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Macrophages and Pancreatic ductal adenocarcinoma

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Abstract

Monocytes and macrophages make up part of the innate immune system and provide one of the first defenses against variety of treats. Macrophages can also modulate the adaptive immune system. Efficient sensing and response to tissue environmental cues highlights the complexity and dynamic nature of macrophages and their plasticity. Macrophages may have divergent roles depending on their polarity and stimulus received. Accumulating evidence demonstrates the critical role played by macrophages in tumor initiation, development, and progression. In this review, we discuss the characteristics of tumor-associated macrophages (TAMs) and their role in pancreatic adenocarcinoma. In addition, we give an overview on recent advances related to the therapeutic implication associated with targeting TAMs in pancreas cancer.

Keywords

macrophages; tumor associated macrophages; pancreas cancer; pancreatic ductal adenocarcinoma

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive tumors and the fourth leading cause of cancer-associated death in the United States [72, 83]. PDAC develops by an adenoma to carcinoma sequence as a result of accumulating genetic alterations, which provide signals (e.g. TGFβ via SMAD4) that promote recruitment of immune cells [8, 80, 89]. Histologically, microscopic pancreatic intraepithelial neoplasms (PanIN) progress from intraepithelial to invasive pancreatic cancer and parallel the accumulating genetic alterations in the adenoma to carcinoma succession of PDAC [8, 33, 50]. Macrophages are one of the early infiltrating immune cells in PanIN lesions and

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continue to persist through the invasive cancer [12]. Moreover, recent studies showing macrophage involvement even at the early stages of acinar cell dedifferentiation to ductal cells or acinar-to-ductal metaplasia (ADM) underscores the critical role these cells play in pancreatic cancer initiation, progression, and metastasis. Tumor macrophage infiltration is associated with poor outcome. Analysis of human pancreatic cancer tissues showed that the number of infiltrating macrophages in tumor tissue from patients with metastases was significantly greater compared to patients with no metastasis [23].

The tumor environment is rich in immune cell infiltrates yet tumors are able to circumvent interference through mechanisms that inhibit immune cell mediated anti-tumor effects. Such modification of immune cell functions creates a favorable microenvironment that allows tumor progression (Figure 1). This review attempts to summarize the knowledge gained during the last few years on the role of macrophages in pancreatic cancer.

Characteristics of tumor associated macrophages (TAMs)

Macrophages are efficient phagocytic cells of the innate immune system originating from bone marrow derived monocytes that constantly reconstitute most of the gastrointestinal tissues including the pancreas under homeostatic and inflammatory conditions [26, 27, 86]. In addition, local proliferation also contributes to maintain tissue resident macrophages. As a result of their plasticity, macrophages make up a heterogeneous population of immune cells with distinct functional and phenotypic characteristics [26]. More recently, classification of macrophages based on their functions, namely host defense, wound healing, and immune regulation, has been suggested [59]. More broadly and for simplicity, macrophages have been classified as classically activated (M1; commonly activated by IFN-γ and TLR ligands, express higher levels of IL-12, IL-23, TNFα, MHCII, IL-6, and inducible nitric oxide synthase or iNOS) or alternatively activated (M2; commonly activated by IL-4 and IL-13, express higher levels of IL-10 and TGFβ) [52, 53]. M1- and M2-polarized macrophages predominate in acute and chronic pancreatitis respectively [84, 86], with tumor-associated macrophages (TAMs) infiltrating solid tumors including PDAC thought to bear similarities to M2s and associated with poor prognosis [41, 89].

TAMs have been studied in different cancer types, play an immunosuppressive role, and are frequently associated with poor prognosis due to their abilities in promoting tumor growth, invasion, angiogenesis, and metastasis as discussed below [6, 90]. In addition to general macrophage markers (F4/80 in mouse, CD68 in human), scavenger receptors such as CD163, CD204, and CD206 have been used to identify TAMs in cancers [30]. Functional properties of TAMs include production of IL-10 and matrix metalloproteinases (MMPs), as well as M1-associated iNOS suggesting heterogeneous or mixed characteristics amongst tumor infiltrating macrophages [29, 82]. Recent study found that TAMs were predominantly M2 and associated with poor prognosis, whereas M1 predominated in the non-tumor inflammatory region surrounding the caner cells, highlighting the importance of spatial localization of TAMs [34].

