Advances in pancreatic cancer stem cells, tumor-associated macrophages, and their interplay

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Cancer stem cells are currently considered to contribute to the aggressiveness and therapeutic resistance of pancreatic cancer, providing new hope for the cure of this disease and resulting in a growing body of mechanistic research. Solid tumors are heterogeneous cellular entities that contain malignant cells and a surrounding nonmalignant stroma. An abundant tumor stroma with a high density of tumor-associated macrophages is a distinguishing characteristic of pancreatic cancer. Accumulating evidence has indicated that tumor-associated macrophages can promote pancreatic cancer progression by inducing angiogenesis, invasion, metastasis, and immune suppression. However, there is limited information about the interactions between tumor-associated macrophages and pancreatic cancer stem cells. In this article, we review the current understanding of the biology of pancreatic cancer stem cells and tumor-associated macrophages and their molecular interplay.

Keywords: Pancreatic cancer; Cancer stem cells; Tumor microenvironment; Tumor-associated macrophages


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Introduction

Despite considerable research efforts, pancreatic cancer (PC) remains one of the most frustrating diseases with an extremely poor prognosis; it is ranked as the fourth leading cause of cancer death in the Western world. The high mortality and the overall five-year survival rate of less than 6% reflect the relative lack of available therapeutic options for PC treatment [¹]. Therefore, new options for pancreatic cancer treatment are urgently needed.

The concept of cancer stem cells (CSCs) established by the study of acute myeloid leukemia (AML) has received wide acceptance. In AML, a subpopulation of cells selected by specific cell surface markers exhibits self-renewal properties and has the potential to differentiate into all of the other cancer cell types [², ³]. Subsequently, CSCs have been identified in different types of solid tumors and shown to be a key determinant in the failure of cancer treatment. The biological progressive characteristics of PC, such as a propensity for early local invasion, distant metastasis, and a highly drug-resistant phenotype, are attributed in part to the presence of CSCs.
The tumor is currently perceived as a complex entity in which tumor cells interact closely with non-tumor cells residing in or infiltrating into the microenvironment, which plays a critical role in the promotion of tumorigenesis and metastasis [4, 5]. A large number of diverse immune cell types that infiltrate tumors can facilitate cancer progression by inducing tumor-promoting inflammation and immune evasion [6]. Among the tumor-infiltrating immune cells, tumor-associated macrophages (TAMs) are a major component of the inflammatory infiltrate in the tumor microenvironment [7]. Increasing evidence supports TAM infiltration as a negative correlate of clinical outcomes in various malignancies, including PC [8-13].

This review describes the recent advances in our understanding of pancreatic CSCs, TAMs, and their interplay. An expansion of our knowledge of the mechanisms underlying the crosstalk between pancreatic CSCs and TAMs will enable us to develop novel therapeutic strategies for PC.

1. Pancreatic CSCs

1.1 The stem cell concept

Tumors from different patients often display heterogeneity in morphology, cell surface marker expression, genetic lesions, cell proliferation kinetics, and response to therapy [14]. The CSC model has been proposed to explain the intratumor heterogeneity. This hypothesis based on the hierarchical organization of the tumor postulates that tumor initiation, propagation, metastasis, and relapse are caused by a tiny subpopulation of CSCs that have the ability to self-renew and engender diverse tumor cells [15]. Over the last decade, emerging evidence supports the presence of CSCs in leukemia and breast cancer. Using flow cytometry, a small subpopulation of tumor cells expressing the specific cell surface markers CD34+/CD38- in leukemia or CD44+/CD24-low in breast cancer could be isolated [3, 16]. In NOD/SCID mice, the isolated subpopulations were able to initiate and form tumors with histological features resembling those of the parent tumors. By contrast, xenotransplantation of the remaining bulk of cells resulted in no tumor growth, even when the cells were injected at much higher doses. Thereafter, many studies have taken similar approaches to demonstrate that other cancers also follow the CSC model, including brain tumors, prostate cancer, head and neck squamous cell carcinoma, colon cancer, and PC [17-22].

