

Mast cells: the JEKYLL and HYDE of tumor growth

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Mast cells accumulate in the stroma surrounding certain tumors, especially mammary adenocarcinoma, and molecules they secrete could benefit the tumor. These include heparin, interleukin-8 (IL-8) and vascular endothelial cell growth factor (VEGF), which induce neovascularization, histamine, which is an immunosuppressant, mitogenic factors, such as platelet-derived growth factor (PDGF), nerve GF (NGF), stem-cell factor (SCF), and proteases, which disrupt the surrounding matrix and facilitate metastases. By contrast, mast-cell mediators detrimental to the tumor include cytokines, such as IL-1, IL-4, IL-6 and tumor necrosis factor- α (TNF- α), which can induce apoptosis of tumor cells, tryptase, which stimulates protease-activated receptors and induces inflammation, and chondroitin sulfate, which could act as a decoy and inhibit metastases. We propose that beneficial and destructive mediators are either released from separate granules or much smaller vesicles regulated by selectively distinct signals, such as tumor-derived oxidized polyamines or nitric oxide from new endothelial cells. This dual role of mast cells could be additive to that of tumor infiltrating macrophages, the 'polarization' of which to M2 type appears to be conducive to tumor growth.

Mast cells are important in allergic and late phase reactions and also in inflammation and T-cell-mediated immunity [1–3]. Despite substantial resources invested in basic cancer research, mortality rates for the most frequent forms of cancer have not been significantly reduced. Mutations alone, such as the presence of breast cancer susceptibility genes, are not sufficient to explain most cancers. Moreover, metastases are the chief cause of morbidity and mortality, which appears to be regulated by stromal proteolytic enzymes [4] and chemokines [5]. The stroma surrounding the tumor is clearly important for its growth and accumulating evidence appears to suggest that local inflammatory processes might actually contribute to the development of malignancy [6]. For instance, tumor-associated macrophages (TAMs) [7] and tumor-associated

fibroblasts (TAFs) are beneficial to tumor angiogenesis and growth [8] through secretion of epidermal growth factor (EGF) [9] and platelet-derived growth factor (PDGF) [10] or indirectly by promoting angiogenesis [9]. In one model of subcutaneous melanoma, both angiogenesis and growth rate correlated with tumor infiltration by macrophages that expressed angiotensin I receptor and vascular endothelial growth factor (VEGF) [11]. There have even been reports in animal models of hybrid macrophage–Cloudman melanoma cells with high metastatic potential. TAMs were also significantly correlated with stages of squamous cell carcinoma invasion [12]. Alternatively, TAMs could be detrimental to the tumor by presenting tumor-associated antigens to cytotoxic T cells [13] and increasing lymphocytic infiltration [7]. TAMs could become functionally 'polarized' to an 'M2' phenotype in response to tumor- and T-cell-derived cytokines, which subvert defense responses and promote tumor growth and metastases [6].

Mast cells accumulate at sites of tumor growth in response to numerous chemoattractants. These could include tumor-derived peptides as well as RANTES or monocyte chemoattractant protein-1 (MCP-1) [14]. Mast-cell infiltration has been implicated in tumor growth and is associated with poor prognosis [15]. Mast cells might be recruited by the tumor for its benefit, they might accumulate in reaction to the tumor, which somehow prevents their degranulation [16], or they might be innocent bystanders. However, this would not explain why they are increased (Figure 1). This potential dual role of mast cells is probable because of their ability to secrete either the content of individual granules [17] or distinct mediators selectively [18], possibly through regulation of specific phosphoproteins [19,20].