The importance of TAMs in PDAC is emphasized by the results coming from several groups showing relationships between number and location of TAMs in surgically resected tumors and patient outcome. Of note, the 5 year survival rate after surgery performed with curative

intent is only about 20% [18]. Thus, the correlation between disease recurrence and survival after surgery represents opportunities to determine factors associated with disease recurrence and survival. Examples include findings that metastasis to lymph nodes found in surgical specimens is highly associated with M2s in the primary tumor and poor outcome [41]; and that high level of M2s in tumors as mentioned above is associated with shorter survival times while a greater ratio of M1s to overall macrophage number is associated with a longer survival after surgery [34]. It is known that extra-pancreatic nerve plexus metastasis of PDAC is associated with a poor outcome. One study showed that there is an association between a high number of M2s in the extra-pancreatic nerve plexus and decreased disease free survival and overall survival in patients undergoing resection of PDAC [74]. These studies confirm the longstanding findings in PDAC that a poor prognosis is associated with neural invasion and lymph node metastasis, and show that these biomarkers of outcome are associated with greater numbers of M2 type of macrophages.

Tumor microenvironment/evasive mechanisms recruiting and promoting TAM

The tumor microenvironment includes proliferating tumor cells, tumor-associated stromal fibroblasts (pancreatic stellate cells or PSCs in the case of PDAC), immune cells, blood vessels, and other tissue cells. The tumor microenvironment is enriched by factors that recruit circulating monocytes and favor generation of TAMs resembling M2 (Figure 1). Such factors include colony-stimulating factor (CSF)-1, chemokines and cytokines such as IL-4, IL-13, TGFβ and IL-10 [3, 15, 52, 71, 73]. CSF-1 promotes myeloid progenitor differentiation into monocytes and macrophages; and regulates proliferation, function, survival and migration of macrophages [43]. CSF-1 has also been implicated in promoting generation of alternatively activated macrophages or M2 [54, 75]. In experimental PDAC models CSF-1/CSF-1R inhibition depleted $CD206^{hi}$ TAMs and altered residual TAMs to support anti-tumor responses [91]. Thus, tumor-derived cytokines and chemokines not only promote macrophage recruitment and survival but also TAM functional properties that enhance tumor growth. In addition, other tumor-derived factors such as vascular endothelial growth factor (VEGF) also promote TAM infiltration as discussed below [87]. Dineen *et al.* showed in an animal model of pancreatic cancer that TAMs express epidermal growth factor receptor (EGFR) 2 where as, macrophages from the animals without tumors did not express the receptor. They also found that VEGF recruits TAMs to the tumor microenvironment and that inhibition of VEGF prevented this effect [20].

CCL2/CCR2 axis is important for monocyte egress from the bone marrow and their recruitment into tissues under homeostasis and inflammation [42, 69, 70]. Of the chemoattractants identified to play a role in tumor monocyte/macrophage recruitment, the CCL2/CCR2 axis has been the best studied. Bone marrow monocyte mobilization and tumor infiltration was shown to depend on CCR2 in PDAC model, and increased bone marrow monocyte mobilization correlated with increased PDAC infiltration with CCR2+ macrophages and was associated with poor survival in patients with PDAC [66]. In addition, CCR2 inhibition led to a decrease in monocyte recruitment, tumor growth and metastasis in an orthotropic model of PDAC. Results from this study have led to the ongoing Phase Ib trial testing CCR2 blockade in combination with chemotherapy in patients with advanced PDAC (NCT01413022).

TAMs are found in high concentration in hypoxic and avascular areas of the tumor [44]. Presence of large areas of hypoxia has been correlated with poor prognosis and resistance to anti-tumor therapies [78]. In a mammary tumor model, TAMs were shown to up regulate VEGF expression in areas of hypoxia [44]. In addition, increased TAMs were present in poorly vascularized areas VEGF-positive as compared to VEGF-negative areas of the tumor and the authors proposed that VEGF might provide macrophage chemoattraction. Hypoxia up regulates VEGF and class-3 semaphorins (Sema3A, a ligand for neruopilin-1 or Nrp1) [10]. Inhibition of Nrp1 in experimental tumor models was shown to inhibit angiogenesis and tumor growth [32, 63]. A recent study using models of PDAC and breast cancer showed that Nrp1-dependent (via Sema3A) and Nrp1-independent VEGF receptor transactivation led to TAM localization and entrapment in hypoxic tumor niches [10]. In addition to the Nrp1 and pro-angiogenic factor VEGF, hypoxia induces macrophages to up regulate hypoxia-inducible factor (HIF)-regulated genes and MMPs that allow them not only to survive in harsh environment but also to favor revascularization of the ischemic sites of the tumor [61]. TAMs are an important source of VEGF especially in poorly vascularized areas [44]. In fact, TAMs in hypoxic tumor regions have been shown to represent a macrophage population (closely resembling M2, expressing HIF-1α) that promotes angiogenesis, suppresses anti-tumor immunity, and metastasis [21, 60].

