Few, but Efficient: The Role of Mast Cells in Breast Cancer and Other Solid Tumors

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ABSTRACT

Tumor outcome is determined not only by cancer cell–intrinsic features but also by the interaction between cancer cells and their microenvironment. There is great interest in tumor-infiltrating immune cells, yet mast cells have been less studied. Recent work has highlighted the impact of mast cells on the features and aggressiveness of cancer cells, but the eventual effect of mast cell infiltration is still controversial. Here, we review multifaceted findings regarding the role of mast cells in cancer, with a particular focus on breast cancer, which is further complicated because of its classification into subtypes characterized by different biological features, outcome, and therapeutic strategies.

Introduction

Mast cells (MC) belong to the innate arm of the immune system, they derive from $CD34^+$ $CD117^+$ pluripotent hematopoietic stem cells within the bone marrow (BM) and complete their differentiation in tissues (1). On the basis of their role, MCs are differentially located in human tissues although they are predominantly abundant in close proximity to vessels (2), epithelia, fibroblasts (3), and nerves (4). MCs store many small secretory granules, whose content allows the classification of MCs in two major types: $MC_{(T)}$ and $MC_{(TC)}$. The former are characterized by granules that are particularly rich in tryptase, they play mainly a role in the immune response and can be found near the external mucosa of the gastrointestinal and respiratory apparatuses (5, 6). Conversely, $MC_{(TC)}$ display secretory granules with tryptase together with chymase and carboxypeptidase, contribute to tissue repair and are sited in the submucosa and connective tissues in close proximity to blood and lymphatic vessels (6). The specific signals responsible for progenitor recruitment and the mechanisms underlying MC differentiation are still poorly understood. MC biology is often studied by employing mouse models, although MC features are not fully conserved between human and mice (7). Hence, in this review, we will indicate whether findings were obtained in mouse models.

MC function is well characterized in allergic reactions and parasite responses, but their role in cancer is less understood and it is still a matter of debate. MCs can be detected both at the margins or infiltrating the tumor and have been reported to be endowed with both protumor or antitumor (8) properties depending on their abundance and localization, the type of stimuli and tumor context. Upon activation, MCs release a wide range of soluble mediators with either proinflammatory (e.g., TNF) or anti-inflammatory (including IL10 and TGFβ) effects (9). Moreover, MCs can differentially promote an inflammatory or immunosuppressive tumor microenvironment (TME) by modulating the functions of diverse immune populations (10), such as $CD8^+$ T cells (11). Moreover, in mouse models, MCs have been shown to regulate myeloid-derived suppressor cells (MDSC; ref. 12), tumor-associated macrophages (13), and regulatory T cells (Treg; ref. 14). MCs can sense environmental modifications and influence stromal (15) and immune components of the TME (16) in a bidirectional cross-talk, which enables them to finely tune the host responses in the presence of developing tumors, ultimately influencing their outcome (17).

The Ambiguous Role of MCs in Cancer

MC presence has been shown to be associated with poor prognosis and aggressive disease in diverse cancer types including Hodgkin lymphoma (18), pancreatic adenocarcinoma (19, 20), hepatocellular carcinoma (HCC, ref. 21), and cholangiocarcinoma (22). In patients with Hodgkin lymphoma, MC infiltration is predictive of worse relapse-free survival rates (18). The same effect has been reported in pancreatic cancers, where presence of tumor-infiltrating MCs is associated with higher grade and reduced survival. Notably, through *in vitro* experiments, it was shown that pancreatic tumor cells could promote MC infiltration, which, in turn, favors cancer cell growth and invasion, and in this manner worsens the outcome of the disease (20). MC number also increases along with carcinogenesis in HCC and intrahepatic cholangiocarcinoma (21). In these tumors, MCs could promote cancer fibrosis and immune response, negatively affecting patients' survival rate. In cholangiocarcinoma, a vicious circle has been described between cholangiocytes and MCs. By employing both *in vitro* and mouse models, it was proposed that MC-derived histamine promotes cholangiocyte proliferation, thus favoring cholangiocarcinoma progression and angiogenesis. Simultaneously, cholangiocytes secrete stem cell factor (SCF), which stimulates MCs via c-Kit (23).

