



# Mast cells and angiogenesis in pancreatic ductal adenocarcinoma

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## Abstract

Mast cells are recognized as critical components of the tumor stromal microenvironment in several solid and hematological malignancies, promoting angiogenesis and tumor growth. A correlation between mast cells infiltration, angiogenesis and tumor progression has been reported for pancreatic ductal adenocarcinoma as well. Mast cells contribute to the aggressiveness of the pancreatic ductal carcinoma enhancing the expression of several pro-angiogenic factors such as vascular endothelial growth factor, fibroblast growth factor-2, platelet-derived growth factor and angiopoietin-1 as well as stimulating the pancreatic cancer cells proliferation by IL-13 and tryptase. The disruption of this pro-angiogenic and proliferative stimulation by inhibiting the mast cells migration and degranulation is under investigation as a potential therapeutic approach in pancreatic ductal adenocarcinoma patients. This review will summarize the literature concerning the mast cells infiltration in the pancreatic ductal adenocarcinoma analyzing its role in angiogenesis and tumor progression.

**Keywords** Angiogenesis · Mast cells · Pancreatic ductal adenocarcinoma

## Mast cells and tumor growth

Mast cells (MCs) originate from progenitor cells in the bone marrow, which move through the circulation and become mature mast cells after homing to different organs under the influence of the local microenvironment [1]. MC progenitors enter the blood and exit into tissues by transendothelial migration and are undetectable in the blood. Indeed, mast cells are found in human mucosal and epithelial tissues throughout the body, in all vascularized tissues except for the central nervous system and the retina [2].

MCs contain inside their secretory granules powerful biologically active molecules including cytokines, histamine,

proteases, and proteoglycans. These are released when MCs are activated, exert sometimes opposing biological effects and affecting the functional profile of different resident tissue cells, like fibroblasts, smooth muscle cells, endothelial cells, epithelial cells and nerve fibers. In addition to degranulation, MCs directly transfer granular content to endothelial cells [3] and communicate with other cells also secreting lipid bodies, microvesicles and exosomes containing a variety of proteins, basic fibroblast growth factor (bFGF) and extracellular RNA [3, 4].

MCs accumulate in the stroma of cancer and participate in tumor rejection producing molecules like interleukin-1, interleukin-4, interleukin-6 (IL-1, IL-4, IL-6) and tumor necrosis factor alpha (TNF- $\alpha$ ) that kill tumor cells. By contrast, MCs can benefit the tumor growth by promoting expansion of its vascular supply, proteinase-mediated degradation of the tumor extracellular matrix and immunosuppression [5]. Moreover, MCs synthesize and release angiogenic cytokines, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), the serine proteases tryptase and chymase, IL-8, transforming growth factor beta (TGF- $\beta$ ), TNF- $\alpha$  and nerve growth factor (NGF) [5]. MC-secreted molecules exert a direct angiogenic effect and stimulate other inflammatory cells to release angiogenic mediators and cytokines as well as extracellular

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matrix-degrading proteases. As tumor growth progresses, MCs recruit eosinophils and neutrophils and activate T and B cell-immune responses, and MC-derived metalloproteinases (MMPs) degrade the interstitial tumor stroma and hence release extracellular matrix-bound angiogenic factors [6].

The tyrosine-kinase receptor Kit (CD117) is up-regulated in tumor cells, and mutations in *c-kit* are associated to the development of gastrointestinal stromal tumor (GIST), in mastocytosis and MC leukemia [7].

MCs express high levels of *c-kit* and stem cell factor (SCF), the ligand for kit is involved in MC development and function [8]. In particular, the kit protein is transferred by exosomes from MCs to cancer cells, inducing PI3K/AKT signaling, migration and cell proliferation [3]. Moreover, SCF enhances tumor growth through increased release of VEGF, IL-6, IL-10 and TNF $\alpha$  [9].

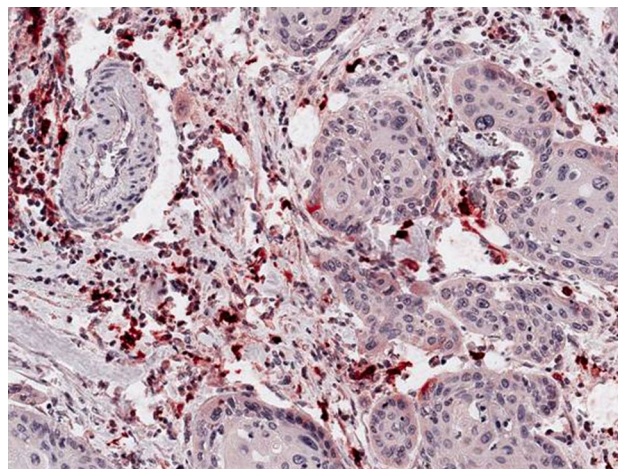
An increased number of MCs have been demonstrated in angiogenesis associated with vascular, hematological and solid tumors in which mast cell accumulation correlates with increased neovascularization, VEGF and FGF-2 expression, tumor aggressiveness and poor prognosis [5].