Alarmins such as high mobility group box protein 1(HMGB1), S100A8, S100A9, serum amyloid A3 (SAA3), and fibronectin have been implicated to recruit myeloid cells to tumor areas [13, 14]. HMGB1 is released from necrotic cells and activated macrophages [81]. Recent study showed that HMGB1 was over expressed in tumor tissues of PDAC patients and associated with poor outcome [45]. However, more studies are needed to understand the role of alarmins in PDAC and their effect on TAM localization as well as function.

Tumor growth and immune evasion is maintained by immunosuppressive factors such as IL-10, TGFβ, and prostaglandin E2 (PGE2) released by TAMs that favor Treg recruitment and inhibit anti-tumor effector $CD8^+$ T cell activities [62]. Several effects of IL-10 have been implicated in promoting TAM recruitment and TAM immunosuppressive properties: i) IL-10 enhances monocyte differentiation into macrophages and suppresses anti-tumor dendritic cell generation; ii) IL-10 suppresses myeloid cells derived IL-12 release, thus enhancing Th2 differentiation and production of high levels of IL-4/13 that further promote TAM generation. Additional tumor evasive mechanisms involve up regulation of CD47 or the “do not eat me signal” [35, 77]. CD47 interacts with macrophage regulatory protein-α and CD47 overexpressing cancer cells are protected from phagocytosis by TAMs [11].

Factors secreted by primary tumors have been shown to mediate formation of pre-metastatic niche in particular organs and lead to organotropism [1, 88]. Some of the secreted factors implicated include VEGF and placenta growth factor or PIGF [37, 38]. Exosomes play an important role as molecular carriers and provide mechanism for cross talk between cells; and more recently tumor derived exosomes have been shown to promote pre-metastatic niche formation [64]. Interestingly, a recent study showed that PDAC-released exosomes were enriched with the chemokine macrophage migration inhibitory factor (MIF) and induced recruitment of TAMs and formation of pre-metastatic niche in the liver [17]. These studies

demonstrate the ability of the primary tumor not only to influence local TAM recruitment but also their recruitment to distant pre-metastatic sites.

Role of TAMs in tumor initiation and progression

Accumulating evidence shows that acinar cells give rise to PDAC, whereas duct and centro-acinar cells are resistant to oncogenic transformation suggesting that acinar cells must convert or dedifferentiate into duct-like cells to give rise to PDAC [9, 19, 39]. Recent study in experimental model of acute pancreatitis showed that macrophage-secreted factors TNFα and CCL5 (RANTES) induce ADM through activation of NFκB, and macrophage depletion blocked generation of pancreatic ADM [47]. Folias and coworkers showed that macrophages play an important role in acinar de-differentiation and pancreatic regeneration in experimental model of acute pancreatitis [22]. In the presence of oncogenic Kras, such regenerative process of ADM can progress into neoplastic transformation [31, 46, 58]. Macrophage infiltration is also noted early on around neoplastic ducts in PanIN lesion and persists around neoplastic epithelium in invasive carcinomas [12]. Thus, TNFα-producing macrophages (M1) likely play an important role in the initiation of pre-neoplastic lesions in the right settings [48].

TAMs have been shown to promote immunosuppressive environment by suppressing adaptive immunity, which provides tumors a survival and growth advantage [51, 55]. TAMs have been shown to secrete chemokines that allow regulatory T cell (Treg) recruitment and provide co-stimulatory signals that inhibit T cell proliferation [40]. Accordingly, increased Treg and decreased $CD8^+$ T cell tumor infiltration is associated with PDAC progression and poor prognosis [34, 76]. Moreover, tumor derived-mucins were shown to bind mannose receptor CD206 on TAMs and enhance immunosuppressive properties of TAMs [2]. Notably, functionally distinct MHC II^{hi} and MHC II^{low} TAMs were identified within normoxic and hypoxic mammary tumor microenvironment respectively, yet both TAMs were inefficient at activating T cells and suppressed T cell proliferation highlighting T cell suppressive characteristics of TAMs [60].

