

Mast Cell-Targeted Strategies in Cancer Therapy

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Keywords

Mast cells · New targets · Anti-cancer therapy

Summary

Mast cells (MCs) are cells that originate in the bone marrow from pluripotent CD34⁺ hematopoietic stem cells. Precursors of MCs migrate through the circulation to their target tissues, completing their maturation process into granulated cells under the influence of several microenvironment growth factors. The most important of these factors is the ligand for the c-Kit receptor (c-Kit-R) namely stem cell factor (SCF), secreted mainly by fibroblasts and endothelial cells (ECs). SCF also regulates development, survival and de novo proliferation of MCs. It has already been demonstrated that gain-of-function mutations of gene c-Kit encoding c-Kit-R result in the development of some tumors. Furthermore, MCs are able also to modulate both innate and adaptive immune response and to express the high-affinity IgE receptor following IgE activation. Among the other IgE-independent MC activation mechanisms, a wide variety of other surface receptors for cytokines, chemokines, immunoglobulins, and complement are also described. Interestingly, MCs can stimulate angiogenesis by releasing of several pro-angiogenic cytokines stored in their cytoplasm. Studies published in the last year suggest that angiogenesis stimulated by MCs may play an important role in tumor growth and progression. Here, we aim to focus several biological features of MCs and to summarize new anti-cancer MC-targeted strategies with potential translation in human clinical trials.

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Introduction

Mast cells (MCs) are derived from the myeloid stem cell and are part of the innate immune system. Although best known for their role in allergy and anaphylaxis, MCs play an important protective role as well, being intimately involved in wound healing and defense against pathogens. MCs are present in most tissues, characteristically surrounding blood vessels and nerves, and are especially prominent near the boundaries between the outside world and the internal milieu, such as the skin, mucosa of the lungs and digestive tract, as well as in the mouth, the conjunctiva, and the nose [1–5].

MCs express a high-affinity receptor for the Fc region of IgE, the least-abundant member of the antibodies. This receptor is of such high affinity that binding of IgE molecules is in essence irreversible. As a result, MCs are coated with IgE, which is produced by plasma cells. MCs release their preformed mediators when they encounter the complement anaphylatoxins C3a and C5a, and organisms that attack humans often produce exogenous factors (e.g. bacteria-derived ADP and mite-derived proteases) that also induce the release of the granule constituents of MCs via different surface receptors. MC degranulation is also stimulated by the activation of its membrane tyrosine kinase receptor, the c-Kit receptor (CD117), by means of the stem cell factor (SCF) [6–8].

MC granules are key functional elements that are characterized by two distinct secretory patterns: exocytosis and piecemeal degranulation. Interestingly, this latter mechanism, representing a slow and selective pathway of cell secretion, has been more frequently observed in MC-infiltrating areas of chronic inflammation, such as tumor tissues. Correspondingly, a link between MCs, chronic inflammation and cancer has long been suggested. Thus, MCs are one of the earliest and major inflammatory cell types recruited into the tumor microenvironment [9, 10].

MCs have been involved in tumor angiogenesis of several human and animal malignancies. It has been well demonstrated that MCs can secrete several classical and non-classical pro-angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), platelet-derived growth factor- β (PDGF- β), IL-6, IL-8, thymidine phosphorylase (TP), and chymase. MCs can secrete other molecules such as tryptase and TNF- α that play a role in angiogenesis, and not in immune response [11–26].

In light of this, our paper aims at examining the specific contribution of MCs to cancer, also shedding light on the existing MC-based therapeutic approaches to be evaluated.

Mast Cells in Cancer

MCs are important in allergic and late-phase reactions, inflammation, and the regulation of adaptive T-cell-mediated immunity: MCs mobilize T cells and antigen-presenting dendritic cells. However, the role of MCs in the tumorigenesis of cancers is not totally clear, and data about their benefit or detriment to tumorigenesis have been controversial, depending on the local stromal conditions [27–29].

Tumor cells produce inflammatory mediators and pro-angiogenic factors, including SCF. Activation of the SCF/Kit pathway is necessary for the maturation, migration, and survival of MCs since they derive from hematopoietic precursors inside the bone marrow and complete their differentiation and maturation within vascularized tissues. The surrounding environment of tumors, through SCF chemotaxis, promotes infiltration and maturation of MCs, which release angiogenic mediators, proteases, and growth factors that support tumor development.

Studies have allowed substantial progress in understanding the role of MCs in tumorigenesis and progression but further studies are necessary to completely elucidate their impact on the pathophysiology of cancer [30–37].

MCs can directly influence tumor cell proliferation and invasion but also help tumors indirectly by organizing its microenvironment and modulating immune responses to tumor cells. MCs are best known for orchestrating inflammation and angiogenesis, and their role in shaping adaptive immune responses has become a focus of recent investigations [38–41].