## Mast cells and angiogenesis in pancreatic ductal adenocarcinoma (PDAC)

PDAC is characterized by a low microvascular density as compared to other tumor types [10]. Therefore, hypoxia-inducible factor 1 alpha (HIF- $\alpha$ ) and VEGF-A expression are increased and correlates with poor prognosis [11, 12]. Another typical feature of PDAC is the presence of an intense fibro-inflammatory reaction, namely desmoplastic reaction, responsible of an high intratumoral pressure and solid stress causing vasculature collapse [5, 13].

Fibro-inflammatory reaction in PDAC is characterized by extracellular matrix deposition and inflammatory cells infiltration, including cancer-associated fibroblasts (CAFs), MCs, macrophages and lymphocytes [5, 14]. Several evidences suggest a role of inflammation in PDAC carcinogenesis [15]; however, the role of inflammation in PDAC is only partially explained.

As far as MC infiltration is concerned, it is more prominent in PDAC than in normal pancreatic tissue as well as in benign pancreatic pathology (Fig. 1) [16, 17], and increased MCs infiltration correlates with higher tumor grade and worse prognosis [18, 19]. Also MC distribution in the tumor tissue seems to have a critical role in clinical features and patients' outcome in PDAC. In particular, high MCs infiltration of the intratumoral border zone is associated with lymph node metastasis, tumor-stage, lymphatic and microvascular invasion and correlate with overall survival (OS) in resected patients [20].



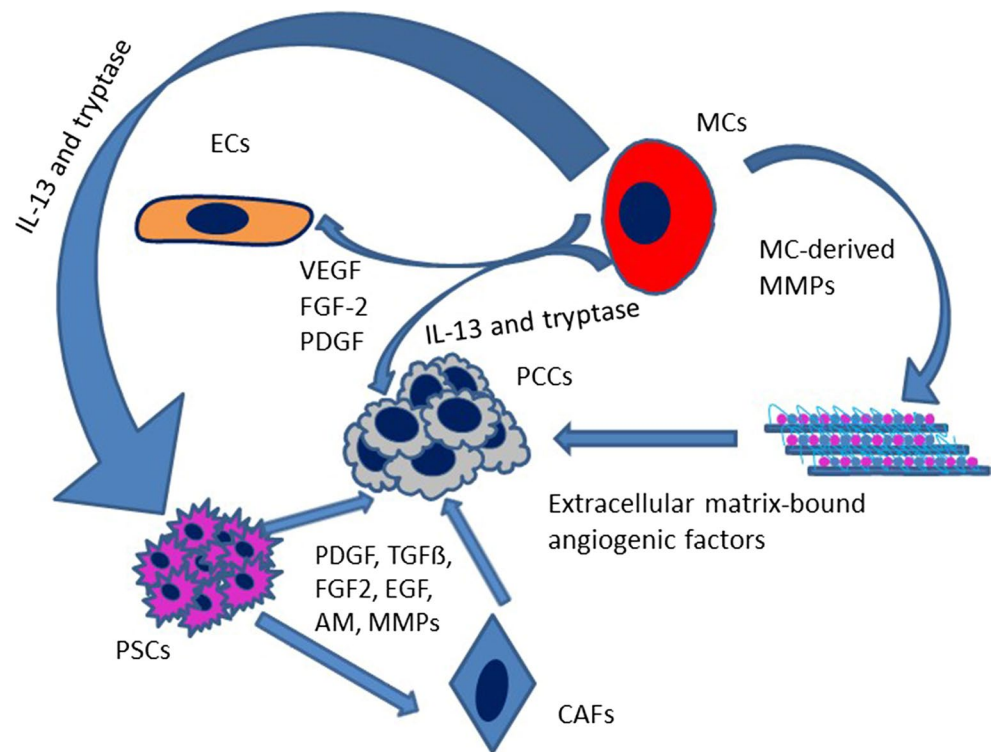
**Fig. 1** A massive infiltration of tryptase-positive mast cells in a biopsic sample of human pancreatic ductal adenocarcinoma

The mechanism by which MCs contribute to the progression of pancreatic cancer is far from being defined (Fig. 2). Pancreatic cancer cells (PCCs) and stellate pancreatic cells (PSCs) recruit and activate MCs. In turn, IL-13 and tryptase released by MCs induce the proliferation of both PCCs and pancreatic stellate cells enhancing the PCC-invasive capacity [21]. Activated PSCs produce PDGF, TGF $\beta$ , FGF2, epidermal growth factor (EGF), connective tissue growth factor, adrenomedullin (AM), MMPs, stroma cell-derived factor-1, secreted protein acidic and rich in cysteine and galectin-1, stimulating angiogenesis and tumor growth [22, 23]. In particular, AM plays a critical role in the crosstalk between PCCs, PSCs and MCs, stimulating the expression of VEGF, monocyte chemoattractant protein-1, bFGF and as a chemotactic factor for MCs [24, 25]. Moreover, PSCs activation induces a hypoxic-fibrotic environment with accumulation of lactic acid, adenosine, prostaglandin E2, interferon  $\gamma$ , and low pH, resulting in MCs release of IL-6 and VEGF-A [26].