Mast-cell biology

Mast cells derive from a specific bone marrow progenitor cell and migrate into tissues where they mature depending on microenvironmental conditions. Mast cells are located perivascularly in close proximity to neurons and have recently been shown to have a crucial role in neuro-inflammatory diseases [21]. Mast cells vary considerably

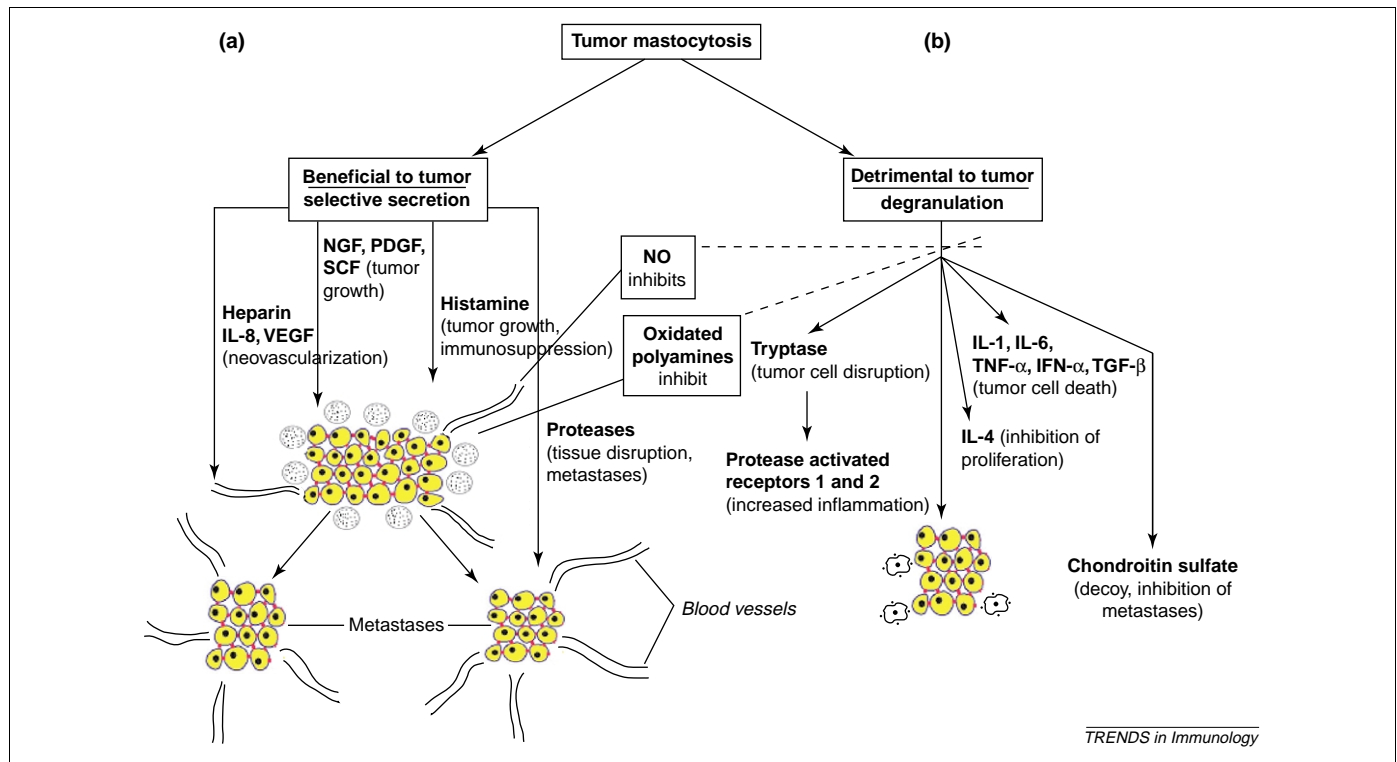


Figure 1. The possible role of the increased number of mast cells in the stroma of certain tumors. (a) Mast cells could be recruited by tumor-derived chemoattractants, such as MCP-1, RANTES and SCF, to selectively secrete molecules beneficial to the tumor; these could include growth factors, histamine, which is mitogenic (H1) and an immunosuppressant (H2), neovascularization agents, such as heparin, VEGF and IL-8, as well as proteases that could permit new blood vessel formation and metastases. Degranulation appears to be blocked by tumor-derived inhibitors, such as oxidized polyamines or NO derived from new blood vessels. (b) Mast cells could accumulate in reaction to the tumor. They could degranulate and secrete molecules, such as IL-4 and TNF- α , which induce apoptosis, IL-1 and IL-6, which are proinflammatory, or tryptase, which could stimulate protease-activated receptor-induced inflammation, as well as chondroitin sulfate, which could block metastases. Abbreviations: IFN- γ , interferon- γ ; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; NGF, nerve growth factor; NO, nitric oxide; PDGF, platelet-derived growth factor; SCF, stem cell factor; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α ; VEGF, vascular endothelial cell growth factor.

in their cytokine and proteolytic enzyme content. At least two easily identifiable types of human mast cells have been reported: connective tissue mast cells that contain tryptase and chymase (TC mast cells), and mucosal mast cells that contain only tryptase (T mast cells). These two cell types differ in the number and type of secretory granules they contain, as well as their responsiveness to stimuli. For instance, TC mast cells contain more heparin, whereas T mast cells contain more chondroitin sulfate; in addition, TC mast cells respond to neuropeptides, whereas T mast cells do not.