1.2 Markers for the identification of pancreatic CSCs

To date, several potential CSC markers have been identified in PC (Table 1). The initial evidence for the existence of CSCs in PC was reported in 2007 by Li et al [22]. The authors used a triple combination of the cell surface markers CD44+/CD24+/epithelial specific antigen (ESA)+ to identify a subpopulation of cancer cells from resected pancreatic tumors, based on prior work demonstrating the CSC properties of the cell subpopulation with a ESA+/CD44+/CD24+low phenotype in breast tumors [16]. The CD44+/CD24+/ESA+ cells were much more tumorigenic than marker-negative cancer cells and possessed the capacity to self-renew and differentiate into diverse types of progeny cancer cells, reflecting the heterogeneity of the patient’s primary tumor. Transplantation of only 100 CD44+/CD24+/ESA+ cells was sufficient to produce tumors in half of the evaluated immunodeficient mice [22]. In addition, clinical data has shown that patients with CD44+ PC have a poor prognosis [23].

Soon afterwards, another study provided evidence that human PC tissues and PC cell lines contained a stem cell population identified by CD133 expression based on prior experiments with brain tumors and colon cancer [24]. Compared with CD133- cells, CD133+ cells exhibited increased tumorigenicity and were more resistant to chemotherapy. During serial passage, CD133+ cells retained the ability to self-renew and generate differentiated nontumorigenic descendants to reconstruct hierarchically organized tumors in an orthotopic mouse model [24]. Notably, there was a partial overlap between

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Table 1. Markers for identification of pancreatic CSCs

<table>
<thead>
<tr>
<th>Category</th>
<th>Markers</th>
<th>References</th>
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<tbody>
<tr>
<td>Surface Molecules</td>
<td>CD44+/CD24+/ESA+</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>CD133+</td>
<td>[24]</td>
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<td></td>
<td>CD44+/CD133+</td>
<td>[28]</td>
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<td></td>
<td>CD133+/CXCR4+</td>
<td>[24]</td>
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<td></td>
<td>c-Met+/CD44+</td>
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<td></td>
<td>ABCG2+</td>
<td>[34]</td>
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<td></td>
<td>Integrin β1+</td>
<td>[35]</td>
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<td>Intracellular Molecules</td>
<td>ALDH1+ or High ALDH1 Activity</td>
<td>[36, 37]</td>
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<tr>
<td></td>
<td>Low 26S Proteasome Activity</td>
<td>[38]</td>
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<tr>
<td></td>
<td>DCLK1+</td>
<td>[39]</td>
</tr>
<tr>
<td>Others</td>
<td>Side Population Cells</td>
<td>[40]</td>
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CD44+/CD24+/ESA+ and CD133+ PC cells. Therefore, it is conceivable that the selection of a combination of these markers may help to identify more pure CSCs. Recently, the combination of CD44 and CD133 has been used to identify CSCs in colon cancer, prostate cancer, and gallbladder carcinoma [25-27]. In the case of PC, a CD44+/CD133+ cell population has also been demonstrated in cell lines and tumor specimens [28, 29]. The CD44+/CD133+ MiaPaCa2 cells, which express high levels of Notch and Bcl-2, exhibited a strong capacity to form tumorspheres and to initiate tumors in a mouse xenograft model [28]. Kallifatidis et al., analyzed 2 cases of PC to study the drug resistance of CSCs and found that the tumor containing CD44+/CD133+ cells was more resistant to chemotherapeutic agents than the other tumor lacking these two markers [30]. Consistent with the previous results, our group showed that CD44+/CD133+ cells isolated from the PANC-1 cell line were capable of forming tumorspheres in vitro, exhibited tumor-initiating potentials in vivo, and profoundly responded to Wnt pathway activation or inhibition [31]. Moreover, using tissue microarray analysis, we have also demonstrated that high expression levels of both CD44 and CD133 were associated with poor outcomes in PC patients [32]. Altogether, these findings suggest that the combination of CD44 and CD133 may mark a genuine CSC population in PC.

The role of CSCs in metastasis remains elusive; however, several features of CSCs enable them to spread and establish secondary tumors at distant sites. Herman et al. reported that CD133+ pancreatic CSCs did not represent a homogeneous cell population but contained a mixture of CD133+/chemokine (C-X-C motif) receptor (CXCR) 4+ and CD133+/CXCR4+ cells [24]. The CD133+/CXCR4+ cells were located at the invasive margin of the tumors, suggesting their higher invasive and metastatic phenotype. Moreover, the chemokine stromal cell-derived factor-1 (SDF-1), the ligand of CXCR4, was shown to enhance the in vitro chemotactic migration of CD133+/CXCR4+ PC cells, whereas this migration could be blocked by CXCR4 neutralizing antibodies. Likewise, eliminating CD133+/CXCR4+ cells by CXCR4-targeting AMD-3100 prevented the development of liver metastases in mice without affecting their tumor-initiating ability [24]. Consistent with these observations, CD133+ PC cells showed enhanced cell proliferation, invasion, and migration, especially when cocultured with primary pancreatic stromal cells expressing SDF-1 [41].