The central role of MCs in the control of innate and adaptive immunity endows them with the ability to tune the nature of host responses to cancer and ultimately influence the outcome of disease and fate of the cancer patient [42].

MCs could stimulate growth, neo-angiogenesis and metastasis of tumors by multiple mechanisms. MCs are involved in innate immunity by releasing TNF- α and interleukins (IL-1, IL-4, IL-6). In addition, MCs express both the major histocompatibility class (MHC) II antigen and its costimulatory molecule, which activate adaptive T- and B-cell responses. Cytokines, secreted by stromal cells, can exacerbate the malignant phenotype of cancer cells. By producing chemoattractant molecules, cytokines recruit inflamma-

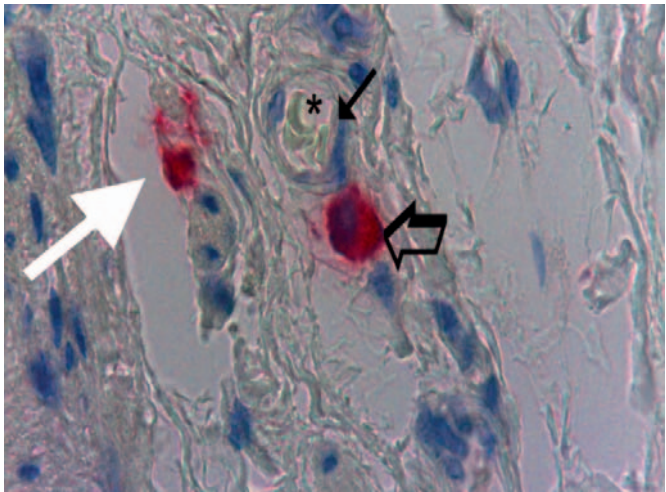


Fig. 1. MCs degranulation in primary colon cancer tissue (white arrow). Mast cells positive to tryptase (black big arrow) and the adjacent microvessels with an asterisk in its lumen (black small arrow).

Table 1. Mediators stored in the granules of MCs

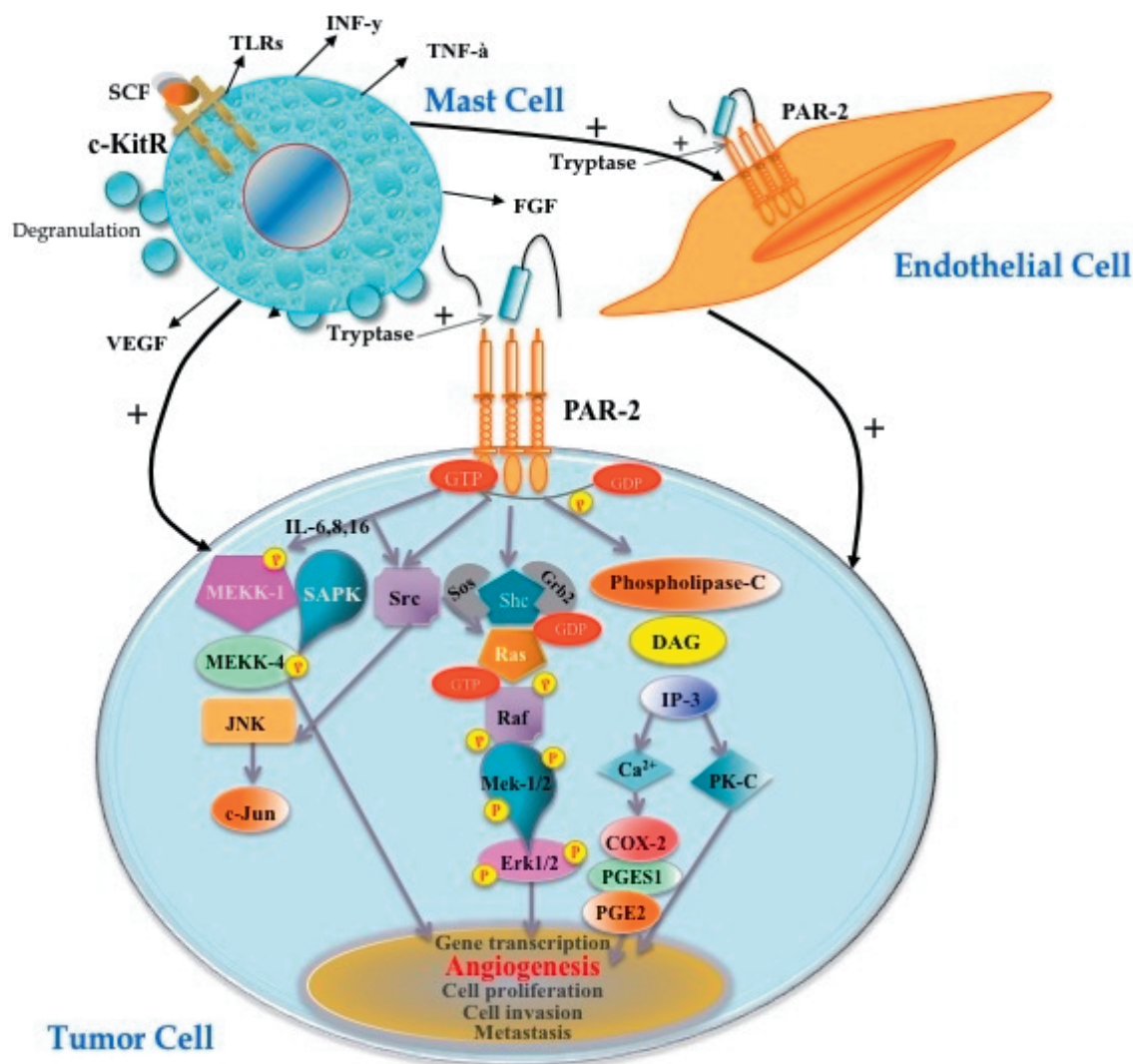
IL-16
TNF- α
Platelet-derived growth factor
IL-6
IL-8
VEGF
GM-CSF
IFN- γ
TGF- β
FGF
Tryptase
M-CSF
Heparin
Chymase
Serotonin
Histamine

