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Paola Pittoni and Mario Paolo Colombo

*Cancer Res* 2012;72:831-835. Published OnlineFirst February 3, 2012.

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## Review

The Dark Side of Mast Cell-Targeted Therapy  
in Prostate Cancer

Paola Pittoni and Mario Paolo Colombo

## Abstract

Tumor development requires accomplices among white blood cells. Other than macrophages, mast cells have been observed to support the outgrowth of certain neoplasias because of their proangiogenic properties. In some tumor settings, however, mast cells may have a protective role, exerted by their proinflammatory mediators. In prostate cancer, no conclusive data on mast cell function were available. Here, we discuss recent work on the role of mast cells in mouse and human prostate cancer, showing that mast cells can behave alternatively as dangerous promoters, innocent bystanders, or essential guardians of tumors, according to the stage and origin of transformed cells. In particular, mast cells are essential for the outgrowth of early-stage tumors due to their matrix metalloproteinase-9 production, become dispensable in advanced-stage, post-epithelial-to-mesenchymal transition, and are protective against neuroendocrine prostate tumor variants. The common expression of c-Kit by mast cells and neuroendocrine clones suggests a possible competition for the ligand Stem cell factor and offers the chance of curing early-stage disease while preventing neuroendocrine tumors using c-Kit-targeted therapy. This review discusses the implications of these findings on the advocated mast cell-targeted cancer therapy and considers future directions in the study of mast cells and their interactions with other c-Kit-expressing cells. *Cancer Res*; 72(4); 831-5. ©2012 AACR.

## Introduction

Immune cells are part of the normal tissue stroma from embryonic life throughout adulthood and become a key component in tumorigenesis when tissues undergo neoplastic transformation (1).

Among stromal cells of immune origin, mast cells have been observed to infiltrate tumor masses since the late 1800s (2). In addition to their established role in allergy and inflammation, mast cells are now known to be capable of releasing molecules able to influence tumor growth. Mast cell mediators are either premade and stored in granules for immediate release (such as tryptase, chymase, and other proteases) or synthesized *de novo* (such as lipid mediators, cytokines, chemokines, and angiogenic factors). In general, the former are secreted in large quantities upon immunoglobulin E (IgE)-induced, high-affinity IgE receptor (FcεRI)-mediated degranulation, whereas the latter are released in a controlled fashion upon cell stimulation. A third alternative mechanism of mediator release, called "piecemeal degran-

ulation," has been described; it involves the release of small aliquots of granule-associated material and has been frequently observed in mast cells infiltrating tumors. This alternative pathway is IgE-independent and can be activated, among other mechanisms, by engagement of c-Kit receptor by its ligand Stem cell factor (SCF) or of pattern recognition receptors on the mast cell surface (3).

According to the tumor setting, mast cells have been associated alternatively with tumor promotion or tumor rejection (4). Mechanisms of tumor promotion involve the release of proangiogenic factors and matrix metalloproteinases (MMP), but also of immunosuppressive cytokines like interleukin-10 (5). Tumor rejection, on the other hand, has been ascribed to the release of proinflammatory cytokines and proteases (like TNF-α and tryptase, respectively).

A correlation between mast cell infiltration and prognosis has been described in different human tumors (6, 7). In prostate cancer, mast cells have been recently indicated as novel independent prognostic markers, and mast cell targeting associated with castration has been suggested as a potential therapy for this disease (8). Nevertheless, previous studies on prostatic biopsies associated high mast cell densities with favorable tumor characteristics and good prognosis (9, 10). A likely explanation for these discrepant findings may come from the observation that prostate cancer is a multifocal disease; each prostate tumor is, in fact, usually characterized by multiple neoplastic foci with heterogeneous characteristics. Therefore, a study aimed at dissecting the relationships between mast cells and prostate cancer cells with different degrees of malignancy was needed to allow a more focused approach to mast cell targeting in cancer therapy.

