

CHAPTER 14

MAST CELLS, ANGIOGENESIS AND CANCER

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Abstract: Mast cells (MCs) were first described by Paul Ehrlich¹ in his doctoral thesis. MCs have long been implicated in the pathogenesis of allergic reactions and certain protective responses to parasites. As most tumors contain inflammatory cell infiltrates, which often include plentiful MCs, the question as to the possible contribution of MCs to tumor development has progressively been emerging. In this chapter, the specific involvement of MCs in tumor biology and tumor fate will be considered, with particular emphasis on the capacity of these cells to stimulate tumor growth by promoting angiogenesis and lymphangiogenesis. Data from experimental carcinogenesis and from different tumor settings in human pathology will be summarized. Information to be presented will suggest that MCs may serve as a novel therapeutic target for cancer treatment.

INTRODUCTION

Mast cells (MCs) are versatile, tissue-homing secretory cells, which were first described by Paul Ehrlich¹ in his doctoral thesis.² MCs have long been implicated in the pathogenesis of allergic reactions and certain protective responses to parasites. They have been linked to various cell-mediated immune reactions, being found in tissues from multiple disease sites and as a component of the host reaction to bacterial, fungal and viral infections. They have also been shown to participate in angiogenesis and tissue repair processes after injury.^{3,4}

Mast Cell Biology: Contemporary and Emerging Topics, edited by Alasdair M. Gilfillan and Dean D. Metcalfe. ©2011 Landes Bioscience and Springer Science+Business Media.

The importance of a possible functional link between chronic inflammation and cancer has long been recognized. As most tumors contain inflammatory cell infiltrates, which often include plentiful MCs, the question as to the possible contribution of MCs to tumor development has been emerging. A major point linking MCs to cancer is the well recognized capacity of these cells to synthesize and release potent angiogenic compounds. Although some evidence suggests that MCs can promote tumorigenesis and tumor progression, there is some clinical evidence, as well as experimental tumor models, where MCs seem to have functions that favour the host. Thus, the involvement of MCs in tumor development is complex and far from being settled.

We will examine the specific involvement of MCs in tumor biology and tumor fate, with particular emphasis on the capacity of these cells to stimulate tumor growth by promoting angiogenesis and lymphangiogenesis. Then, data from experimental carcinogenesis and from different tumor settings in human pathology will be reviewed. The last section will be dedicated to drugs affecting MC number.

MAST CELLS IN EXPERIMENTAL CARCINOGENESIS

Involvement of MCs in experimental carcinogenesis has increasingly attracted the interest of researchers. Development of squamous cell carcinoma in a HPV (human papilloma virus) 16-infected transgenic mouse model of epithelial carcinogenesis has provided experimental evidence in favour of an early participation of MCs in tumor growth and angiogenesis.⁵ Accumulation of MCs was observed at the hyperplastic and dysplastic stages and in the invasive edge of tumor growth. They were typically localized around capillaries and epithelial basement membrane, where MC degranulation resulted in the release of the MC-specific proteases, tryptase and chymase. MCs were absent within the tumor mass itself. Infiltration of MCs and activation of matrix metalloproteinase (MMP)-9 coincided with the angiogenic switch in premalignant lesions. Addition of chymase alone was sufficient to stimulate an angiogenic phenotype when coincubated with a hyperplastic skin sample *in vitro*, while tryptase was shown to play a prominent role in tissue remodelling.

Gounaris et al⁶ developed a transgenic mouse model over-expressing an inducible, stabilized β -catenin in intestinal enterocytes, as well as the APC ^{$\Delta 468$} as an independent hereditary model of polyposis. In both mice, developing polyps were rapidly infiltrated by CD34⁺ MCs; and histological staining confirmed the presence of both connective and mucosal type MCs in developing tumors. The latter MC type was prevalent at the site of newly developing polyps, while the former was prevalent in the stroma at later stages of tumor development. Treatment with antitumor necrosis factor alpha (TNF α) antibodies resulted in fewer polyps, reduced polyp growth and decreased MC infiltration. Reconstitution of W^{sh}/W^{sh} MC-deficient mice with CD34^{-/-} CD43^{-/-} bone marrow also resulted in a decrease in polyp growth, as compared to mice reconstituted with wild type bone marrow. Overall, these data suggest that MCs are an essential component for preneoplastic polyp development as well as a possible target for therapeutic intervention.

In a pancreatic β -cell tumor model, *in vivo* activation of Myc was shown to induce several chemokines in developing tumors, including chemokine ligand 2 (CCL2), monocyte chemotactic protein 1 (MCP-1), and CCL5. This latter chemokine triggered rapid recruitment of MCs to the tumor site, recruitment that is absolutely required for macroscopic tumour expansion.⁷ MCs were the only inflammatory cells increased in the

vicinity of tumor cells at the early stage of tumor growth and their infiltration correlated with the expansion of islet tumors. Macrophages and neutrophils were also recruited after the onset of tumor angiogenesis. Treatment of established β -cell tumors with disodium cromoglycate (cromolyn), an inhibitor of MC degranulation, rapidly triggered hypoxia and cell death of tumor and endothelial cells. The authors concluded that MCs are necessary for tumor angiogenesis and growth; and suggested that inhibition of MC function might prove to be therapeutically useful in restraining the growth of pancreatic cancer. In an *in vivo* transplantation assay in nude mice, angiopoietin-1 (Ang-1) secreted by primary murine MCs promoted marked neovascularization.⁸ The MCs studied were bone marrow mast cells (BMMCs) from C57BL/6 mice and two mouse plasmocytoma cell lines. Greater angiogenesis was demonstrated when BMMCs were transplanted into mice together with plasmocytoma cells, than when each of the cell types was transplanted alone. The use of Ang-1-neutralizing antibodies significantly reduced the growth of plasmocytoma containing MC. Overall, these data demonstrate that MC-derived Ang-1 promotes growth of plasmocytomas by stimulating neovascularization.