However, the phenotypic expression of mast cells is not limited to those two types and is not fixed because T mast cells can develop into CT mast cells given the appropriate microenvironmental conditions, such as the presence of stem-cell factor (SCF), nerve growth factor (NGF), interleukin-6 (IL-6) and IL-4. Moreover, addition of IL-5 to human umbilical cord blood-derived cultured mast cells was recently shown to augment IgE-induced production of distinct cytokines, such as tumor necrosis factor- α (TNF- α), macrophage inflammatory protein-1 α (MIP-1 α) and granulocyte-macrophage-colony stimulating factor (GM-CSF), but without histamine [22]. In addition to IgE and antigen, the anaphylatoxins (C3a, C5a), the cytokines (IL-1, SCF), hormones (CRH) and neuropeptides (SP, NT, CGRP, NGF) can trigger mast-cell activation leading to secretion of numerous mediators but through different pathways. For instance, IL-1 and SCF [23] induce selective

release of IL-6 without histamine and without degranulation. Moreover, in certain diseases, such as scleroderma and interstitial cystitis [24], mast cells could be almost totally depleted of their granule content, without classic degranulation, rendering them undetectable by light microscopy (phantom mast cells) [25]. Mast cells, therefore, have the ability to be one of the most versatile cells in the body, owing to their ubiquitous location and the plethora of biologically active molecules produced. Some of these molecules are also synthesized by TAMs [6] and it is interesting that these, as we propose for mast cells, can secrete molecules that are either detrimental (M1-type) or beneficial (M2-type) to the tumor (Table 1). The tumor stroma microenvironment could not only alter the phenotypic behavior of mast cells but might also alter its secretory processes. For instance, acidity created by H₂O₂ was shown to inhibit allergic degranulation but enhance IL-4 production [26]. Nitric oxide (NO) generated from new vessels could inhibit mast-cell degranulation [27] as could oxidized polyamines secreted by the tumor [28].

Mast cells: beneficial to the tumor?

Mast cells appear to be able to promote tumor development through many different ways (Figure 1a): they could facilitate tumor angiogenesis through heparin-like molecules, and heparin could further permit neovascularization and metastases through its anti-clotting effects. Moreover, vascular endothelial cell growth factor [VEGF;