Furthermore, patients with PDAC have higher serum tryptase activity compared with benign pancreatic pathologies, and increased levels of serum tryptase correlate with higher intratumoral microvessel density contributing to endothelial cell growth and tube formation via up-regulation of angiopoietin-1 expression [27].

Moreover, MCs express other pro-angiogenic factors, including VEGF-A, FGF-2, platelet-derived growth factor; tryptase-positive MCs as well as MCs expressing FGF-2 significantly correlate with IMD in PDAC [17, 28]. Finally, MCs isolated from PDAC tissue produce VEGF-C, which stimulate lymphangiogenesis, explaining at least in part the correlation between MC infiltration and lymph node metastasis [17].

**Fig. 2** MCs contribute to the progression of PDAC by several mechanisms. MCs express pro-angiogenic factors such as VEGF, FGF-2, PDGF and MC-derived MMPs promote the release of extracellular matrix-bound angiogenic factors. PCCs and PSCs recruit and activate MCs and at the same time IL-13 and tryptase released by MCs induce activation and proliferation of PCCs and PSCs. Activated PSCs produce several pro-angiogenic factors and stimulate CAFs and PCCs



## Therapeutic perspectives

Several phase II and III clinical trials have been conducted in PDAC using anti-angiogenic inhibitors [14, 29]. A multicenter phase II trial in patients with metastatic PDAC achieved a 21% ORR and a median OS (mOS) of 8.8 months with the combination gemcitabine plus bevacizumab [30].

PDAC development is suppressed in genetically MC-deficient mice [19, 21], and blocking MCs migration and degranulation inhibits PDAC growth and promotes increased survival in vivo [21]. Treatment of an orthotopic model of PDAC with AMD 3100, a CXCR4 antagonist blocking MC migration in vivo [21], reduces tumor size and increases survival with a reduced MCs migration in the tumor site [21]. A phase I study evaluating continuous 7-day IV infusion of AMD 3100 in PDAC patients is ongoing [31].

The MCs stabilizer cromolyn induces a significant tumor shrinkage in vivo [32]. However, cromolyn is a weak inhibitor of human MC secretion and is poorly absorbed, so it is unlikely to be effective in PDAC patients [32]. Targeting the molecules secreted by MCs is another potential strategy [33, 34]. Inhibitors of trypsin-like serine proteases, including gabexate mesylate, nafamostat mesylate and tranilast, reduce invasiveness and proliferation of PCCs through inhibition of tryptase release and decreasing the tryptase-mediated activation of PAR-2 on the surface of PCCs and endothelial cells [33].

MCs cytoplasmic granules contain also a considerable amount of histamine and PCC express histamine

receptor (HR)s [34]. Histamine or agonist binding to H1HR expressed on PCC line induces proliferation by up-regulating nerve growth factor expression and promotes metastasis by decreasing cellular adhesion and increasing MMP2 activity [35]. The block of H1HR using specific receptor antagonist results in increased cell adhesion and decreased motility [36].

Masatinib, a potent oral tyrosine-kinase inhibitor of c-kit, Lyn and Fyn that reduces proliferation, differentiation and degranulation of MCs, is active on gemcitabine-refractory pancreatic cell lines [37]. In a phase III study, the addition of masatinib to gemcitabine resulted in a significant increase in mOS in two subgroups of poorly prognosis PDAC patients. In particular, masatinib increases the mOS of about 6 months in the group of patients over-expressing the acyl-CoA oxidase-1. Moreover, accordingly with the correlation between MCs infiltration and abdominal pain in PDAC patients, masatinib increased mOS of 2, 6 months in the subgroup of patients with higher baseline pain [38].

## Conclusions

An intense crosstalk between MCs and PCCs contributes to the PDAC progression. Specifically, the PCCs induce MCs migration and MCs subsequently promote angiogenesis and tumor growth. Although, the specific MC functions that promote the invasiveness are far from being defined, the disruption of the interactions between tumor microenvironment



and PCCs is a growing therapeutical strategy. In particular, drugs that reduce proliferation, differentiation and degranulation of MCs are under investigation. Further clinical trials are mandatory to evaluate the efficacy of these treatments.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Human and animal rights** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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