**Authors' Affiliations:** Molecular Immunology Unit, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS "Istituto Nazionale dei Tumori," Milan, Italy

**Corresponding Author:** Mario P. Colombo, Fondazione IRCCS Istituto Nazionale dei Tumori, via Amadeo 42, 20133 Milan, Italy. Phone: 39-0223902252; Fax: 39-0223902630; E-mail: mario.colombo@istitutotumori.mi.it

doi: 10.1158/0008-5472.CAN-11-3110

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## Key Findings

The study by Pittoni and colleagues (11) exploited the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model (12). In TRAMP mice, tumor progresses from prostatic intraepithelial neoplasia to advanced cancer, characterized by multiple adenocarcinoma lesions ranging from well to poorly differentiated, similarly to the human counterpart. As in humans, aggressive anaplastic neuroendocrine tumors may arise following radiation, chemotherapy, or hormone ablation (13).

The work originated from the observation of prostates from TRAMP mice containing many mast cells in their stroma; in particular, few mast cells were found in healthy areas, but they increased in number around neoplastic foci. Notably, mast cells had a density peak around areas of well-differentiated adenocarcinoma but were lacking in areas of poorly differentiated adenocarcinoma. This trend was maintained in human prostate biopsies and suggested intriguing connections between mast cells and prostate cancer cells, with well-differentiated and poorly differentiated adenocarcinoma either "beloved" or "neglected" by mast cells, respectively (11). As a means to better understand these interactions, new tumor cell lines with different degrees of malignancy were derived from TRAMP tumors, encompassing well-differentiated or poorly differentiated characteristics. Well-differentiated lines had an epithelial morphology and expressed epithelial markers (high E-cadherin and cytokeratins), whereas poorly differentiated lines had a mesenchymal morphology and displayed the transcriptomic signature of epithelial-to-mesenchymal transition (EMT). Both cell-line types grew efficiently *in vivo*, when injected s.c. or intraprostate in wild-type (WT) mice, but only nodules derived from well-differentiated tumors were infiltrated with mast cells. Accordingly, well-differentiated but not poorly differentiated tumors secreted large amounts of SCF, a previously described mechanism for mast cell recruitment adopted by tumors of various origins (14).

To assess functionally whether mast cells influenced the growth of well-differentiated and poorly differentiated tumors, the representative cell lines were injected into WT mice that, concomitantly, received the mast cell stabilizer drug sodium cromoglycate (cromolyn) and, as a second approach, were injected into mast cell-deficient Kit<sup>W-sh</sup> mutant mice. Both pharmacologic inhibition and genetic ablation of mast cells resulted in a growth failure of well-differentiated but not poorly differentiated tumor cell lines. Reconstitution of mast cell-deficient mice with WT bone marrow-derived mast cells rescued well-differentiated tumor growth, showing that mast cells are both necessary and sufficient for the development of well-differentiated prostate tumors. On the other hand, poorly differentiated nodules seem not to require mast cells for continuous growth.

The search for the key factor mediating mast cell protumoral activity identified MMP-9, which was very highly expressed in poorly differentiated lines, but absent in well-differentiated cells lines. Because MMP-9 had been previously described as an essential player in the tissue remodeling required for tumor invasion and angiogenesis (15), the logical

hypothesis was that mast cells might supply the need for MMP-9 of well-differentiated tumors, allowing their growth. Mast cells activated by FcεRI-dependent and -independent stimuli have been shown to secrete active MMP-9 by conventional and piecemeal degranulation (11, 14, 16). Reconstitution of Kit<sup>W-sh</sup> mice with bone marrow-derived mast cells from MMP-9 knockout donors showed that, although efficiently recruited, these mast cells were unable to support tumor take. This meant that early-stage prostate tumors depend on and exploit mast cells for MMP-9 provision to invade the nearby tissue and recruit new blood vessels. Along with neoplastic progression, tumors undergo EMT, become able to self-produce MMP-9, and, therefore, are mast cell independent (Fig. 1, top).

