis.

MCs release several polypeptide growth factors, including FGF-2, VEGF, TGF- β , TNF- α and IL-8. These cytokines are involved both in normal as well as tumour-associated angiogenesis. The spectrum of cytokines expressed appears to vary depending on the maturity state of the MCs and of the tissue of residence. Qu et al. [36] demonstrated that FGF-2 is localized in the cytoplasmic and extruded granules of MC in several human tissues. Grutzkau et al. [37] demonstrated the expression of VEGF in the human MC line HMC-1 and in human skin MC.

Nerve growth factor (NGF), also contained in MC secretory granules, induces endothelial cell proliferation *in vitro* and angiogenesis *in vivo* in the chick embryo chorio-allantoic membrane (CAM) assay [38, 39].

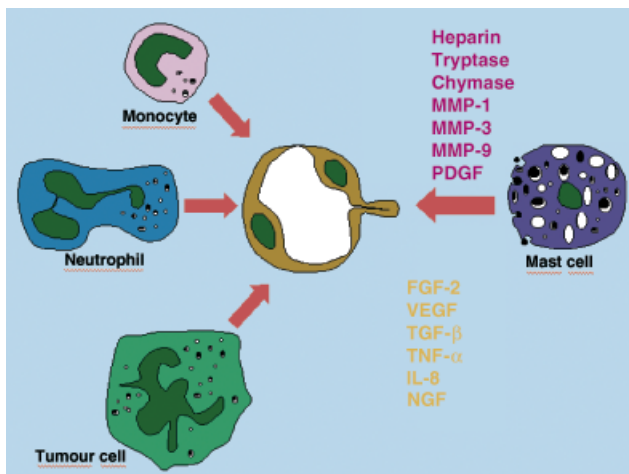


Fig. 1. Interplay between mediators released from mast cells and other cells participating in the angiogenic response.

Mast cells in experimental tumour angiogenesis

Accumulation of MCs appears to be related to the secretion of angiogenic factors from the neoplastic cells themselves. Kessler et al. [40] demonstrated that tumour angiogenesis factor elicited a vasoproliferative response when implanted upon the CAM of the chick embryo. This response was first observed stereomicroscopically 2–3 days after implantation and a 40-fold increase in MC density was observed in the vicinity of the implants by 24 h.

Poole and Zetter [41] demonstrated that rat peritoneal MCs migrate in response to conditioned medium from several tumour cell lines. The active chemoattractant(s) in this conditioned medium appeared to be peptide(s) with a molecular weight of 300–1000. They proposed that the chemoattraction of MCs by tumour-derived peptides may be an important early event in tumour neovascularization.

Starkey et al. [42] investigated the role of host MCs in tumour-associated angiogenesis by comparing the angiogenic response of genetically MC-deficient W/W^v mice and MC-sufficient +/+ litter mates to subcutaneously growing B16-BL6 tumours. The response was slower and initially less intense in W/W^v. Fewer W/W^v than +/+ mice developed spontaneous lung metastases. Bone marrow repair of the MC deficiency restored the incidence of haematogeneous metastases to approach that of +/+ mice. These results demonstrate a role for MC *in vivo* during tumour angiogenesis and also suggest a role for host MC in haematogeneous metastases.

Coussens and Werb [43] reported that expression of HPV 16 early-region genes in basal keratinocytes of transgenic mice elicits a multi-stage pathway to squamous carcinoma. Infiltration by MCs and activation of MMP-9 in MCs and leucocytes coincided with the angiogenic switch in premalignant lesions. MC release specific proteases, MCP-4 (chymase) and MCP-6 (tryptase). MCP-4 activates progelatinase A, itself a component of MC granules, releases sequestered angiogenic factors, such as VEGF and FGF-2 from neoplastic skin, and thus induces angiogenesis. MCP-6 induces normally quiescent fibroblasts to participate in the formation of neoplastic stroma.