In other types of tumors, MC role is less clear and could depend on their localization. This is the case of colorectal cancer where MC infiltration has been defined as a favorable independent prognostic factor (24), although the presence of a high number of MCs localized in the peritumoral is associated with poor prognosis (19, 25). Moreover, MCs were shown to be crucial for the development of preneoplastic polyps. In fact, it was reported that MCs are enriched in the polyp masses of patients and, at least in mouse models, are necessary to transform premalignant lesions in colon cancer (26). Also in prostate

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Cancer Res 2022;82:1439–47

doi: 10.1158/0008-5472.CAN-21-3424

cancer preclinical models, MCs display a different effect depending on their tissue compartment localization (27). Intratumor MCs inhibit angiogenesis and tumor growth, whilst peritumor MCs promote the expansion of human prostate tumors. During the onset of castrate-resistant prostate tumors, MCs are mobilized to the peritumoral area where they contribute to tumor relapse (27). Hence, their inhibition could be exploited to enhance the effects of castration in this setting. In melanoma, MCs have been associated with better patients' survival (28), but also with poor prognosis (29) and resistance to immune therapy (30) as shown in patients and through mouse models. In breast carcinomas, the comprehension of the role of MCs is made even more complicated by the high intertumor and intratumor heterogeneity and by the diverse outcomes, which characterize the different breast carcinoma subtypes (31).

Relation between MC Density and Breast Carcinoma Subtypes

Breast carcinoma is currently the most common type of tumor in women with about 2 million new cases every year (32). Breast carcinoma is a highly heterogeneous disease in terms of phenotypical features and tumor aggressiveness, making patients' outcome and response to therapy extremely variable (33). In the clinical practice, treatment decision is commonly based on histopathologic markers that is, expression of hormone receptors (HR), HER2, and Ki67, which define different breast carcinoma subtypes: luminal A and B, HER2-positive, and triple-negative (TN) breast carcinomas. Luminal breast carcinomas are usually characterized by better prognosis, while HER2-positive breast carcinomas and TNBCs show a more aggressive behavior and unfavorable prognosis (34). The efficacy of the treatment is not only influenced by the expression levels of these receptors, but also by the quality and quantity of immune infiltrate (35).

Recently, numerous studies have employed CIBERSORT (36) to infer the proportions of 22 immune cell subsets and evaluate the association between the abundance of the diverse immune subpopulations and the clinical outcome of solid tumors (37). CIBERSORT was employed to analyze the gene expression profiles of almost 11,000 tumors and verify whether differences in the immune infiltrate depend on the molecular subtype (38). This work allowed to evaluate the effect of innate immunity in cancer since, until then, the association between immune infiltration and clinical outcome was generally limited to adaptive immunity. Moreover, results confirmed the complexity between MC infiltration, cancer cell molecular profiles and clinical features.

Other studies aimed at determining whether specific molecular profiles of breast carcinomas are characterized by a different density of infiltrating MCs (39–42). MC abundance has been compared between highly HR-positive (>50%) cancers versus tumors with low expression of HRs (<5%; ref. 41) finding an increased number of MCs (detected by Giemsa and Alcian blue staining) in the former group, mainly in the peritumoral zone. Glajcar and coworkers investigated the density of tryptase- and chymase-expressing MCs in different molecular subtypes of breast carcinoma, according to the molecular classification of St Gallen 2013 International Expert Consensus (43), to evaluate their association with standard prognostic markers (39). This study showed that low- and intermediate-grade breast carcinomas are characterized by high density of MCs both infiltrating the tumor and at the invasive margins. A statistically significant higher presence of both chymase- and tryptase-positive MCs was observed in luminal (estrogen or progesterone receptor positive; ER^+ or PR^+) compared with non-luminal (ER^- and PR^-) tumors (**Fig. 1**). The *in silico* analysis of gene expression profiles of the molecular taxonomy of breast carcinoma international consortium (METABRIC) database through CIBERSORT algorithm (36) confirmed that there is a significantly higher infiltration of MCs in luminal tumors, particularly luminal A, compared with more aggressive HER2-positive and TNBC subtypes (40).

Different Breast Carcinoma Molecular Subtypes, Diverse Prognostic/Predictive Value of MCs?

Luminal/HR-positive

Immune infiltrate is differently associated with survival probability (44) based on the expression of HRs. Because MCs are more abundant in HR-positive breast carcinoma tumors, which are characterized by a better prognosis, it has been hypothesized that MCs could be endowed with a favorable prognostic value (**Table 1**). Accordingly, MCs are negatively associated with the proliferation rate, identified by a lower expression of Ki67 (39). The positive correlation between MC presence and expression of ER, or the ER-target gene *BCL2*, was confirmed also showing a negative correlation of MCs with the TNBC marker EGFR (45). In accordance to this, we found that *in vitro* MCs reduce the activation of EGFR and cMET receptors (40), which are both regulators of the basal program and are highly expressed in TNBCs. MCs were shown to inhibit cMET also by cleaving its ligand, HGF, into an NK4-like inhibitory molecule (46). Altogether these findings support the hypothesis that MCs could not only be recruited more efficiently by ER-positive breast carcinoma cells, but could also promote their luminal phenotype by favoring the expression and activity of ER, while simultaneously inhibiting the function of basal receptors such as cMET and EGFR (**Fig. 1**). In preclinical settings, MC presence has been associated to increased aggressiveness in luminal B models. In fact, breast carcinoma growth and metastasis formation were increased in a C57BL/6 MMTV-PyMT mouse model, when compared with C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ (Wsh) mice, which lack MCs due to an inversion of the c-Kit promoter (47).