The same correlation between mast cell infiltration, MMP-9 provision, and tumor stage was found in both TRAMP and human prostate cancers as well, supporting the idea of targeting mast cells for the therapy of early-stage prostate cancer. Unfortunately, the limits of such an approach came to light when tested directly in TRAMP mice, in which the independent development of multiple tumor foci with different pathologic features can occur. Genetic ablation or pharmacologic inhibition of mast cells in these mice induced, surprisingly, high incidence of otherwise rare anaplastic cancers. Transcriptional profiling revealed the neuroendocrine nature of these tumors, a prostate tumor variant that originates directly from prostate stem cells (17). Finding the c-Kit receptor expressed on neuroendocrine tumors, as well as on prostate stem cells (18) and mast cells, brought up the possibility that mast cells are involved in the homeostatic control of prostate stem cells.

## Implications

The above-described findings have several implications affecting both cancer therapy and basic science. The most immediate therapeutic implication is that the enthusiasm toward mast cell targeting for the cure of prostate cancer should, at least in part, be dampened. The inhibition of mast cells may in fact be efficacious in patients with early-stage neoplastic lesions, but it would be completely useless for those with more advanced disease. Rather, a potentially effective therapeutic approach targeting both early-stage and advanced prostate cancer is the inhibition of MMP-9. Although MMPs can be specifically inhibited by endogenous tissue inhibitors of metalloproteinases (TIMP), the possibility of developing TIMP mimetics is hampered by their large size and short half-life *in vivo*. Therefore, several synthetic MMP inhibitors were developed (such as batimastat, marimastat, and prinomastat), mainly comprising small molecules with a chelating group binding the catalytic zinc atom at the MMP active site (19). Although promising at the preclinical level, these drugs failed the challenge of clinical trials in patients with advanced cancer, mainly because of their broad activity toward multiple MMPs and associated toxicity. Moreover, because MMPs are involved in multiple stages of tumor progression, it is expected that their inhibition would decrease the rate of progression, with minimal