In addition to cell surface markers, the cellular molecule aldehyde dehydrogenase (ALDH) 1 involved in drug resistance has been used to identify pancreatic CSCs. ALDH1 is a detoxifying enzyme that catalyzes the oxidation of intracellular aldehydes, and plays a role in early differentiation of stem cells through its function in the conversion of retinol to retinoic acid [42, 43]. In PC, ALDH1+ cells were reported to be highly tumorigenic, initiating tumor development at low cell numbers and undergoing epithelial-mesenchymal transition (EMT) [36, 37]. PC cells with high ALDH1 activity were resistant to chemotherapy-induced cell death and exhibited a significant tumorigenicity irrespective of CD133 expression [44]. Furthermore, increased expression of ALDH1 in primary human PC specimens was associated with worse survival [37].

Like CXCR4 and ALDH1, c-Met has been recognized as a functional marker of pancreatic CSCs based on its physiological and pathological functions. PC cells expressing high levels of c-Met (c-Methigh) were capable of forming tumorspheres, but this was not the case in cells

### Table 2. Associations between CSCs and TAMs

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Descriptions</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Brain</td>
<td>- CSCs-derived chemoattractants induced macrophage infiltration and polarization.</td>
<td>[114, 115]</td>
</tr>
<tr>
<td>Oral</td>
<td>- CD163+ TAMs correlated with poor prognosis and CSCs.</td>
<td>[117]</td>
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<tr>
<td>Breast</td>
<td>- TAMs stimulated CSC proliferation and self-renewal through HAS2.</td>
<td>[118]</td>
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<td></td>
<td>- TAMs might promote cancer metastasis through cell fusion, and the hybrids might gain a CSC phenotype.</td>
<td>[119]</td>
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<tr>
<td></td>
<td>- Depletion of TAMs suppressed the generation of CSCs and metastatic potential by modulating the NFkB/miR-448 circuit.</td>
<td>[120]</td>
</tr>
<tr>
<td></td>
<td>- TAMs regulated murine CSCs through a paracrine EGFR/STAT3/Sox-2 signaling pathway.</td>
<td>[121]</td>
</tr>
<tr>
<td>Liver</td>
<td>- TAMs promoted CSC-like properties via TGF-β1-induced EMT.</td>
<td>[116]</td>
</tr>
<tr>
<td>Colon/Lung</td>
<td>- The release of MFG-E8 and IL-6 by TAMs protected CSCs from chemotherapy and promoted their tumorigenicity in colon cancer and non-small cell lung cancer.</td>
<td>[122]</td>
</tr>
<tr>
<td>Stomach</td>
<td>- CSCs promoted TAMs to produce OPN, which in turn facilitated the tumorigenicity of colon cancer via the CD44-OPN interaction.</td>
<td>[123]</td>
</tr>
<tr>
<td>Pancreas</td>
<td>- Targeting TAMs eliminated CSCs, relieved immunosuppression, and improved therapeutic responses.</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>- The presence of CD44+/CD133+ CSCs positively correlated with CD204+ TAMs, which predicted a poor outcome.</td>
<td>[32]</td>
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lacking c-Met expression. Within the c-Met\textsuperscript{high} cell population, cells coexpressing c-Met and CD44 were more tumorigenic than any other cells and able to form tumor xenografts that histologically and phenotypically resembled the original patient tumors. Knockdown of c-Met by short hairpin RNAs or blockade of c-Met activity by the pharmacologic inhibitor XL184 reduced the CSC population, sphere formation, tumor growth, and metastasis, suggesting that c-Met is a therapeutic target for eliminating pancreatic CSCs.