Hart et al.,⁹ using W/W^v mice, demonstrated a direct correlation between MC density in the dermis and susceptibility to ultraviolet-B-induced systemic immunosuppression. These mice, which are homozygous for the W (white spotting) mutation and therefore severely MC deficient, are unresponsive to ultraviolet-induced immunosuppression unless first injected with MC precursors at the irradiated site.⁹ The W locus encodes the KIT tyrosine kinase receptor that binds stem cell factor (SCF).¹⁰ The MC products involved in ultraviolet-induced immunosuppression are believed to be TNF α and histamine.¹¹

These data lend support for a crucial role of MCs in early carcinogenesis in different experimental settings.

MAST CELL RECRUITMENT TO TUMOR

Recent literature identifies MCs as critical regulators of inflammation and the immunological response in the tumor microenvironment. Chronic inflammation induces both proliferation of resident tissue MCs and recruitment of MCs and their precursors from outside the lesion.^{12,13} During tumor development, circulating MC precursors migrate into the neoplastic mass and form one of the major stromal cell populations. Recruitment and activation of MCs in the tumor infiltrate is mainly mediated by tumor-derived stem cell factor (SCF) through its receptor KIT on MCs.¹⁴ In a hepatocarcinoma model, MCs failed to migrate into SCF-knockdown tumors and anti-KIT antibodies abolished the migration of MCs into tumors, leading to decreased tumor growth. Low concentrations of SCF efficiently induced the chemotactic migration of MCs and the release of active MMP-9 into the local environment. Higher SCF concentrations promoted activation of recruited MCs in the tumor context, which were induced to release pro-inflammatory factors including interleukin (IL)-6, TNF- α , vascular endothelial growth factor (VEGF), Cox-2, i-NOS and CCL-2.¹⁴ The intervention of SCF-activated MCs in the tumor microenvironment increased the transcription of the IL-17 gene and the number of IL-17-producing cells in the tumor mass. In turn, IL-17, a potential candidate for regulating the tumor inflammatory reaction through the production of IL-9, would attract more MCs to the site of inflammation.¹⁵ TNF- α and other pro-inflammatory factors released by MCs can similarly increase the activity of NK- κ B and AP-1 in tumor cells.¹⁴ NK- κ B and AP-1 may, in turn, favour the proliferation of tumor cells by inducing the expression of cyclines, the survive of tumor

cells by the blockade of apoptosis and the invasiveness of tumor cells by inducing the production of macrophage migration inhibitory factor (MIF) and EMMPRIN.^{16,17}

MAST CELLS AND IMMUNE TOLERANCE

A new paradigm of immunological participation for MCs has recently been proposed to explain their involvement in the dynamics of tumor development and progression. Although immune surveillance works at an early stage of tumorigenesis, established tumors primarily induce immune tolerance by creating sites of immune privilege and by inducing a shift of the immune balance from activation to tolerance.^{18,19} MCs have recently been proposed to be mechanistically involved in the negative modulation of immune surveillance in the tumor microenvironment (Fig. 1). MCs have thus been found to play a critical role in the suppression of immune reactions.²⁰ These cells not only produce inhibitory cytokines, such as IL-10,²¹ but also are essential in promoting the immune tolerance mediated by regulatory T (Treg) cells. Indeed, MCs serve as enforcers for Treg cells, turning down the immune system's reaction to a skin allograft, possibly through IL-10 secretion.²² Ultraviolet-B irradiation which represents the most important skin immunosuppressor and initiator of cutaneous malignancies, activates MCs.^{23,24} Upon