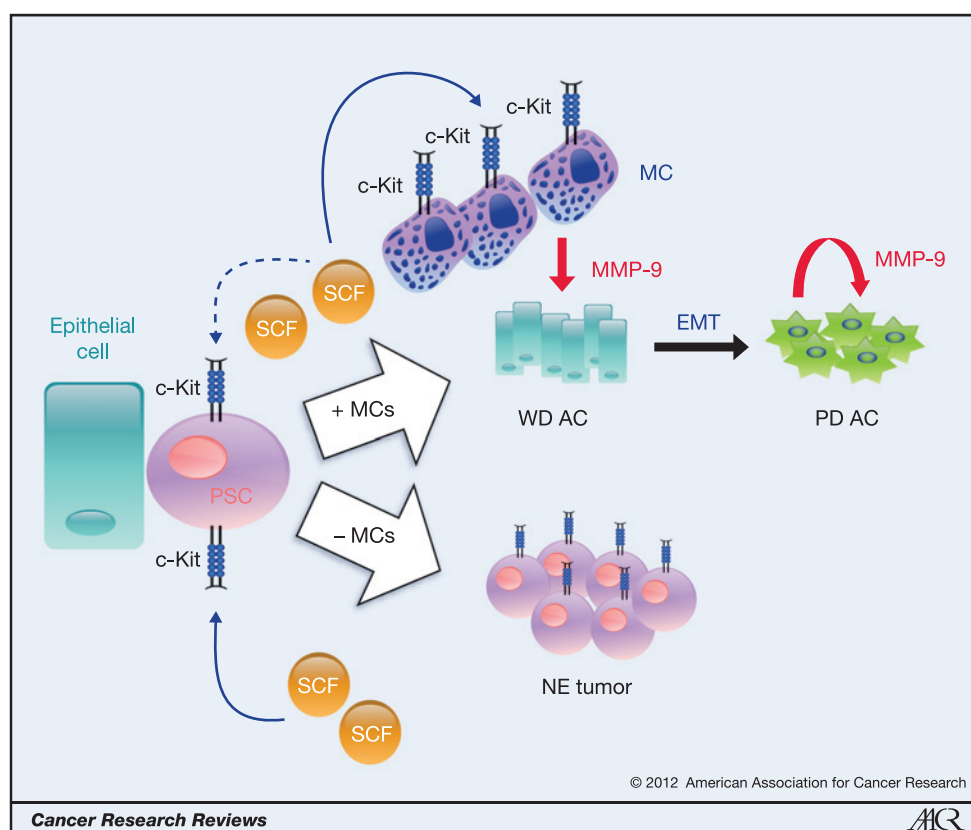


**Figure 1.** Proposed model for mast cell (MC) involvement in prostate carcinogenesis. In the transformed prostate, both epithelial (luminal and basal) cells and prostate stem cells start to proliferate. Transformed epithelial cells form focal areas of hyperplasia and recruit mast cells through SCF secretion. In the presence of functional mast cells, all SCF in the prostatic environment is sequestered by c-Kit receptor on mast cells. Mast cells produce MMP-9, which enables well-differentiated (WD) adenocarcinoma (AC) outgrowth by favoring angiogenesis and invasion to the surrounding tissue. Upon tumor progression, well-differentiated adenocarcinoma undergoes EMT and foci of poorly differentiated (PD) adenocarcinoma are originated. Poorly differentiated adenocarcinoma produces MMP-9 autocrinally, thus becoming independent from mast cells. In the absence of functional mast cells (i.e., when mast cells are inhibited and/or genetically ablated), SCF in the prostatic environment is no longer sequestered and becomes available for binding to c-Kit receptor on prostate stem cells. Upon enhanced c-Kit signaling, prostate stem cells continue to proliferate, undergo neuroendocrine (NE) differentiation, and progress to neuroendocrine tumors, which grow fast and quickly invade the surrounding tissue.

effects at late stages of disease (20). A more rational selection of the appropriate cancer type and stage might lead to more positive outcomes.

The second caveat to mast cell targeting is that it may favor the occurrence of aggressive neuroendocrine tumor variants in patients who would otherwise survive for a long time. Fortunately, this issue could be circumvented because the c-Kit receptor is a "druggable" target existing on both mast cells and neoplastic neuroendocrine cells. Imatinib and similar compounds are tyrosine kinase inhibitors able to inhibit c-Kit and other tyrosine kinases (21) and have been used in the clinic for the last 10 years for the therapy of chronic myelogenous leukemia and gastrointestinal stromal tumors (22). The use of imatinib to block mast cells, which rely on c-Kit for their migration and activation at the tumor site, would be the desirable approach, because the potential development of neuroendocrine tumors, which overexpress c-Kit to grow, would be prevented as well. In the past, some clinical trials have assessed the efficacy of imatinib in patients with advanced prostate cancer, albeit

with inconclusive results (23–27); however, in those cases, patients were selected according to platelet-derived growth factor receptor expression, as the putative imatinib target on tumor cells. As mentioned above for MMP inhibitors, it is likely that a selection of patients with an earlier tumor stage would have yielded more positive results and, in our opinion, would deserve the consideration of clinicians.