Table 1. Comparison of mast cell and macrophage mediators of tumor growth

Mediators		Main pathophysiologic effects
Beneficial effects on cancer		
Mast cells	Macrophages (M2)	
<i>Biogenic amines</i>		
Histamine		Vasodilation, mitogenesis, immunosuppression
<i>Chemokines</i>		
MCP-1, RANTES	CCL17, 22, 24, 16, 18	Chemoattraction for mast cells
<i>Cytokines</i>		
IL-8	IL-1ra, IL-10, IL-13	Neovascularization
<i>Enzymes</i>		
Chymase		Tissue damage
Kinogenases		Synthesis of vasodilatory kinins
Tryptase		Tissue damage, metastases
<i>Growth factors</i>		
CSF, NGF, PDGF, SCF		Tumor growth
VEGF		Angiogenesis, neovascularization
<i>Proteoglycans</i>		
Heparin		Angiogenesis, NGF stabilization, patent vessels
Detrimental effects on cancer		
Mast cells	Macrophages (M1)	
<i>Chemokines</i>		
IL-8, MCP-3, MCP-4	CXCL8	Leukocyte chemoattraction
<i>Cytokines</i>		
IL-1, -2, -3, -5, -6, -9, -10, -13, -16	IL-1	Inflammation, leukocyte migration
IL-4	IL-6	Tumor cell apoptosis
IFN- γ	IL-12	Inflammation, leukocyte proliferation and activation
TNF- α	TNF- α	Inflammation, tumor cell death
<i>Growth factors</i>		
GM-CSF, TGF- β		Inflammatory cell proliferation
<i>Proteoglycans</i>		
Chondroitin sulfate		Acts as decoy and prevents metastases
<i>Arachidonic acid products</i>		
LTB ₄		Leukocyte chemotaxis
PAF		Platelet activation and serotonin release
PGD ₂		Vasoconstriction
LTC ₄		Vasoconstriction

Abbreviations: CSF, colony stimulating factor; GM-CSF, granulocyte-monocyte-CSF; IFN- γ , interferon- γ ; IL-8, interleukin-8; LTB₄, leukotriene B₄; MCP-1, macrophage inflammatory factor-1; NGF, nerve growth factor; PAF, platelet activating factor; PDGF, platelet-derived growth factor; PGD₂, prostaglandin D₂; SCF, stem cell factor; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α ; VEGF, vascular endothelial cell growth factor.

vascular permeability factor (VPF)] is secreted in response to Fc ϵ RI crosslinking from mouse bone marrow-derived and human cultured mast cells [29], as well as from human leukemic mast cells (HMC-1) [30]. Mast cells also generate and secrete IL-8, which is an angiogenesis factor as well as a tumor cell chemotactic factor and tumor mitogen. In fact, mast-cell-deficient W/W^v mice exhibited a decreased rate of tumor angiogenesis [31]. Ongoing experiments in our laboratory use the mast-cell-deficient Ws/Ws rats [32] to investigate if the carcinogen nitroso methyl urea (NMU) can induce mammary gland tumors. Mast cells secrete growth factors, such as PDGF, SCF and NGF. They also secrete histamine that could induce tumor cell proliferation through H1 receptors identified in human malignant carcinoma, while suppressing the immune system through H2 receptors.

A considerable number of papers have recently addressed the potential role of histamine in promoting or inhibiting tumor growth *in vivo*. This question was prompted by some *in vitro* findings suggesting that certain compounds with anti-histaminic activity might promote proliferation of cultured cancer cells; however, further analysis of those papers disputed those contentions. In fact, terfenadine inhibits growth of human cancer cells [33]. More recent laboratory and clinical data concerning histamine are also confusing. For instance, high histamine

concentration inhibited human primary melanoma-cell proliferation, presumably by acting through H1 receptors, an action enhanced by IL-2, whereas low amounts through H2 receptors increased proliferation [34]. However, addition of histamine to other drug regimens did not alter outcomes or side effects. The predominance of the evidence suggests that H2-receptor antagonists might be beneficial by blocking histamine-induced immunosuppression. Ranitidine, used as adjuvant therapy, prolonged survival of colorectal cancer patients [35]; it also had a weak effect on the growth of melanoma cells in mice, whereas cimetidine was more effective. Another H2 receptor antagonist, famotidine, given pre-operatively for 14 days, enhanced tumor infiltrating lymphocytes and increased metastatic lymph node reactive changes in breast cancer in humans [36]. Nevertheless, pre-operative administration of cimetidine did not influence tumor-cell proliferation [37].