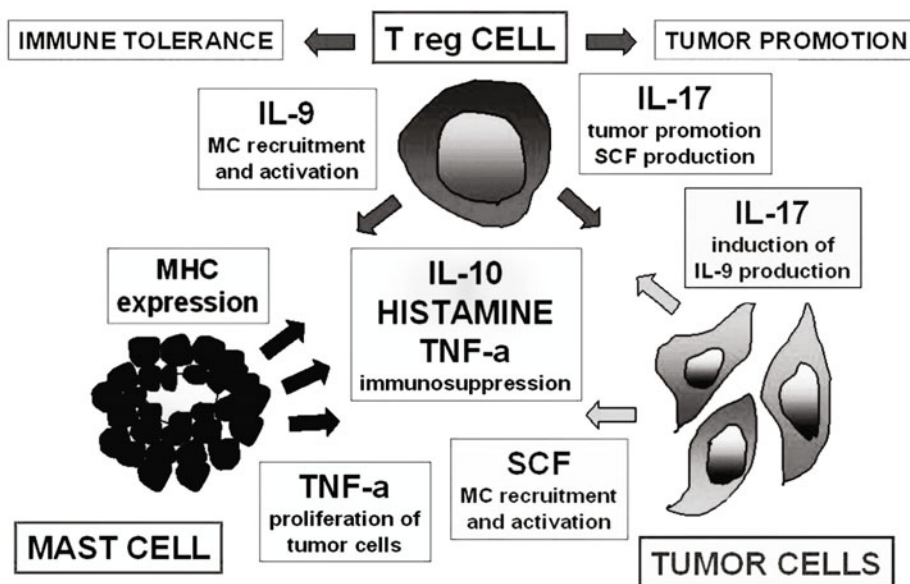


Figure 1. The immuno-suppressive role of MCs in the tumor environment. MCs infiltrating the tumor stroma favor expansion and activation of regulatory T cells which, in turn, stimulate immune tolerance and tumor promotion. SCF-mediated recruitment of MCs by tumor cells leads to MC activation and release of immunosuppressive and tumor-promoting molecules such as IL-10, histamine and TNF- α . Upon activation, regulatory T cells release SCF and IL-9, which expand the MC population. MCs serve as enforcers for regulatory T cells, possibly by MHC-mediated activation. Abbreviations: T reg cell, regulatory T-cell; SCF, stem cell factor; TNF- α , tumor necrosis factor- α ; MHC, major histocompatibility complex; IL-9, IL-10, IL-17, interleukin-9, -10, -17.

irradiation of the skin, *trans*-urocanic acid in the epidermis isomerizes to *cis*-urocanic acid, which stimulates neuropeptide release from neural c-fibers. These neuropeptides, in turn, trigger secretion of histamine, TNF- α and other mediators from MCs, leading to suppression of the cellular immune system.^{11,25} Using MC-deficient W/W^v mice, a direct correlation has been demonstrated between MC density in the dermis and susceptibility to ultraviolet-B-induced systemic immunosuppression.⁹ In a skin transplantation model of allograft tolerance in the mouse, MCs were crucial for graft acceptance, as MC-deficient C57BL/6-*Kit*^{W-sh/W-sh} mice showed an inability to induce tolerance.²² Activated Treg cells in the tolerant tissue produced high levels of IL-9, a cytokine which seems important in MC recruitment, growth and activation. This cytokine appears to be a crucial factor in mediating regional tolerance, as neutralization of IL-9 greatly accelerates allograft rejection in tolerant mice.²² In a mouse model of tumorigenesis, SCF-activated MCs exacerbated immunosuppression in the tumor microenvironment.¹⁴ MCs were shown to promote a decrease in mRNA for the immune activating factor IL-2, while inducing an increase in mRNAs for the immune suppressor factors IL-10, transforming growth factor beta (TGF- β) and forkhead box p 3 (Foxp3) in the tumor. The percentage of Treg cells in the total T-cell number in the tumor mass was also increased because CD4⁺CD25⁻ T cells can be converted into CD4⁺CD25⁺ regulatory T cells by TGF- β induced expression of Foxp3.²⁶

Another mechanism of potential physiological and pathological significance in MC-driven Treg cell expansion might be linked to the lipopolysaccharide (LPS) and interferon (IFN)- γ -induced expression of MHC class II on MCs. The expression of MHC-II grants MCs the ability to process and present antigens directly to T cells. In fact, MCs preferentially expand antigen-specific Treg cells over naïve T cells which may help explain allograft tolerance induction by MCs.²⁷ MCs are poor stimulators of naïve T cells in vitro. However, one consequence of MHC-II expression on MCs may be to activate regulatory Treg cells and thus dampen the immune response. Activation of Treg cells by MCs may also thus contribute to the protective effect of MCs on skin allografts.^{22,27} A recent publication supports the role of MC-Treg cell interactions in promoting immunosuppression and tumor development. The density of peritumoral MCs and Treg cells was evaluated to predict the prognosis of hepatocellular carcinoma.²⁸ It was found that higher peritumoral MC numbers were associated with poorer clinical outcome. Peritumoral Treg cells were positively correlated with MC density and inversely related to hepatocellular carcinoma outcomes. Further, MC number in combination with Treg cell number could predict prognostic outcomes more effectively than MC numbers alone. Thus, MCs and Treg cells may cooperate with each other in hepatocellular carcinoma to result in a poorer prognosis.²⁸

In conclusion, the pivotal role of MCs in the induction of an acquired condition of immune tolerance appears to be well established in various experimental settings. The secretion of immunosuppressive mediators by MCs seems to be critical for sustaining tolerance.

MAST CELLS AND TUMOR GROWTH IN HUMANS

As noted, MCs accumulate at sites of tumor growth in response to numerous chemoattractants, in particular SCF secreted by tumor cells.^{14,29} MCs are endowed with a vast array of mediators, some of which have promoting and others, inhibitory effects on malignancies.³⁰ Degradation of the extracellular matrix (ECM) plays a critical role in tumor expansion and metastasis. Dabbous et al³¹ showed that MC degranulation is associated

with disruption and lysis of the tumor ECM, either directly through the action of their enzymes, or indirectly through modulation of the collagenolytic activity of fibroblasts, macrophages and tumor cells.^{32,33} Tryptase activates latent MMP and plasminogen activator, which, in turn, degrade the ECM.³² MCs cause apoptosis in various target cells³⁴ and induce the accumulation of tumor associated inflammatory cells *in vivo*.³⁵