HER2-positive

In HR-positive luminal tumors, the expression of ER is obviously a positive predictive marker of responsiveness to hormonal-based interventions, but recent data from neoadiuvant studies suggest that ER status displays an opposite effect in patients with HER2-positive breast carcinoma treated with anti-HER2 therapy (48, 49). Trastuzumab, the standard of care for the treatment of HER2-positive breast carcinomas (34), is often effective in combination with chemotherapy, but some patients do not respond and eventually progress (50). To overcome resistance to trastuzumab, several novel HER2-targeting agents have been developed, including the tyrosine kinase inhibitor (TKI) lapatinib, and the mAb pertuzumab (51). However, ER activation could represent an escape pathway that promotes cell survival and resistance to therapy (52, 53). Because ER expression hampers the efficacy of anti–HER2-based therapy and given the capacity of MCs to increase ER level and activity (40), MCs may negatively affect the response to anti-HER2 therapy also via ER stimulation. The analysis of breast carcinoma transcriptomes confirmed that the presence of a high fraction of MCs correlates with worse disease-free survival (DFS) (54) and overall survival (OS). These observations have been confirmed by independent works that highlighted a negative effect of MCs in HER2-positive breast carcinoma patients' outcome (38, 54, 55). Patients treated with trastuzumab and displaying a high risk of relapse [trastuzumab risk model (TRAR)-high] (56) express higher levels of