tory cells into tumor sites, influencing them in a way that ultimately promotes cancer progression (fig. 1) [42–46].

MCs usually express Toll-like-receptors (TLRs), a class of proteins that play a key role in the innate immune system. TLRs are a type of patterns recognition receptor (PRR) and recognize molecules that are broadly shared by pathogens, collectively referred to as pathogen-associated molecular patterns (PAMPs) [47]. TLRs together with the IL-1 receptors, form a receptor superfamily known as the ‘IL-1 receptor / toll-like receptor superfamily’. All members of this family have in common a so-called TIR (Toll-IL-1 receptor) domain [48]. TLR signaling is divided into two distinct signaling pathways: i) the MyD88-dependent pathway with the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and mitogen-activated protein kinase (MAPK), and ii) the TIR-domain-containing adapter-inducing IFN- β (TRIF)-dependent pathway with activation of serine/threonine-protein kinase-1 (TBK1) and receptor-interacting serine/threonine-protein kinase 1 (RIPK-1). They also have different adaptors

Fig. 2. Mediators, released after MCs activation of c-KitR/SCF-mediated, have angiogenic activity stimulating both human vascular endothelial and tumor cell proliferation in paracrine manner, helping tumor cell invasion and metastasis. c-KitR = c-Kit receptor; PAR-2 = proteinase-activated receptor-2; SCF = stem cell factor; FGF = fibroblast growth factor; VEGF = vascular endothelial growth factor; TLRs = Toll-like-receptors; NHERF-1 = Na⁺/H⁺ exchanger regulatory factor-1; MEKK-1 = mitogen-activated protein kinase/extracellular signal-related kinase-1; MEKK-4 = mitogen-activated protein kinase/extracellular signal-related kinase-4; JNK = c-Jun N-terminal kinase; c-Jun = Jun protooncogene; SAPK = mitogen-activated protein kinase-9; GEF = rho/rac guanine nucleotide exchange factor; Rho = rhodopsin transcription termination factor; SOS

= SON of sevenless protein; Grb2 = growth factor receptor-bound protein 2; Shc = Shc transforming protein kinase; Ras = Ras protein kinase; Raf = Raf protein kinase; MEKK-1/2 = mitogen-activated protein kinase/extracellular signal-related kinase-1/2; Erk = Elk-related tyrosine kinase; DAG = diacylglycerol; IP-3 = inositol triphosphate; PK-C = protein kinase-C; COX-2 = cyclooxygenase-2; PGE2 = prostaglandin E2; PGES-1 = prostaglandin E synthase-1; PK-A = protein kinase-A.



to respond to activation and are located sometimes at the cell surface and sometimes at internal cell compartments. Following their activation by pathogen ligands, several reactions are possible: inflammation, tumor growth, and angiogenesis [49]. However, the ability of TLRs agonists to cause MC degranulation, migration and cytokine/chemokine production is controversial. In fact, some researchers have been unable to demonstrate degranulation after stimulation of MCs with TLR ligands [50].