Heparin enhances both the activity and production of collagenase *in vitro* and release plasminogen activator from endothelial cells.^{36,37} MCs are a major source of histamine, which could induce tumor cell proliferation through H1 receptors identified in human malignant carcinoma, while suppressing the immune system through H2 receptors.³⁸ High histamine concentrations inhibit human primary melanoma-cell proliferation, presumably by acting through H1 receptors, an action enhanced by IL-2. Low amounts of histamine acting through H2 receptors increased this cell proliferation.³⁹ The incidence of metastases, as well as the appearance of tumors, correlates inversely with the tissue histamine level.⁴⁰ MCs may also exert direct mitogenic effect on tumor cells through additional mediators. For example, mast cell-derived fibroblast growth factor (FGF)-2 and IL-8 are directly mitogenic to melanocytes and melanoma cells.^{41,42}

Histological examination of many kinds of tumors has identified MCs in the inflammatory infiltrate. For instance, MC density in benign gastric ulcers was found to be much higher than in control subjects⁴³ and MC accumulation is also increased in well-differentiated gastric cancers when compared with controls. These observations suggest a role for MCs in the shift between inflammation and cancer. However, poorly-differentiated gastric adenocarcinomas showed lower MC density than well-differentiated adenocarcinomas.⁴³ MCs have also been detected in bilateral multiple spindle cell lipomas of the tongue.⁴⁴ It has similarly been suggested that MCs may be responsible for a fibrotic reaction occurring in the solitary sclerotic neurofibroma of the skin.⁴⁵ By electron microscopy, MCs were seen in close contact with fibroblasts and abundant collagen fibers were noted in the lesions. MCs have been found in great numbers at the periphery and in between the tumor islands of basal cell carcinomas.⁴⁶ In addition, MCs in basal cell carcinoma have been found to express VEGF, IL-8 and CCL5 (RANTES), suggesting an active MC involvement in the tumor microenvironment.⁴⁷ An increased number of MCs has been detected in the mononuclear cell infiltrate of telangiectasia macularis eruptiva perstans lesions,⁴⁸ in a case of conjunctival myxoma,⁴⁹ and in synovial sarcoma.⁵⁰

CORRELATION WITH PROGNOSIS

In many tumor settings, the number of infiltrating MCs has been shown to correlate with the clinical tumor stage and to represent a significant prognostic factor. A progressive increase in MC number associated with a increasingly poor prognosis has been shown during the progression of human melanoma and oral squamous carcinoma.^{51,52} Rojas et al⁵³ demonstrated a higher number of MCs in squamous cell carcinoma of the lip, as compared to normal lip. Sharma et al⁵⁴ reported a higher number of MCs in nodular sclerotic-type Hodgkin's lymphoma (HL) than in other types of HL, and the number of MCs was higher in fibrotic areas than in cellular areas. Molin et al^{55,56} observed a worse prognosis for a nodular sclerosing HL exhibiting a high MC number. Fukushima et al⁵⁷ demonstrated an increased number of MCs in fibrotic areas in diffuse large B-cell lymphoma (DLCL) lymph nodes. The greatest number of MCs among T-cell lymphomas was observed in

angioimmunoblastic T-cell lymphoma.⁵⁸ Dave et al⁵⁹ utilized gene array to study the relationship between prognosis and a specific gene expression profile and concluded that the length of survival among patients with follicular lymphoma correlated with the molecular features of nonmalignant immune cells present within the tumor at the time of diagnosis. One of the genes observed to correlate most negatively with survival was microphthalmia-associated transcription factor (MITF), a transcription factor which has been found to be highly expressed in MCs and to play a critical role in the regulation of several key MC-specific genes.⁶⁰ Tournilhac et al⁶¹ demonstrated that MCs may support tumor cell expansion in Waldenstrom's macroglobulinemia through constitutive CD154-CD40 signalling. Moreover, the use of a CD154-CD40 signal inhibitor partially inhibited MC mediated bone marrow lymphoplasmacytic proliferation and/or tumor colony formation.

Nonomura et al⁶² demonstrated in prostate cancer that MC counts were higher around cancer foci in patients with higher Gleason scores than in those with low Gleason scores. The MC number correlated with clinical stage and multivariate analysis revealed that MC count was a significant prognostic factor. By contrast, high intratumoral MC density was associated with favourable tumor characteristics and a good prognosis in prostate cancer.⁶³ These contradictory findings are consistent with a role for MCs in the immunological host-defense reaction to prostate cancer.

Gomes et al⁶⁴ detected an increased MC density in actinic cheilitis and in squamous cell carcinoma of the lip compared to normal oral mucosa, suggesting a role for MCs in the development and progression of these lesions. Similar findings were reported by Lago Costa et al.⁶⁵ In another study, cyclooxygenase (COX)-2 over-expression, tryptase-positive MCs and proteinase-activated receptor (PAR)-2 were investigated in normal lip biopsies and in lip biopsies with actinic cheilitis.⁶⁵ MC-derived tryptase was found to induce COX-2 expression by the cleavage of PAR-2. Increased epithelial co-expression of COX-2 and PAR-2, as well as an increase in the subepithelial density of tryptase-positive MCs were found in actinic cheilitis compared to normal lip. COX-2 overexpression was found to be a significant predictor of actinic cheilitis and to be correlated with both tryptase-positive MCs and PAR-2 expression. The authors concluded that tryptase may contribute to COX-2 up-regulation by epithelial PAR-2 activation during early lip carcinogenesis.⁶⁶ However, the number of tryptase- and KIT-positive MCs was found to be decreased in oral squamous cell carcinoma biopsies by Oliveira-Neto et al.⁶⁷