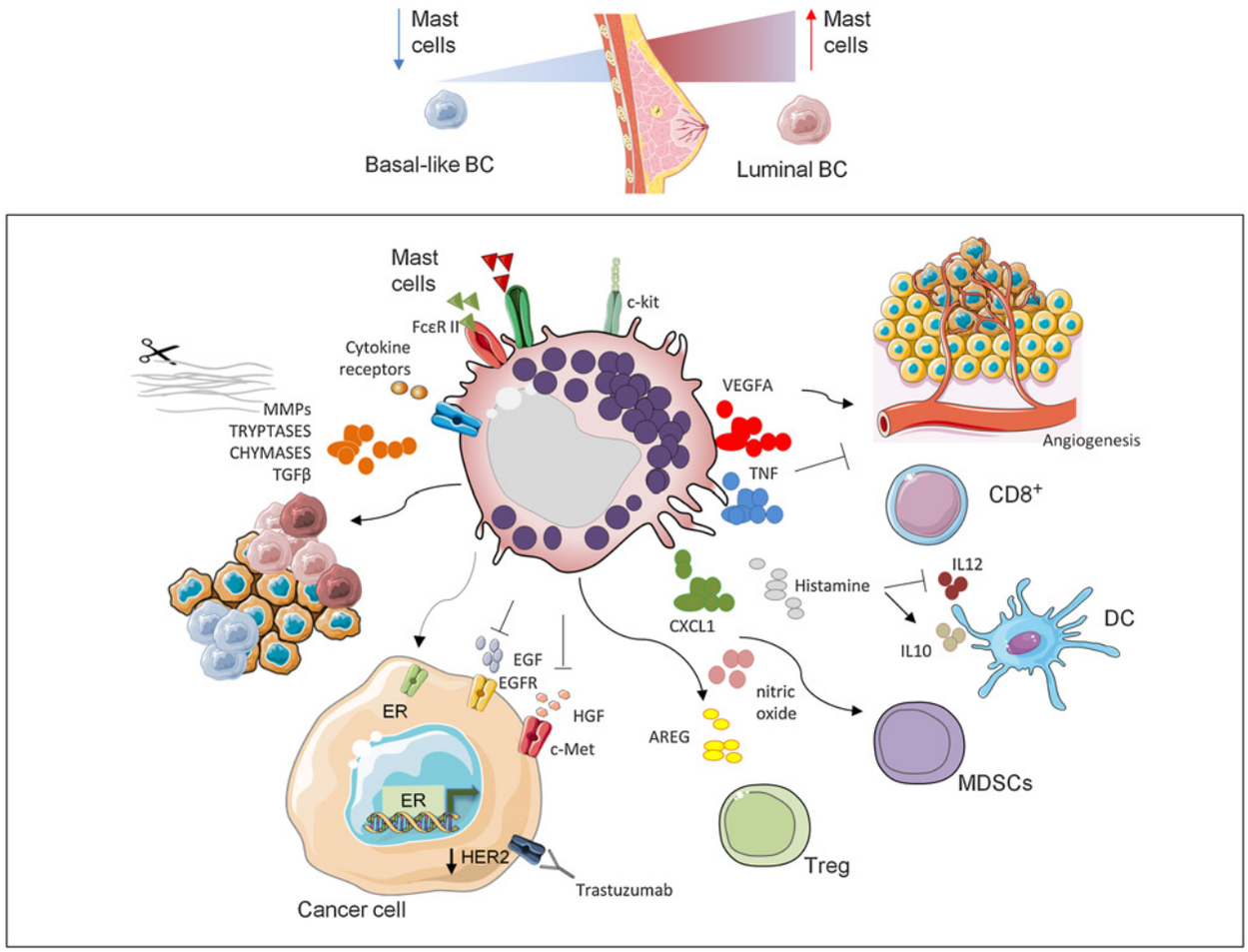

Figure 1.

Multiple roles of MCs in breast tumors. Diverse breast carcinoma subtypes are differently infiltrated by MCs with a higher presence in luminal compared with HER2-positive tumors and TNBCs. The release of diverse factors for example, VEGFA, TNF, CXCL1, histamine, nitric oxide, and AREG together with the interaction with various populations of the immune system, including $CD8^+$, DC, MDSC, and Tregs, lead MCs to mold the TME in a different manner and play both protumor and antitumor roles according to the tumor context. Because of their capability to shape the TME with tryptases, chymases, and through the release of TGFβ and MMPs, MCs influence tumor aggressiveness. Finally, MCs also affect the therapeutic response by limiting the efficacy of anti-HER2 treatment through the stimulation of the survival escape route favored by ER activity and reducing the activity of the basal markers EGFR (45) and cMET (40, 46). BC, breast carcinoma.

genes associated to MCs. Furthermore, the MC-related gene carboxypeptidase A (CPA3) correlates with ER expression (*ESR1*) and ER-activated genes, for example, *PGR*, *BCL2*, and *SCUBE2*, unveiling a novel possible link between MCs and clinical resistance to trastuzumab therapy (40).

TNBC/basal-like

In contrast with data obtained from HER2-positive tumors, Bense and colleagues reported that activated MCs are associated with a higher pathologic complete response (pCR) rate in TNBC patients, thus supporting the role of MCs as favorable predictive markers in this breast carcinoma histotype (54). It has been shown that MCs are recruited by tumors, and TNBC cells then stimulate their activation and degranulation (57). In this scenario, activated MCs appear to mold a tumor stroma characterized by antitumor rather than supportive features (57). In accordance to the potential positive prognostic value of MCs in breast carcinomas, other studies depict the possible association between MC infiltrates and low tumor grade (41, 45, 58). The presence of MCs in the peritumoral stroma improves the prognosis of breast carcinomas with long-term follow-up, particularly in the node-negative subset, supporting an important biological role for MCs in mammary tumors (58). Moreover, the value of MC presence as an independent prognostic factor of good prognosis in invasive breast carcinomas was validated in a large tissue microarray study of 4,444 cases (45). Herein, Kaplan–Meier survival curves showed that MC presence is a favorable prognostic marker in the entire set of analyzed invasive breast carcinomas. Conversely, Okano and colleagues described an association between Annexin A1, a protein that has been reported in various studies to be predictive of a significantly shorter OS in patients with TNBC (59), with MCs. Here, MCs have also been linked with various aggressive phenotype features of TNBC, such as epithelial-to-mesenchymal transition (EMT) and angiogenesis (60, 61). MCs were shown to negatively correlate with therapy efficacy in inflammatory (I) breast carcinoma (11) and to be associated with no-pCR in a durvalumab/olaparib/paclitaxel trial enrolling patients with HER2-negative breast carcinoma, both HR-positive and TN (62).

Table 1. Role of MCs in the outcome of breast carcinoma.