The implications of these data for basic science are also important. When mast cells were chronically stabilized or genetically ablated in TRAMP mice, the probability of neuroendocrine cancer development increased, indicating that functional mast cells somehow inhibit the occurrence of this tumor variant. Neuroendocrine prostate tumors have been shown to arise independently from epithelial adenocarcinoma (28), directly from the preferential neuroendocrine differentiation of prostate stem cells (17). The mechanisms at the basis of tumor inhibition by mast cells could be diverse. mast cells may exert an antitumoral effect that could be either direct or mediated by other stromal cells; alternatively, tumors may have an advantage over dysfunctional mast

cells because a competition between neoplastic cells and mast cells may be in place. Even though the first hypothesis is reasonable and deserves further investigation, the latter seems more attractive and draws inspiration from the observation that both mast cells and neuroendocrine tumor cells express c-Kit. c-Kit has recently been described as a marker for prostate stem cells, in which its engagement by SCF seems necessary for prostate regeneration (18). c-Kit expression is maintained along with the neuroendocrine differentiation of transformed prostate stem cells and is a common feature of neuroendocrine tumors arising in various organs (29). Expressing c-Kit, transformed prostate stem cells in young TRAMP mice could sense changes in the concentration of SCF in the microenvironment, which may occur in both mast cell-deficient and cromolyn-treated mice. The mechanism of action of cromolyn, though not yet completely clear, involves the induction of cell membrane stiffening, which prevents mast cell degranulation. It is conceivable that at the tumor site, mast cells are not activated via FcεRI-dependent pathways, typical of allergic reactions, but rather by other signals, such as plasma membrane debris released by necrotic tumor cells or, more appropriately in our setting, SCF. SCF is, indeed, capable of inducing direct mast cell degranulation (30). c-Kit is a rapidly cycling receptor, which is immediately internalized upon SCF binding (31); for this reason, membrane stiffening induced by cromolyn would not only block SCF-induced degranulation, but also affect mast cell migration toward the tumor and SCF sequestration by mast cells. SCF not properly scavenged by prostatic mast cells may, therefore, be sensed by prostate stem cells and provoke an increased signaling, altering their homeostasis and inducing, concomitantly with transgene-driven transformation, an early onset of neuroendocrine tumors.

We suggest that mast cells may exert an unexpected role controlling prostate homeostasis through competition with prostate stem cells. The concept of compensatory mechanisms existing between c-Kit-dependent cellular populations is corroborated by our data showing that mast cell reconstitution normalizes the enhanced granulocytosis characterizing Kit<sup>W-sh</sup> mice (21). Moreover, a similar mechanism of competition has been recently described between metastatic prostate

tumor cells with different degrees of differentiation and hematopoietic stem cells (HSC) in the bone marrow niche (32); even in this case, and as proposed also by others (33), c-Kit can be implicated, because both HSCs and prostate tumor cells with a stem cell phenotype express c-Kit in the bone marrow cytokine milieu rich in SCF.

## Future Directions

The mechanism used by mast cells to control neuroendocrine tumors deserves further investigation. Coculture experiments with bone marrow-derived mast cells and WT/TRAMP prostate stem cells will assess the potential competition between the 2 cell populations for SCF ligation. As a corollary, it will be tested whether increased SCF/c-Kit signaling in transformed prostate stem cells results in augmented neuroendocrine tumor formation. If this hypothesis is confirmed, other important research directions will become of extreme interest, such as the following: (i) the role of controllers exerted by mast cells may be expanded to other c-Kit-expressing tumors, such as Merkel carcinoma or gastrointestinal stromal tumors; (ii) interesting relationships based on competition for SCF may be envisaged between mast cells and other c-Kit-expressing cells, such as dendritic cells or HSCs; and (iii) if the dysregulation of prostate stem cell homeostasis in TRAMP prostates results in a higher occurrence of neuroendocrine tumors, the ablation of other cells and/or molecules known to control the homeostasis of prostate stem cells may end up with the development of such tumor variants.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Grant Support

This work was supported by grants from Fondazione Italo Monzino, the Italian Ministry of Health, and Associazione Italiana Ricerca sul Cancro (to M.P. Colombo). P. Pittoni is supported by a fellowship from Fondazione Italiana Ricerca sul Cancro (FIRC).

Received September 15, 2011; revised October 31, 2011; accepted November 8, 2011; published OnlineFirst February 3, 2012.

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