Aaltomaa et al⁶⁸ found a positive correlation between survival and increased MC number in a study of 187 breast cancer biopsies. Dabiri et al⁶⁹ analyzed the correlation between MC number in breast cancer and patients' prognosis in a study of 438 patients. They found a strong correlation between the presence of MCs and a favourable prognosis. Perivascular tumor-associated MCs in mammary adenocarcinoma could secrete several cytokines and proteolytic enzymes that could be detrimental to the tumor cells. For instance, IL-4, which binds to IL-4 receptors expressed by human breast carcinoma cells, could lead to apoptosis in breast cancer.⁷⁰ However, the histamine content of human breast cancer tissue is much higher than adjacent normal tissue and could act as a local immunosuppressant.⁷¹ Moreover, the mean level of serum tryptase in women with breast cancer is three-times higher than in healthy women.⁷² Kankkunen et al⁷³ similarly observed that the significant increase in MC counts in breast carcinoma versus benign lesions is due to tryptase-containing MCs. In benign lesions, the number of MCs exhibiting tryptase activity was similar to that of chymase containing MCs. Malignant tumors, however, had 2-3 times more tryptase-containing, than chymase-containing, MCs and the tryptase

activity was significantly higher than in benign lesions. Moreover, in malignant lesions, tryptase-containing MCs were concentrated at the tumor edge, whereas chymase-containing MCs were not increased in this area. Breast cancer patients with metastases in the axillary nodes revealed greater numbers of MCs in all nodes examined, compared with patients without metastasis.⁷⁴ Della Rovere et al,⁷⁵ however, argued that MCs may play a protective role against breast cancer cells. They found that there was a highly significant increase of MCs in infiltrating ductal breast cancers having high hormone receptor content with respect to both hypo-hormonal cancers in the same location and to controls. In another study, stromal MCs were found to correlate to low grade invasive breast carcinoma and estrogen receptor positivity.⁷⁶

Welsh et al⁷⁷ analyzed the presence of MCs in the tumor stroma of 175 patients with surgical resected non small cell lung carcinoma. They demonstrated that both macrophage and MC infiltration of the tumor islets was associated with a marked increase in 5-year survival, independently of other favourable prognostic factors including stage. Chan et al⁷⁸ studied samples of ovarian cancer from 44 patients and demonstrated that patients with tumors with higher microvascular density had a higher mean survival compared to those with tumors with low MC density or low microvessel density. Cinel et al⁷⁹ showed a significant correlation between high MC density and the presence of myometrial invasion in endometrial carcinomas. An increase in the number of tumor infiltrating tryptase-positive MC after IL-2 preoperative induction therapy predicted an improved clinical outcome in patients with malignant pleural mesothelioma. This highlights the critical role of the local inflammatory response in mesothelioma cancer progression.⁸⁰

ANGIOGENESIS FACTORS STORED IN MAST CELLS

MCs synthesize and store large amounts of molecular compounds exerting remarkable activity upon different aspects of angiogenesis. Heparin stimulates endothelial cell proliferation and migration *in vitro*.^{81,82} *In vivo*, however, it has been reported to stimulate,⁸³⁻⁸⁵ inhibit⁸⁵⁻⁸⁷ or have no effect^{88,89} on angiogenesis. These differences seem to be related to the molecular size and degree of sulphation of the heparin employed. Heparin acts as a soluble form of the low-affinity FGF-2 receptor,⁹⁰ displacing FGF-2 in the biologically active form and allowing FGF-2 rapid interaction with endothelial cells.⁹¹

Histamine has an angiogenic effect through both H1 and H2 receptors.⁹² It may also increase the permeability of newly formed microvessels during tumor angiogenesis and hence increase the leakage of plasma proteins and deposition of fibrin. Degradation products of fibrin are angiogenic *in vivo*.⁹³

MC synthesize and store large amounts of MMP-2, MMP-9 and serine-proteinases of two subclasses: tryptase and chymase.⁹⁴⁻⁹⁶ Given the ability of MMP-2 and MMP-9 to degrade Type IV, V, VII and X collagens and fibronectin,⁹⁷ the major components of the interstitial stroma and subendothelial basement membrane, these facts suggest that MCs may contribute to the *in situ* progression of invasive and metastatic solid tumors.⁹⁷ Tryptase and chymase are involved in angiogenesis after their release from activated MC granules. Their proteolytic activities degrade ECM components, release matrix-associated growth factors,⁹⁸ and act indirectly by activating latent MMP⁹⁹ and plasminogen activators.³² Blair et al¹⁰⁰ have demonstrated the angiogenic potential of tryptase *in vitro* and its important role in neovascularization. Muramatsu et al^{101,102} used the hamster sponge-implant model to show that angiogenesis is induced by angiotensin

II and inhibited by chymase inhibitors. These observations suggest that MC-derived chymase is an important mediator of MC-dependent angiogenesis.