Patients' tumor type	Prognosis	Detection	Localization	Mechanism proposed
Basal-like	Negative	CIBERSORT	NA	MC associated with CAF-derived high-risk score (63)
HER2-negative (TNBC and luminal)	Negative	5 genes MC signature (95)	NA	MC associated with no-pCR in durvalumab with olaparib and paclitaxel patients (62)
HER2-positive	Negative	CIBERSORT	NA	Activated MCs are reduced in TRAR-low patients of the NeoALTTO trial (55)
Luminal B, HER2-enriched and basal-like BC	Negative	CIBERSORT	NA	Activated MCs are included in a immunorisk score (IRS) signature, which correlates with reduced OS (44)
TNBC	Negative	CIBERSORT	NA	ANXA1 is associated with activated MC and is predictive of reduced OS (60)
Inflammatory breast cancer	Negative	Tryptase	Intratumoral	MC infiltration associated with poor response (pCR) to neoadjuvant chemotherapy (11)
pan-BC	Negative	Tryptase	Intratumoral/ peritumoral	MC distribution depends on HR and HER2 expression; intratumor MC is associated with worse prognosis (97)
Ductal invasive carcinomas	Negative	Tryptase	Interstitial/ periglandular	Periglandular MC position are more numerous in G3 compared with G1/G2 tumors and control samples (102)
pan-BC	Negative/ Positive	CIBERSORT	NA	Activated MCs are associated with worse DFS and OS in HER2-positive BC, while MC are linked with pCR in TNBC (54)
pan-BC	Negative	MC-dependent genes signature	NA	A MC-dependent genes signature predicts recurrence-free survival (98)
Nonspecified	Negative	Tryptase	Metastases	MC-rich primary tumors are more prone to form metastases (71)
pan-BC	Positive	Tryptase- and chymase	Intratumoral/ invasive margins	MC density is associated with lower tumor grade, higher ER and PR expression, lower proliferation (39)
pan-BC	Positive	Toluidine blue	NA	MCs correlate with HR positivity and reduced Ki67 (42)
pan-BC	Negative	MCT	NA	MCs interact with HLA-G+ BC cells favoring invasion and metastasis (108)
pan-BC	Negative	Tryptase	NA	Role of MC tryptase in angiogenesis (103)
pan-BC	Negative	Tryptase	Intratumoral/ peritumoral	MCs interact with lymphatic vessels favoring lymphovascular invasion in a subtype-specific manner (101)
pan-BC	Negative	Tryptase/Toluidine blue	Intratumoral/ peritumoral	MCs contribute to stromal remodeling during BC progression (15)
pan-BC	Positive	c-Kit	NA	MC infiltration is an independent good prognostic marker (45)
High and low ER	Positive	Alcian blue/Giemsa	Peritumoral	Mast cells exhibit cytolytic activity against tumor cells (41)
pan-BC	Negative	Tryptase	Peritumoral	MC tryptase in the peritumoral tissue may promote breast cancer invasion (72)
pan-BC	Negative	Toluidine blue	Peritumoral	MCs could promote angiogenesis (104)
pan-BC	Positive	Tryptase	Peritumoral	MC heparin inhibits primary and metastatic tumors (8)

Preclinical tumor model	Effect on tumor	Detection	Localization	Mechanism proposed
Luminal A, B, and basal	Negative	Toluidine blue	Stromal	MC degranulation responsible for antibiotic-dependent increased growth (109)
Luminal B	Negative	Toluidine blue/ Real time on MC genes	Peritumoral	MCs favor primary tumor growth and mets formation in MMTV-PyMT mice and tumor engraftment in NeuT-derived BC cell line (40)
Normal mammary tissue	NA	Tryptase	Ducts and lymph nodes	MC infiltration increased in C57BL/6 mammary tissue upon exposure to cigarette smoke (105)
Luminal B	Negative	Toluidine blue	Peritumoral	MMTV-PyMT mammary growth and metastasis are increased in the presence of MCs (47)
Luminal B and TNBC	Negative	Tryptase/ Toluidine blue	NA	MCs remodel TME and metastatic niche to promote mets through SCF/cKit interaction in BC with arthritis (113)
Basal and luminal	Negative	Toluidine blue	Peritumoral, tumor-stroma interface	MCs secrete IL6-activating fibroblasts and promoting tumor progression (16)
Basal	Negative	Tryptase	NA	MC tryptase protects tumors from blood clotting and hypoxia (111)

Note: Both clinical and preclinical studies are listed together with the subtype/model investigated, the effect, the detection method and localization of MC, and a brief description of the mechanism/effect observed. Papers are listed in reverse chronological order.
Abbreviations: BC, breast carcinoma; CAF, cancer-associated fibroblast; NA, information not available/not specified.

Moreover, by identifying a cancer-associated fibroblast signature predictive of OS, it was shown that a high-risk score correlated to increased MC infiltration in basal-like breast carcinomas (63).