Mast cells and macrophages are rich in metalloproteases that contribute the majority of proteolytic components necessary for tumor invasiveness [4]. Mast cells could disturb the normal stroma-epithelium communication, as was shown for matrix degradation at sites of tumor invasion in rat mammary adenocarcinoma. [38] Mast cells could also regulate the permeability of the blood-brain barrier (BBB) and promote brain metastases;

in particular, acute stress can activate mast cells and increase BBB permeability that is mast-cell-dependent [39]. These findings are of importance in view of the fact that acute stress could increase metastases and >30% of breast cancer patients develop brain metastases with poor associated prognosis [40]. Bone metastases and bone lesions could also involve mast cell-derived molecules, such as IL-6. For instance, increased serum IL-6 correlated with the extent of cancer and with worse survival in patients with metastatic breast cancer [41]. In fact, serum IL-6 was increased, unlike tryptase, in systemic mastocytosis patients with bone involvement.

The only way mast cells could be helpful to the tumor is if secretion of beneficial molecules from mast cells could occur selectively without degranulation. This mechanism appears to be possible because differential release of serotonin without histamine had been reported over 20 years ago [18]. In addition, IL-6 could be secreted without histamine *in vitro* [23]. Moreover, VEGF (VPF) was secreted without parallel serotonin from bone marrow-derived mouse mast cells [29]. Such processes have been termed 'differential release,' 'intragranular activation' or 'piecemeal degranulation' [42]. We recently showed that IL-1 could induce selective secretion of IL-6 from human cultured mast cells without degranulation through a unique vesicular shuttle [43]. IL-1 can also stimulate secretion of VEGF [44], as well as promote angiogenesis and tumor growth [44]. This 'alternative activation model' could be similar to what is known of monocyte activation that leads to the generation of macrophages, which could in turn acquire different functional roles. The mast cell could change both its differentiation and secretory ability status, so that it no longer conforms to its classic anaphylactic degranulation, known in allergic diseases. This possibility could depend on several conditions. Tumors might be more or less sensitive to mast cell-derived molecules. The timing of mast-cell infiltration might also be crucial because early recruitment might be essential for the initiation of delayed hypersensitivity reactions [45] but might become detrimental later. The state of mast-cell differentiation and activation might also be important because of the type of molecules it could synthesize and store, as well as its responsiveness to triggers.

Mast cells: detrimental to the tumor?

Mast cells could increase at sites of breast cancer and associated lymph nodes in reaction to the tumor and might participate in tumor rejection (Figure 1b). Certain findings suggest that where tumor burden is high, mast cells might be inhibited from degranulation by tumor-derived blockers, such as oxidized polyamines [28].

Perivascular tumor-associated mast cells in mammary adenocarcinoma could secrete several cytokines and proteolytic enzymes that could be detrimental to the tumor cells (Figure 1). Tryptase could stimulate protease-activated receptors (PAR-1 and -2), also activated by thrombin and trypsin, respectively, and induce widespread inflammation [46]. Cytokines, such as IL-4, which binds to IL-4 receptors (IL-4Rs) expressed by human breast carcinoma cells [47], could lead to apoptosis in breast

cancer [47]. TNF- α could also induce tumor-cell death [48]. Heparan sulfate proteoglycans could block binding of heparin to the cell surface and prevent neovascularization [49]; for instance protamine, which binds to heparin and neutralizes its anticoagulant properties, could induce selective thrombosis of blood vessels within mammary adenocarcinoma [50]. Moreover, treatment of mice bearing mammary adenocarcinoma with the mast-cell 'stabilizer' disodium cromoglycate (cromolyn) led to clotting of blood vessels and hypoxia [51]. Cancer cells express sulphated glycosaminoglycans (s-GAGs), chondroitin sulphate (CS) and heparin/heparan sulphate, which accumulate in mammary gland tumors and in metastatic lesions; [52] tumor cells metastasize through binding of their surface glycoproteins to other cellular elements and to the interstitial matrix [53]. Secretion of chondroitin sulfate from mast cells could stop tumor cells from metastasizing by acting as a decoy [53]. Exogenous administration of CS inhibited metastasis of ovarian carcinoma [53].