MCs release polypeptide growth factors including FGF-2, VEGF, TNF- α , TGF- β and IL-8. These cytokines are involved in normal, as well as tumor-associated angiogenesis. The spectrum of cytokines expressed appears to vary depending on the maturity state of the MC and the tissue of residence. Qu et al¹⁰³ demonstrated that FGF-2 is localized in the cytoplasmic and extruded granules of MCs in several human tissues. Grutzkan et al¹⁰⁴ demonstrated the expression of VEGF in the human MC line HMC-1 and in human skin MCs. Nerve growth factor (NGF), also contained in MC secretory granules, induces endothelial cell proliferation in vitro and angiogenesis in vivo in the chick embryo chorioallantoic membrane (CAM) assay.^{105,106}

MAST CELLS IN EXPERIMENTAL TUMOR ANGIOGENESIS

Kessler et al¹⁰⁷ demonstrated that tumor angiogenesis factor (TAF) elicited a vasoproliferative response when placed upon the CAM of the chick embryo. The action of TAF might depend upon recruitment of MCs. Poole and Zetter¹⁰⁸ demonstrated that rat peritoneal MCs migrate in response to conditioned medium from several tumor cell lines. The active chemoattractant(s) in this conditioned medium appeared to be peptide(s) with a molecular weight of 300-1,000. They proposed that the chemoattraction of MCs by tumor-derived peptides may be an important early event in tumor neovascularization. Starkey et al¹⁰⁹ investigated the role of host MCs in tumor-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 tumors. The response was slower and initially less intense in W/W^v mice. Fewer W/W^v mice compared to ^{+/+} mice developed spontaneous lung metastases. Bone marrow repair of the MC deficiency restored the incidence of haematogenous metastases to approach that of ^{+/+} mice. These results demonstrate a role for MCs in vivo during tumor angiogenesis and also suggest a role for host MCs in hematogeneous metastases.

We have demonstrated that isolated MCs or their secretory granules, but not degranulated MC, induce an angiogenic response in the CAM assay.¹¹⁰ Addition of anti-FGF-2 or anti-VEGF antibodies reduced the angiogenic response of both MCs and their secretory granules by 50% and 30% respectively. These data provide 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 TUMOR ANGIOGENESIS

In the uterine cervix, tryptase-positive MCs increase in number and vascularization increases in a linear fashion from dysplasia to invasive cancer.¹¹¹ We have demonstrated that angiogenesis in human endometrial carcinoma is highly correlated with MC tryptase-positive cell counts and that these parameters increase with tumor progression.¹¹² An association of VEGF and MCs with angiogenesis has been demonstrated in laryngeal carcinoma,¹¹³ in non small cell lung carcinoma where most intratumor MCs express VEGF,¹¹⁴⁻¹¹⁶ and in melanoma where MCs express both VEGF¹¹⁷ and FGF-2.¹¹⁸ Lastly, a

prognostic significance has been attributed to MC and microvascular density in squamous cell cancer of the oesophagus,¹¹⁹ squamous cell carcinoma of the oral cavity⁵² and in melanoma.⁵¹ Imada et al¹¹⁴ studied 85 cases of Stage I non small cell lung carcinoma and demonstrated a higher number of tryptase-positive MCs compared to cases of squamous cell carcinoma. A high correlation was observed between intratumor MC counts and microvessel counts; and double immuno-staining showed that most intratumor MCs expressed VEGF.

In melanoma, MC accumulation around the margin of tumors has been observed to peak just as the tumor acquires the angiogenic phenotype and peri-tumor MC counts correlated strongly with microvascular density, melanoma progression and prognosis.^{51,117,118} Furthermore, in melanoma, MCs were closer to each other and to vessels.¹²⁰ This close association between MCs and the endothelium may indicate that MCs are involved in the maintenance and long lasting functional integrity of the endothelium.

Vascular density has been shown to be associated with a worse prognosis in Merkel cell carcinomas.¹²¹ Other factors associated with a worse outcome included tumor size, the presence of lymphovascular invasion and tumor MC count. The prognostic significance of microvessel density and MC density correlated with the survival of Thai patients with primary colorectal cancer.¹²² A significant positive correlation was found between microvessel density and MC density in the areas of highest vascular density. Patients with tumors of low microvascular density and low MC counts had significantly longer survival rates than those with hypervascular and high MC counts. Significant positive correlations between the microvessel density and MC counts in primary colorectal cancer were found also by Gulubova and Vlaykova.¹²³ Patients with hypovascular tumor tissues had significantly longer survival than those with hypervascular tumor biopsies. An analogous significant correlation was observed for tryptase-positive MC density: patients with low MC density had significantly better prognosis compared to those with high MC density. We have demonstrated that angiogenesis increases in parallel to the number of tryptase-positive MCs in lymph nodes from patients with breast cancer and that their values are significantly higher in lymph nodes with micrometastases as compared with those without metastasis.¹²⁴ In addition, significant correlations have been found between the number of tryptase-positive MCs, the area occupied by MCs positive for tryptase, the microvascular density and the endothelial area in early breast cancer patients.¹²⁵

Angiogenesis in benign lymphadenopathies and B-cell non-Hodgkin's lymphomas (B-NHL), measured as microvessels counts, was correlated with the total and MC tryptase-positive counts. Both increased in step with the increase in malignancy grades.^{126,127} In NHL, the cellular expression of VEGF and FGF-2, as well as MC and vessel counts, has been assessed.⁵⁸ The number of MCs were greater in T-cell lymphomas than in B-cell lymphomas; and in all NHL a significant correlation was found between vessel count and number of MCs in healthy lung and between vessel count and number of VEGF-expressing cells. Double fluorescence staining for VEGF mRNA and MC tryptase revealed that MCs expressed VEGF mRNA. These data suggest that MCs intervene in angiogenesis in these lesions by expressing VEGF. Glimelius et al¹²⁸ evaluated the relationship between the number of MCs and the microvessel count in tissue samples from NHL-involved lymph nodes by immunohistochemistry and did not find any correlation between a high microvessel count and the number of MCs.