Effects of MC-Released Soluble Factors on Breast Carcinoma Outcome

MCs contain a large cargo of soluble factors whose release and composition could determine the activity of MCs in tumors. For instance, MC-related antitumor effect could be a consequence of ROS induction (64) or caused by release in the TME of cytotoxic mediators, as TNF (65). The expression of TNF is lower in cancer compared with non-cancer tissues, indicating that tumor cells could negatively affect MCs by reducing TNF and hence hindering their antitumor activities (66). The importance of MC-derived TNF is also supported by the observation that TNF levels are increased in responder or stable patients with lung cancer, compared with patients with progressive disease (66). In contrast, MC-derived proangiogenic factor VEGFA, whose abundance is positively correlated with microvessel density (17, 29), is linked to the protumoral effect of MCs (67). The expression of TNF and VEGFA in MCs is mutually exclusive and their levels vary across different cancer types. This observation could contribute to explain why MCs act in a different manner according to the type of cancer cells (66). MCs expressing a high VEGFA:TNF ratio appear to display a dominant proangiogenic effect in agreement with the observed correlation between MC abundance and vessel formation (68). MCs release numerous other angiogenic factors, including endothelin-1, GMCSF, CXCL8, and CCL2 (69). Barkaway and colleagues have recently described a new link between MCs and aged vasculature in mouse models (70). They found that MC quantity increases in aged organs and promotes tissue damage through the production of high levels of the inflammatory chemoattractant CXCL1, which stimulates the reverse transendothelial migration of neutrophils. This effect still needs to be investigated in the context of tumors, but could potentially impact cancer outcome by favoring the accumulation of MDSC.

The protumoral effect of MCs could also be mediated by the release of other factors such as TGFβ, which stimulates EMT, several proteases including matrix metalloproteinases (MMP; refs. 71, 72), for example, MMP9, which contribute to MC-mediated shaping of the extracellular matrix (ECM), as well as chymase and tryptase that modify pro-MMPs to their active forms (73). Furthermore, MCs are the major source of histamine, whose activity has been largely investigated in allergic reactions (74). However, there is increasing evidence showing that histamine is endowed also with immune modulating activities ultimately affecting cancer outcome. Upon MC activation, the endogenous production of histamine was shown to suppress the immune response and to contribute to breast carcinoma growth in mouse models (75). Moreover, the *in vitro* binding of histamine to H2 histamine receptors stimulated human monocyte-derived dendritic cells (DC) to synthesize IL10 (76) and, in patient-derived xenograft tumor models, prevented the production of IL12, which is responsible for Th1 expansion (77). This event causes DC-driven polarization of $CD4^+$ T cells toward a Th2 phenotype (78). The protumoral effect of histamine has also been reported in patients with cholangiocarcinoma where it contributes to tumor growth (79). In this scenario, MC-mediated release of histamine increases cancer progression and angiogenesis by enhancing the expression of VEGF (23).

In breast carcinomas, MC-released IL4 has an ambiguous effect because of its dual role both in promoting cancer cell dissemination or induction of apoptosis of breast carcinoma cells. Specifically, an increase of IL4, which depends on HR status, and its positive correlation with resistance to apoptosis have been described supporting the idea that IL4 is a negative prognostic factor in patients with breast carcinoma (80). Conversely, it has been reported that IL4 inhibits growth and induces apoptosis of human breast carcinoma cell lines, such as MCF7 and MDA-MB-231 (81).

Cross-talk between MCs and Other Immune Populations in Breast Carcinoma and Beyond

Several studies reported the capability of tumor-infiltrating MCs to shape the immune landscape (**Fig. 1**) either towards an antitumor or a protumor microenvironment (82), thus modulating the response to diverse therapeutic treatments. Because the composition of infiltrating immune cells differs among breast carcinoma subtypes, this may contribute to explain why MCs have a diverse impact in different breast carcinoma types. In particular, Dudeck and colleagues described the effect of MC TNF on T-cell priming. This mechanism could eventually influence their antitumor activity in the context of cancer. This work highlighted the potential of MC-derived TNF to amplify $CD8^+$ DC functionality and linked MCs with T-cell and DC modulation (65). TNF is known to be beneficial for tumor shrinkage (83), to sensitize breast carcinoma cells *in vitro* and *in vivo* to chemotherapy and radiotherapy (84), and to play a critical role in mouse DC functionality and T-cell priming (65). These observations indicate that MCs promote a proinflammatory microenvironment through the release of TNF, affecting the efficacy of immunotherapy and vaccination strategies, at least in mouse models (65).

Collectively, these observations indicate that MCs support $CD8^+$ T-cell activity, in disagreement with what suggested in inflammatory (I) breast carcinomas (11). In this context, MCs were shown to prevent treatment efficacy through their interaction with other immune subpopulations. More specifically, MCs, identified by tryptase staining, resulted more abundant in nonresponders where they were found in close proximity with $CD8^+$ T cells, $CD163^+$ macrophages, and tumor cells (11).