Angiogenesis is critical for tumor progression and metastasis. As discussed in the above, TAMs are important source of VEGF and can regulate angiogenesis. Hypoxic tumor microenvironments recruit TAMs that promote vascularization and suppress anti-tumor responses favoring tumor escape [10, 21, 60]. In addition to TAM-derived MMPs that degrade extracellular matrix proteins and promote metastasis [13, 28], a recent study showed that TAM-derived cathepsins promote new blood vessel formation and enhance tumor invasiveness [24]. Based on these findings, the authors proposed a model in which macrophage cathepsins cleave E-cadherin, contribute to ECM degradation, and decrease cell-to-cell contacts thereby creating ways for cancer cells to migrate.

Similar to angiogenesis, lymphangiogenesis is an essential step for tumor spread as evidenced by the clinical information presented earlier. Two pathways have been proposed for macrophages mediated lymphangiogenesis. These include secretion of pro-lymphangiogenic factors by the TAMs and trans-differentiation of TAMs into lymphatic vessel structures [56, 68]. In both human and experimental tumors, TAMs have been reported to express lymphatic vessel endothelial hyaluronan receptor, a major lymphatic

vessel marker [67, 92]. In experimental models of prostate cancer and pancreatic insulinoma, such TAMs have been reported to directly integrate into peritumoral lymphatic vessels [67, 92]. Recent lineage tracing in dermal lymphatic vessel experiments support macrophages as regulator of lymphatic endothelial cell proliferation however do not support macrophages or myeloid cells as lymphatic endothelial progenitor cells [25]. Interestingly, a recent murine orthotropic lung carcinoma study showed that tumor derived TNFα activated TAMs to produce VEGF-C, and VEGF-C in turn induced lymphatic vessel tip cell formation, lymphatic endothelial cell proliferation, and migration [36]. This study demonstrates an inflammatory-mediated cross talk between tumor cells, TAMs, and lymphatic endothelial cells in promoting metastasis. Although it seems clear that TAMs influence lymphangiogenesis, it remains to be further determined whether they trans-differentiate into lymphatic endothelial cells in PDAC.

Epithelial-to-mesenchymal transition (EMT) is an important process via which primary tumors progress into metastatic state [5]. The tumor cells up regulate mesenchymal cell markers, lose intrinsic polarity, and lose cell-cell junctions by down regulating epithelial cell markers such as E-cadherin [16]. Macrophages contribute to metastasis by producing chemotactic and other factors as well as by promoting degradation of ECM barriers. More recently, in a co-culture system M2-polarized TAMs where shown to promote EMT phenotypic changes in pancreatic cancer cell lines Panc-1 and BxPC-3 [49]. This effect was mediated via macrophage TLR4 activation since knockdown of TLR4 or inhibition of TLR4/IL-10 signaling abolished the pancreatic cancer cells EMT. In addition to the role in EMT, TAMs have also been implicated to promote cancer stemness, a hallmark for cancer cells to self-renew, differentiate into tumor cells, migrate and regrow [57, 84]. Moreover, TAMs were shown to directly induce tumor-initiating cell properties in PDAC by enhancing STAT3 activation and targeting TAMs led to better chemotherapeutic responses [57].

Therapeutic Implication

As a result of macrophage involvement from tumor initiation to invasion and metastasis, there is a lot of interest in targeting TAMs. Many preclinical and experimental studies initially focused in depleting macrophages, but with more recent studies identifying functional and phenotypic heterogeneity among TAMs and differences in their micro environmental localization, therapeutic strategies that re-educate TAMs to those with anti-tumor responses have led to interesting results. A landmark study-using antibody based CD40 agonist showed beneficial effects in experimental models and patients with PDAC [85]. CD40 is expressed by many antigen-presenting cells (APCs) and the CD40 agonistic antibodies activate APCs and promote anti-tumor responses [79]. Commonly CD40 activation licenses dendritic cells in promoting cytotoxic T cell activity against tumors. The effect of agonistic CD40 antibody on PDAC highlighted important yet not previously well-appreciated generation of tumoricidal TAMs and activation of extratumoral macrophages that enhanced anti-tumor T cell responses [4, 85].