Angiogenesis represents an essential step of disease progression in other haematological malignancies. In Waldenström's macroglobulinemia (WM), the bone marrow microvessel density is increased in 30-40% of patients, but this seems to have no impact on survival.¹²⁹

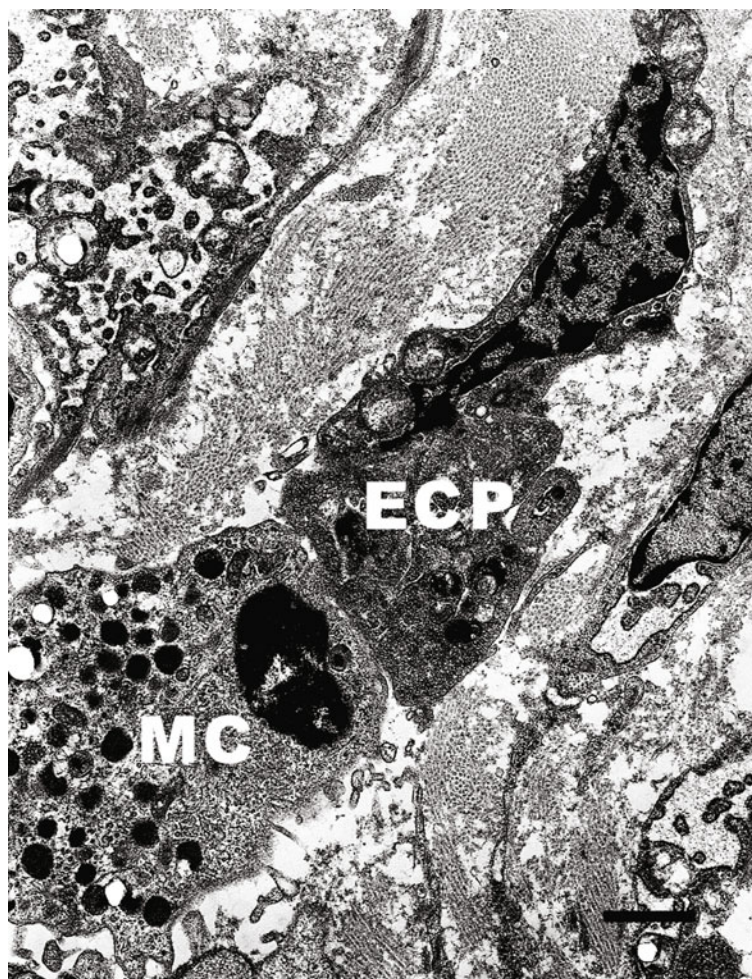


Figure 2. Electron micrograph of a human MC from the lymph node in a patient with B-cell non-Hodgkin's lymphoma. This MC is embedded in an area of intense angiogenesis and establishes a close contact with an endothelial cell precursor (ECP). Bar = 1 μ m.

Angiogenic cytokines produced by MCs, such as angiogenin, VEGF and FGF-2, are increased in the serum of WM or IgM- monoclonal gammopathies of undetermined significance (MGUS) patients. 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 MGUS. Both parameters increase simultaneously in active MM.¹³⁰ In both B-NHL and MM, MCs rest near or around blood or lymphatic capillaries (Fig. 2). Their ultrastructural picture includes piecemeal degranulation of their secretory granules, unlike the IgE-mediated massive degranulation that occurs during immediate hypersensitivity reactions.^{126,130-132} This morphology is typical of the slow degranulation that takes place in delayed hypersensitivity reactions and chronic

inflammation.¹³³ It may reflect slow but progressive release of angiogenic factors which favor chronic and progressive stimulation of MC degranulation.¹³⁴

Bone marrow samples of patients with myelodysplastic syndromes display a high correlation between microvessel counts and both total and tryptase-positive MCs. In addition, both parameters increase simultaneously with tumor progression.¹³⁵ 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 the clinical outcome.^{136,137}

DRUGS AFFECTING MAST CELL NUMBER

Preliminary studies using anti-KIT antibodies,¹⁴ anti-TNF- α antibodies⁶ or the MC stabilizer disodium cromoglycate (cromolyn)⁷ in mouse models, have demonstrated promising results even if administered after the initiation of tumor development. Treatment of mice bearing mammary adenocarcinoma and pancreatic cancer with cromolyn led to clotting of blood vessels, hypoxia and tumor cell apoptosis.^{7,72} Unfortunately, cromolyn is a weak inhibitor of human MC secretion and is poorly adsorbed, so it is unlikely to be effective in treating cancer in humans.

The first tyrosine kinase inhibitor introduced into the clinic, STI571 (imatinib mesylate, Gleevec), has inhibitory activity against the signalling cascade activated through KIT (CD117).¹³⁸ This inhibitory activity is the basis of the use of this drug against gastrointestinal stromal tumors (GIST), most of which harbour a KIT mutation.¹³⁹ STI571 is also in use for some varieties of mastocytosis, although some KIT activating mutations involved in mastocytosis are resistant to its inhibitory activity.¹⁴⁰ Ranitidine, an H₂ receptor antagonist, when used as adjunctive therapy, prolonged survival of colorectal cancer patients.¹⁴¹ Another H₂ receptor antagonist, famotidine, given pre-operatively, enhanced tumor infiltrating lymphocytes and increased metastatic lymph node reactive changes in breast cancer in humans.¹⁴² Molica et al¹⁴³ demonstrated that the reduction in the extent of bone marrow angiogenesis observed after sequential therapy with low doses of subcutaneous alantzumab after a clinical response to fludarabine induction therapy, was associated to a reduction in the number of MCs.