Breast cancer

An increased number of mast cells has been noted in rat mammary tumors when the carcinogen, cis-hydroxyproline, is used in Buffalo rats [54]. Interestingly, rat mammary adenocarcinoma induced by 7,12-dimethylbenz (α)anthracene (DMBA) is also associated with a high number of mast cells, however, these are resistant to degranulation [55]. Similar findings were obtained from human mammary adenocarcinoma biopsies, in which accumulated mast cells in an area of intense tumor infiltration appeared to be intact (Figure 2a,b). However, mast cells in an area of marginal tumor growth appear to be degranulated (Figure 2c). For instance, patients with longer survival have a significantly higher number of mast cells in their axillary lymph nodes. Moreover, it was recently reported that downregulation of VEGF expression was insufficient to resist mammary carcinogenesis and that an enhanced immune response, as evidence by intermammary lymph node enlargement with mast-cell infiltration, might be more important [56]. The location of mast cells and their numbers in relation to tumor cells might, therefore, be important, as also recently shown for TAMs. The histamine content of human breast cancer tissue is much higher than adjacent normal tissue and sufficient to act as a local immunosuppressant [57]. Moreover, the mean level of serum tryptase in women with breast cancer is three-times higher than in healthy women [51].

Recently, using tissue recombination techniques, it was shown that mammary carcinogenesis in Wistar-Furth rats occurs only when the stroma of the mammary gland (fat pad) is exposed to the NMU [58]. The earliest effects of carcinogen administration also involved stroma infiltration of mammary adenocarcinomas with mast cells (T.C. Theoharides, unpublished). Disruption of the normal flow of information between stroma and parenchyma could permit neoplastic progression [59]. Manipulation of the microenvironment, as with stromal matrix metalloproteinases, rather than the target cell, promotes mammary tumorigenesis [60]. Irradiated mammary gland stroma promoted carcinogenesis of unirradiated epithelial cells