MCs are then activated either by direct contact or by cancer cell-derived triggers to release selectively various pro-cancer and pro-angiogenic mediators (table 1) [51–54].

One of these pro-angiogenic mediators is tryptase, stored in MC secretory granules. It is an agonist of the proteinase-activated receptor-2 (PAR-2) in vascular endothelial cells that stimulates their proliferation. Protease-activated receptors (PARs) belong to the G protein-coupled receptor family. Four forms of PARs have been reported (PAR-1 to PAR-4). Proteases cleave the N-terminus to generate a tethered ligand, which interacts and activates the receptor.

Signaling via PAR-2 elicits activation of the major members of the MAPK phosphorylation family and contributes to a pro-malignant transcriptional program and stimulates oncogenic protein synthesis. PAR-2 activation also leads to the production of pro-angiogenic factors, such as VEGF, IL-8, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and macrophage colony-stimulating factor (M-CSF) (fig. 2) [55–59].

Targeting Mast Cells in Cancer Therapy

The precise role of MCs in tumor development and progression will be of critical importance for the development of new targeted therapies in human cancers.

Although MCs offer an attractive target in tumor for new therapies, still limited data are available in literature for the real clinical use in cancer patients care. From a therapeutic point of view, in vitro/in vivo tumor models treated with inhibitors of MC degranu-

Table 2. All studies mentioned above that have considered MCs target in cancer therapy

Reference	Approach	Models	Target
Oldford et al., 2010 [66]; Wei et al., 2012 [67]	drug therapy	in vitro/in vivo	TLR-activated MCs agonists in melanoma, lung and brain tumors
Schmidt et al., 2007 [68]	clinical trial phase I/II	in vivo/human	TLR-2/6 agonist MALP-2 in combination with gemcitabine in pancreas carcinomas
Ammendola et al., 2014 [13]; Ranieri, 2012 [69],	drug therapy	in vitro/in vivo	MC degranulation factors in solid tumors
Leporini et al., 2015 [70]	drug therapy	in vitro/in vivo	MCs positive to tryptase or c-Kit-R in gastric cancer and bone metastases

lation presented decreased growth, vascularization, and metastases. According to these data, targeting MCs is currently under investigation. Actually, therapies with tyrosine kinase inhibitors (imatinib and masitinib) for c-Kit receptor-targeted action and MC tryptase inhibitors (gabexate mesylate, nafamostat mesylate and tranilast) represent the most used clinical treatments against MCs in cancer [8, 60–65].

Experimental in vivo/in vitro results showed the novel anti-tumor effect of TLR-activated MCs agonists in melanoma as well as in lung and brain tumors [66, 67]

An interesting phase I/II trial on 10 patients explored safety and efficacy of the TLR-2/6 agonist MALP-2 in combination with gemcitabine in patients with incompletely resectable pancreas carcinomas. Results showed a mean survival of 17.1 ± 4.2 months and a median survival of 9.3 months [68].

Preliminary in vivo/in vitro results were obtained by other researchers. Their data suggested that therapeutic targeting of MCs degranulation factors could be a novel strategy to inhibit tumor growth and neo-angiogenesis [69].

Finally, some authors focused on the potential use of MCs targeting agents, such as MC tryptase inhibitors (gabexate mesylate, nafamostat mesylate) or c-Kit-R tyrosine kinase inhibitors (imatinib, masitinib) as possible new anti-angiogenic and antiresorptive strategies for the treatment of gastric cancer patients affected by bone metastases [70]. The studies mentioned above are summarized in table 2.

Conclusions

The role of MC infiltrates in tumors is still unclear and merits particular attention. Unveiling complex interactions between MCs, microenvironment and tumors could provide insights into the understanding of disease pathogenesis. This may open new avenues in expanding the arsenal of targeted therapies aiming to induce tumor growth arrest and tumor cell response to chemotherapy. From a conceptual point of view, we think that, if MCs may contribute to stimulate tumor progression and angiogenesis, the concomitant inhibition of MCs or one of its degranulation factors may be a novel strategy worthy of further investigation. For these reasons, we speculate that a combination chemotherapy of tryptase inhibitors or c-Kit receptor inhibitors and classical cytotoxic drugs could potentially exert a synergistic anti-tumor effect. In this context, novel agents killing MCs might be evaluated in adjuvant clinical trials as a new anti-cancer approach.

Authors' Contributions

Michele Ammendola, Girolamo Ranieri, and Giuseppe Sammarco contributed to think up the design of manuscript and to perform the critical review of the literature; Maria Luposella, Rosa Patruno, Rosario Sacco, Cosmo Damiano Gadaleta, and Giovambattista De Sarro contributed to literature research, data analysis, and language revision. All authors wrote the manuscript.

Disclosure Statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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