Dietary supplementation of silymarin, a naturally derived polyphenolic antioxidant, exerts a beneficial role in N-nitrosodiethylamine (NDEA)-induced liver cancer in Wistar albino male rats.¹⁴³ NDEA administered rats showed increased MC density, along with upregulated expression of MMP-2 and MMP-9. Silymarin treatment inhibited the increase in MC density and downregulated the expression of MMP-2 and MMP-9 as revealed by Western blotting and immunocytochemistry. Thus, silymarin may exert beneficial effects on liver carcinogenesis by attenuating the recruitment of MCs and thereby decreasing the expressions of MMP-2 and MMP-9.¹⁴⁴

CONCLUSION

MCs are found in multiple tissues and their presence in association with human cancer has been established for many years. However, multiple reports have described both a positive and a negative correlation between MC number and prognosis in various human tumor types (Fig. 3). These discrepancies are also observed in experimental studies, and

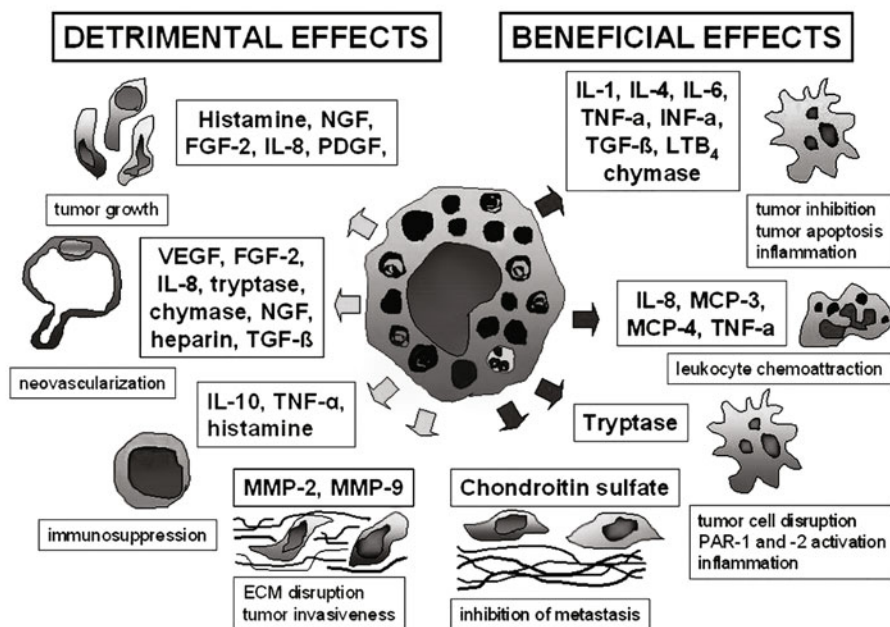


Figure 3. The dual role of MCs in tumor fate. MCs may release cytokines and growth factors in the tumor stroma, such as FGF-2, NGF, PDGF, IL-10 and IL-8, with detrimental effects to the host by stimulating tumor cell expansion. MCs are a major source of histamine, which can induce tumor cell proliferation through H1 receptors, while suppressing the immune system through H2 receptors. In addition, MCs synthesize and store angiogenic factors as well as matrix metalloproteinases, which promote tumor vascularization and tumor invasiveness, respectively. MCs may also generate immunosuppression by releasing IL-10, histamine and TNF- α . By contrast, MCs may promote inhibition of tumor cell growth, tumor cell apoptosis and inflammation by releasing cytokines such as IL-1, IL-4, IL-6 and TNF- α . In particular, IL-8, in particular, is effective in leukocyte chemoattraction. Chondroitin sulphate may inhibit tumor cell diffusion; and tryptase causes both tumor cell disruption and inflammation through activation of protease-activated receptors (PAR-1 and -2). Abbreviations: FGF-2, fibroblast growth factor-2; NGF, nerve growth factor; PDGF, platelet derived growth factor; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α ; INF- α , interferon- α ; LTB $_4$, leukotriene B $_4$; MCP-3, MCP-4, mast cell protease-3, -4; MMP-2, MMP-9, matrix metalloproteinase-2, -9; VEGF, vascular endothelial growth factor; IL-1, IL-2, IL-6, IL-8, IL-10, interleukin-1, -2, -6, -8, -10.

could be due to differences in the mouse model used and the stage of carcinogenesis investigated.

MCs are recruited in tumor development and play a key role in both angiogenesis and tissue remodelling by promoting both tumor initiation and growth. In addition, as tumor growth progresses, MCs recruit immune cells; or alternatively suppress antitumoral responses. Reports addressing MCs specifically as mediators in tumor immunosuppression come from Huang and coworkers.¹³ They mobilize MCs to infiltrate tumors by means of SCF. Tumor-derived SCF dependent MC infiltration and activation resulted in remodelling of the tumor microenvironment and tumor growth.

MCs could be a new target for the adjuvant treatment of tumors, through the selective inhibition of angiogenesis, tissue remodelling and tumor-promoting molecules, permitting the secretion of cytotoxic cytokines and preventing MC-mediated immune suppression. Moreover, some of the new targeted anticancer therapies have pronounced effects on

MCs; in fact, it may be that some of their antitumor effects are closely related to their effect on MCs.

ACKNOWLEDGEMENTS

Supported in part by MIUR (PRIN 2007), Rome and Fondazione Cassa di Risparmio di Puglia, Bari, Italy.

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