Histidine-rich glycoprotein (HRG), which is down regulated in tumors including in pancreas cancer as compared to normal tissues, was shown to support tumor progression and vascularization [7, 65]. Rolny and co-investigators showed a critical role for HRG in

reprograming TAMs from pro-angiogenic and immunosuppressive M2 phenotype into TAMs with M1 marker expression and anti-tumor properties leading to inhibition of tumor growth and metastasis [65]. The investigators went on to show that HRG effect was mediated via a member of the VEGF family placenta growth factor (PIGF), where PIGF deletion in macrophages reproduced the anti-tumor and vascular normalization effects of HRG. Thus HRG offers a potential therapeutic target for reeducating TAMs and suppress tumor evasion.

As mentioned above CSF-1/CSF1R inhibition in experimental PDAC led not only to depletion of TAMs but also to re-education of residual TAMs that promoted antigen presentation and enhanced anti-tumor T cell response [91]. Similarly CCR2 inhibition decreased TAMs and tumor-initiating cells in pancreatic tumors, improved chemotherapeutic and anti-tumor T cell responses [57]. These experimental studies show that targeting TAMs in PDAC improves responses to T-cell checkpoint immunotherapy and likely to provide means to tackle therapeutic resistance mediated by the tumor.

Conclusion

Macrophages are a heterogeneous population with remarkable plasticity, as a result they become an important evasive target for tumors, where pro-inflammatory macrophages provide signals for tumor initiation and anti- and immunosuppressive-macrophages promote tumor growth and metastasis. Hence, macrophage polarization state and spatial localization within the tumor microenvironment influences tumor development and progression. Better understanding of these factors will have significant impact in macrophage directed therapies and means via which macrophages can be reprogramed to promote tumoricidal activities and suppress tumor escape.

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Highlights

- Macrophages infiltrate pancreatic adenocarcinomas
- Presence of tumor-associated macrophages (TAMs) correlates with poor prognosis
- TAMs are involved in pancreas cancer development and progression
- Targeting TAMs offers a novel therapy against pancreas cancer

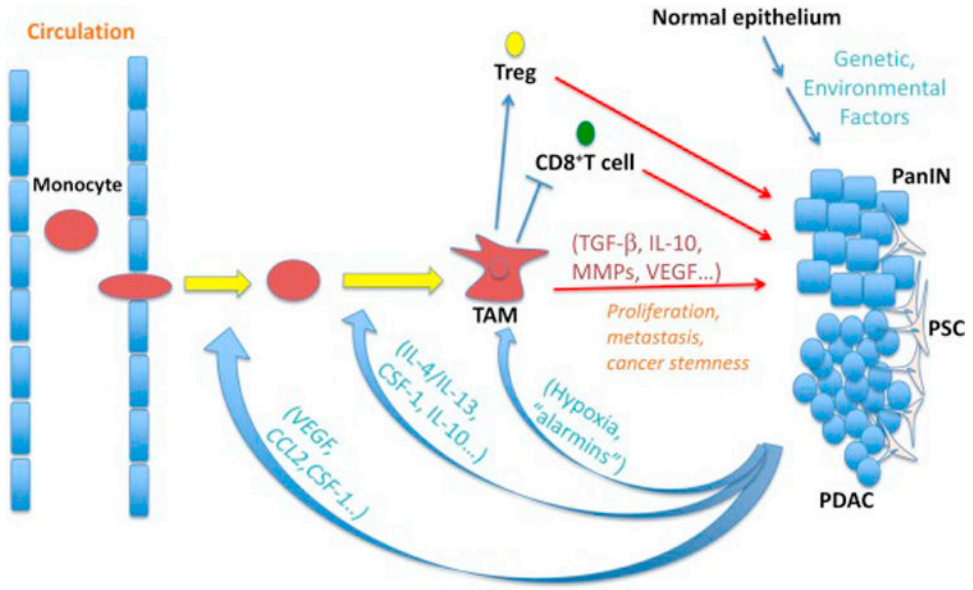

Figure 1. Tumor associated macrophages (TAM) in pancreatic cancer

Macrophages play an important role in pancreas cancer development and progression. CCL2, chemokine (C-C motif) ligand 2; CSF-1, colony stimulating factor 1; IL, interleukin; MMPs, matrix metalloproteinases; PDAC, pancreatic ductal adenocarcinoma; PanIN, pancreatic intraepithelial neoplasia; TGFβ, transforming growth factor beta; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.