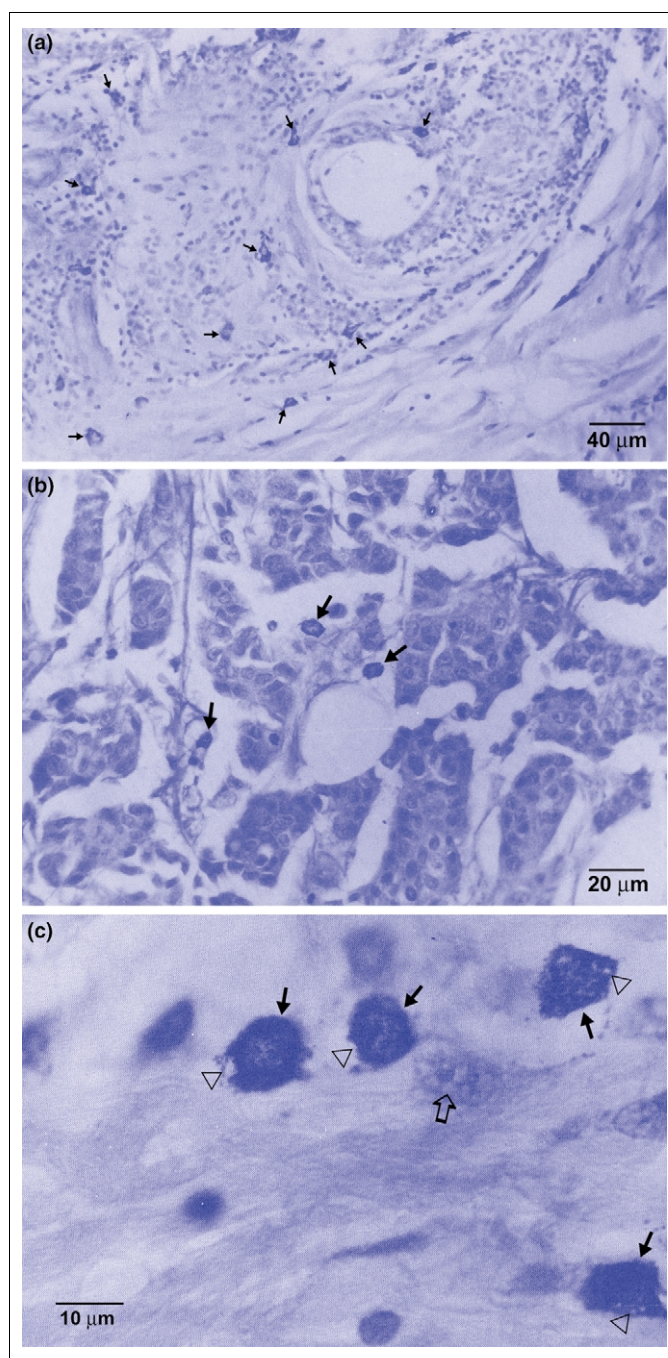


Figure 2. Photomicrographs of human breast cancer biopsies. (a) Light micrograph characterized by intense infiltration of ductal adenocarcinoma with numerous intact mast cells (arrows) stained with acidified toluidine blue (scale bar, 40 μ m). (b) Higher magnification showing intact periductal mast cells (arrows) among infiltrating adenocarcinoma cells (scale bar, 20 μ m). (c) High magnification of a marginal area around breast adenocarcinoma, in which the number of mast cells (black arrows) is greater than that of the cancer cells (open arrow). Note that the mast cells show areas of degranulation (open triangles) and one tumor cell appears not to be viable (open arrow) (scale bar, 10 μ m).

[61]. Endometrial stroma cells also regulate epithelial cell growth *in vitro* [62]. Such stromal–epithelial-cell interactions could regulate chemical carcinogen-induced cancer-cell proliferation and differentiation.

Basal-cell carcinoma and melanoma

Mast cells have been repeatedly noted to accumulate around basal-cell carcinoma lesions and are thought to

contribute to cancer growth by inducing immunosuppression [63]. In addition, mast cells in basal-cell carcinoma express VEGF, IL-8 and RANTES.

Mast-cell accumulation has also been noted repeatedly around melanomas, especially invasive melanoma [64,65]. In fact, mast-cell accumulation was correlated with increased neovascularization, mast-cell overexpression of VEGF, tumor aggressiveness and poor prognosis. Moreover, c-kit product and SCF splice variants were detected in melanoma and could present new forms of mast-cell growth factors related to melanoma growth. Mast-cell secretion blockers have also been shown to interfere with melanoma growth *in vitro*.

Neurofibromatosis

Mast cells have been noted around peripheral nerves and nerve tumors. An increased number of mast cells has been reported around neurofibromatosis type 1 (NF1) lesions, in which c-kit and SCF have been implicated in mast-cell proliferation [66]. It is interesting to note that the NF1 tumor suppressor gene product modulates both melanocyte and mast-cell growth [67]. The histamine-1 receptor antagonist ketotifen reduces NF1 growth [68]. A recent publication reports that in NF1 patients and NF1^{+/−} mice, mast cells are recruited and create an environment that permits initiation of neurofibroma tumor formation [69]. This beneficial action might be at least partly due to increased angiogenesis.

Concluding remarks

Mounting evidence indicates that mast cells accumulate around tumors and could either promote or inhibit tumor growth depending on the local stromal conditions. Mast cells might, therefore, act as a new target for the adjuvant treatment of solid tumors, such as mammary adenocarcinoma or melanoma, through the selective inhibition of tumor-promoting molecules but permitting secretion of cytotoxic cytokines. Certain natural substances could fulfill these inhibitory requirements [70,71].

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