Several mouse studies described the engagement of MCs in the regulation of T-cell activities, including their recruitment and activation (85), as well as the impact on Tregs (86), which is bidirectional (87, 88). Nonetheless, the influence of MCs on T-cell functions is still controversial. In some studies, MCs have been shown to hinder the immune evasion mediated by Tregs in favor of the development of an effective antitumor immunity (14). In particular, it has been demonstrated that MCs are involved in the pathogenesis of diverse inflammatory conditions, for example, airway hyperreactivity and autoimmune encephalomyelitis, because they are able to counteract the immune suppression mediated by Tregs through the release of IL6, thus enabling their switch toward Th17 differentiation (14). In other works, it was described an opposite effect and MCs were shown to favor the immunosuppressive action of Tregs (86, 89). It has been reported that the stimulation with EGF-like growth factor amphiregulin (AREG) markedly enhanced the functions and efficiency of FOXP3+ Tregs, which express EGFR under inflammatory conditions (89). Notably, AREG is a predictive marker of poor therapy efficacy, particularly in patients with colorectal cancer bearing unmutated K-Ras (89–91), and it is highly upregulated upon MC activation (92). Therefore, MC-produced AREG may have a critical role for Treg functions and its release may represent a possible mechanism linking MCs and Tregs at the site of inflammation.

Other findings describing the cross-talk between MCs and Tregs support an effect of MCs on the response to anti-PD1 immune checkpoint inhibitor in murine melanoma models (30). A higher presence of MCs colocalizing with FOXP3+ Tregs was found in tumor tissue sections upon anti-PD-1 administration. By using the multitargeted receptor TKI sunitinib, able to deplete MCs and Tregs, authors assessed a complete regression of tumors in combination with anti-PD-1 therapy. MC infiltration in tumors was also confirmed by CIBERSORT analysis of three independent RNA sequencing datasets of patients with melanoma treated with anti-PD-1 or immune checkpoint therapies. In another trial (93), MC presence was increased in anti-PD-1 nonresponder patients (30). These results show that MC infiltration is associated with the presence of FOXP3+ Tregs, along with the downmodulation of HLA-class I on tumor cells, lack of $CD8^+$ T cells and subsequent ineffectiveness of the anti-PD-1 treatment.

Another mechanism by which MCs could promote immune evasion is constituted by their capability to interact with MDSC. MDSC are the key regulators of immunesurveillance escape given their capability to suppress T-cell responses (94). In colon carcinoma, MC presence increases the recruitment and activity of MDSC, supported by increased release of nitric oxide, which results in a proportional inhibition of T-cell proliferation and consequent tumor-induced tolerance (94). Concerning this aspect, it has been demonstrated that MC and MDSC cross-talk is mediated by CD40L-CD40 and this axis results in the suppression of tumor-specific T-cell response in prostate cancer models (12).

Evaluating MC Infiltration in Human Tumors

Tissue MCs can be detected *in situ* by metachromatic staining, such as toluidine blue, or by specific IHC to detect c-Kit (CD117) and tryptases (7). Alternatively, MC density can be inferred by gene expressing profile of whole tumors (95) exploiting deconvolution software as CIBERSORT (36). *In situ* detection of MCs displays the advantage that also the localization and the shape of MCs can be appreciated providing crucial information on their effect (96). In fact, several reports agree that MC predictive/prognostic value differs according to their localization: around tumor margins (41) or infiltrating the tumor mass (97). Moreover, the distance from other immune cell populations also affects the local immune microenvironment, possibly impacting on breast carcinoma response to therapy (11). MCs are endowed with immunomodulator activities in breast carcinoma and other solid tumors modifying the TME by either direct contact or secreting soluble factors. Therefore, the use of IHC or metachromatic staining allows to evaluate the spatial distribution of MCs, pending some limitations. In theory, the whole tumor should be analyzed because the distribution of MCs within the tumor is heterogeneous, more than one marker needs to be employed because no truly specific marker for MCs (e.g., c-Kit) is available and, finally, degranulated MCs are often difficult to detect.