We have demonstrated that isolated MCs and their secretory granules, but not degranulated MC, induce an angiogenic response in the CAM assay [44]. Addition of anti-FGF-2 or anti-VEGF antibodies reduced the angiogenic response of both MC and their secretory granules by 50% and 30%, respectively. These data support the evidence that the angiogenic properties of MCs depend on the angiogenic molecules contained in their secretory granules, and indicate that FGF-2 and VEGF are the angiogenic cytokines primarily and perhaps synergistically responsible for this vasoproliferative activity.

Mast cells in human solid tumour angiogenesis

MCs play a role in tumour growth and angiogenesis [45] and exert cytotoxicity on tumour cells by immunologic mechanisms [46]. At present, it is not known if MCs are involved in 'sprouting' angiogenesis or in 'non-sprouting' angiogenesis, or in both.

MCs are associated with breast cancer [47–49], haemangioma and haemangioblastoma [50], colorectal cancer [51] and uterine cervix cancer [52]. Tryptase-positive MCs increased in number and vascularization increased in a linear fashion from dysplasia to invasive cancer of the uterine cervix [53]. An association of VEGF and MCs with angiogenesis has been demonstrated in laryngeal carcinoma [54], in small lung carcinoma, where most intratumoural MCs express VEGF [55–57], and in melanoma, where MCs express both VEGF [58] and FGF-2 [59]. Lastly, a prognostic significance has been attributed to MCs and microvascular density in squamous cell cancer of the oesophagus [60] and in melanoma [61].

Mast cells in human haematological tumour angiogenesis

In contrast with solid tumours, the role of angiogenesis in the growth and survival of haematological malignancies has only recently been realized [62].

Angiogenesis in benign lymphadenopathies and B cell non-Hodgkin's lymphomas (B-NHL), measured as microvessels counts, is correlated with the total and MC tryptase-positive counts, and both increase in step with the increase in working formulation malignancy grades [63, 64].

Bone marrow angiogenesis, evaluated as microvessel area, and MC counts are highly correlated in patients with inactive and active multiple myeloma (MM) and in those with monoclonal gammopathies of undetermined significance. Both parameters increase simultaneously in active MM [65].

In both B-NHL and MM, MCs rest near or around blood or lymphatic capillaries. Their ultrastructural picture includes a typical morphological semilunar feature, or piecemeal partial degranulation of their secretory granules, unlike the IgE-mediated massive degranulation that occurs during immediate hypersensitivity reactions [63, 65–67]. This morphology is typical of the slow degranulation that takes place in delayed hypersensitivity reactions and chronic inflammation [68, 69]. Semilunar appearance may reflect slow but progressive release of angiogenic factors favouring chronic and progressive stimulation of MC degranulation.

Fukushima et al. [70] found a significant correlation in NHL between vessel count and the number of both MC and VEGF-expressing cells. Double fluorescence staining of VEGF mRNA and MC tryptase revealed that MC expressed VEGF mRNA.

Bone marrow samples of patients with myelodysplastic syndromes display a high correlation between microvessel counts and both total and tryptase-positive MCs, and that both parameters increase simultaneously with tumour progression [71]. There is also a correlation between the extent of angiogenesis and the number of tryptase-positive MCs in patients with early B cell chronic lymphocytic leukaemia and tryptase-positive MCs predict their clinical outcome [72, 73].

Concluding remarks

Inhibition or destruction of tumour cells have long been the prime goal of cancer therapy. Treatment of these cells and modulation of their microenvironment may prove a better approach.

The balance or imbalance of inducers and inhibitors of angiogenesis secreted by inflammatory cells may favour a tumour's progression or regression. The circumstances in which MCs are a critical source of angiogenic factors *in vivo*, and in such cases, what signals regulate their production and secretion are all matters that need to be determined as a prelude to the elaboration of new therapeutic strategies associated with their presence and activation.

Finally, a more accurate insight into the role of MCs may be obtained from the use of activation markers, along with consideration of local, microenvironmental stimuli in the regulation of their functions.

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