On the other hand, MC signatures are increasingly used to quantify the presence of MCs in tumor tissues (98) and their activation state. CIBERSORT represents the most employed signature to estimate tumor-infiltrating immune populations and includes about 50 genes suitable to quantify the level of MC infiltration, also discriminating between resting and activated MCs (36). Resting MCs are generally less abundant in breast tumors compared with normal tissues, vice versa activated MCs are increased in breast carcinoma tissues. Nevertheless, these two MC statuses are associated with different cancer outcomes (38), sometimes even opposite, suggesting that the presence of MCs may not have a negative impact on tumors *per se*, but their activation is somehow linked to a more aggressive phenotype. This was first described in a pan-cancer analysis (37) and then applied specifically to breast carcinoma. Notably, the negative impact on OS and DFS of activated MCs is not observed in every breast carcinoma subtype, but it is significant in HER2-positive tumors (54). The same work supports the idea that activated MCs display even a positive effect on pCR in TNBC. As already mentioned, many genes, which are commonly exploited to identify MCs, are not really MC-specific and differently localized MCs are characterized by a diverse gene expression profile. Hence, a number of works tried to identify MC-specific genes also able to identify their particular activation states/subsets (99). As already mentioned, MC VEGFA:TNF ratio was shown to be prognostic (66). MCs represent the only cell type able to store preformed TNF in their granules (100), and hence display antitumor activities, while being capable of protumor effects via angiogenesis promotion (67, 101–105). Accordingly, different groups described the prognostic values of MC-related signatures (106) in diverse tumor types (12, 28, 107).

Pharmacologic Approaches to Target MCs in Preclinical Models

Because MCs represent key components of immune tumor infiltrate and have a crucial role in inflammation and angiogenesis (1), their targeting represents a possible strategy for therapeutic purposes (108). Several compounds for example, cromolyn sodium (109), nedocromil and lodoxamide are able to stabilize MCs hereby preventing their degranulation as well as the release of their mediators (110). It has been reported that the administration of cromolyn in TRAMP mice, a murine model of prostate cancer, increased the development of aggressive neuroendocrine areas, suggesting a protective effect of MCs in this tumor type (17). In mouse models of breast carcinoma, treatment with cromolyn induced an increase of blood clotting and hypoxia in subcutaneous 4T1 mammary adenocarcinoma cell line tumors supporting a role of MCs in the inhibition of hypoxia and blood clotting, which likely occurs via release of heparin, chymase, and tryptase (111). The inhibition of MCs by cromolyn sodium in cholangiocarcinoma determines the block of histamine release and, consequently, results in the reduction of tumor proliferation, angiogenesis and expression of mesenchymal markers (23).

In an attempt to inhibit the activity of MCs in angiogenesis, VEGFA was blocked through the employment of FDA-approved antiangiogenic drugs that either target VEGF or its receptors (112). MC presence negatively affects the efficacy of antiangiogenic therapy (AAT) through the release of matrix-degrading granzyme B. By using a pancreatic tumor model, authors found that the absence of MC increased the antitumor efficacy of anti-VEGF-R2 antibody DC101, hence indicating that MCs hinder the sensitivity of tumors toward AAT. The mechanisms involved in this event are related to the expression of ECM-degrading proteases, specifically granzyme B, which is responsible for the release of proangiogenic factors, such as FGF-1 and GMCSF from the ECM (112). Another potential therapeutic approach to inhibit MCs in cancer is achievable through the pharmacologic targeting of the c-Kit receptor tyrosine kinase (113), which is essential for MC homeostasis, by the employment of TKI compounds including imatinib, dasatinib, and sunitinib, which inhibit the catalytic activity of both wild type and mutated, for example, D816V (114), c-Kit. The targeting of c-Kit/SCF interaction is also promising for the treatment of patients affected by cholangiocarcinoma because it results in the disruption of the cross-talk between MCs

and cholangiocarcinoma cells with consequent reduction of tumor progression (23).

Conclusions

Initially neglected, MCs are progressively becoming crucial players in cancer, because increasing evidence supports their capability to affect outcome and therapy efficacy. Nonetheless, despite the many works that have been published in the last year, the ultimate role of MCs in tumors is far from being understood. Findings are characterized by apparently contradictory data, which actually are consequent of the plastic nature of MCs that are extremely sensitive to microenvironmental cues to which they suddenly respond. Hence, the effect of MCs cannot be limited to the dichotomy presence/absence, but it is caused, at least in breast carcinoma, by their activation and degranulation state, localization, secretion of cytokines and/or proteases, density, proximity to other immune and cancer cells. MCs could so represent an important tool to manipulate and predict cancer outcome, but, before they can be employed as prognostic/predictive markers or even as targets for novel therapeutic approaches, a deeper characterization of their biology and the identification of specific profiles associated to their activation and localization are still necessary.

Authors' Disclosures

No disclosures were reported.

Acknowledgments

D. Lecis and M.P. Colombo are supported by Associazione Italiana per la Ricerca sul Cancro (IG 2020 ID 24309 and IG 2020 IG 24363, respectively). M.T. Majorini is supported by a Fondazione Pezcoller-SIC fellowship.

Received October 8, 2021; revised December 17, 2021; accepted January 13, 2022; published first January 19, 2022.

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