Several studies have also attempted to separate tumorigenic cells from pancreatic tumors using some other distinctive markers or methods. A side population, a subset of cells isolated by their ability to efflux Hoechst 33342 dye, can exclude chemotherapy drugs due to the presence of ATP-binding cassette (ABC) transporters. Side population cells isolated from PC cells have been reported to harbor CSC-like properties that are associated with the aggressiveness, metastasis, and therapeutic resistance of PC. Given that the phenotype of side population cells often correlates with the ABC transporter ABCG2, ABCG2 has been considered a marker of pancreatic CSCs. Using a fluorescence reporter system to monitor the protease activity, PC cells with a low activity of 26S proteasome displayed CSC properties. The CSC characteristics of β\textsubscript{3}+ tumor cells were mediated by the interaction of integrin β\textsubscript{3} with KRAS and subsequent activation of the NF-κB signaling pathway. Doublecortin and Ca\textsuperscript{2+}/calmodulin-dependent kinase-like (Dclk) 1 could reportedly distinguish CSCs from normal stem cells in the intestine of Apc\textsuperscript{Min/+} mice. Similar to the results in intestinal adenomas, Dclk1\textsuperscript{+} cells with CSC-like properties were also identified in PC, even in premalignant pancreatic intraepithelial neoplasia (PanIN).

1.3 Signaling pathways in pancreatic CSCs

Recent characterization of CSCs has led to the identification of several key signaling pathways in CSC maintenance that may serve as attractive therapeutic targets. The self-renewal of normal stem cells has been shown to be regulated by embryonic signaling pathways, including Hedgehog (Hh), Notch, and Wnt/β-catenin. Notably, these embryonic pathways have been reported to be upregulated in CSCs and to contribute to CSC functions. While the Hh pathway is required for pancreatic morphogenesis and cellular differentiation, dysregulation of Hh signaling has been considered as a key event in the pathogenesis of PC. Transgenic mice overexpressing SHh, a ligand of Hh signaling, in the pancreatic endoderm developed PanIN-like lesions that contained KRAS mutations and overexpressed HER-2/neu, revealing that the Hh signaling pathway has an early role in pancreatic tumorigenesis. Recent evidence also demonstrated that SHh and several other Hh signaling components were...
highly expressed in pancreatic CSCs, but not in normal pancreatic stem cells and normal pancreatic ductal epithelial cells [22, 53-56]. The natural compounds sulforaphane, epigallocatechin-3 gallate, and quercetin have been reported to impede EMT and the self-renewal capacity of pancreatic CSCs by attenuating the Hh signaling pathway [55-58]. Blockade of the Hh signaling pathway with cyclopamine, a specific inhibitor of Smoothened protein, not only retarded the proliferation of pancreatic CSCs but also decreased ABCG2 expression, resulting in a reversal of the chemoresistance of pancreatic CSCs [54]. IPI-269609, an orally bioavailable small-molecule Hh inhibitor, strongly inhibited metastatic spread and tumor initiation in murine xenograft models of human PC through the selective depletion of tumorigenic ALDHbright cancer cells [59]. Interestingly, a recent study reported that chloroquine, an inhibitor of autophagy, could target pancreatic CSCs by suppressing CXCR4 as well as Hh signaling independently of autophagy and inhibiting Hh signaling in the stroma, which supports CSCs and non-CSCs in a paracrine manner [60]. Taken together, these findings support the critical role of the Hh signaling pathway in sustaining the stemness of pancreatic CSCs.

The Notch signaling pathway is a key regulator of cell fate specification and progenitor maintenance during pancreatic development [61]. Additionally, the Notch pathway appears to play an important role in the regulation of pancreatic CSCs. Several studies showed that pancreatic CSCs expressed high levels of Notch1 and Notch2 [28, 40]. Forced overexpression of Notch1 or ligand activation of the Notch pathway increased the formation of PC spheres consistent with expression of the CSC surface markers CD44 and ESA [62, 63]. The ability to form tumourspheres in PC was inhibited by RNA interference-mediated silencing of Jagged-1, a Notch ligand, or Hes1, a downstream target of the Notch signaling pathway [63, 64]. Similar results were obtained by disrupting Notch signaling with the γ-secretase inhibitor, which depleted the pancreatic CSC population and impaired CSC function [63-65]. Notch signaling also contributes to the regulation of EMT. Acquisition of the EMT phenotype in gemcitabine-resistant PC cells was found to be consistent with upregulation of Notch2, Notch4, and Jagged-1, and inactivation of Notch signaling by small interfering RNA methodology partially reversed the EMT phenotype, resulting in mesenchymal-epithelial transition [66]. Collectively, these findings indicate that the Notch pathway is involved in the self-renewal of pancreatic CSCs and the EMT process.

The Wnt/β-catenin signaling pathway is known for its diverse roles in embryonic development, stem cell self-renewal, and tumorigenesis [67]. Emerging evidence indicates that activation of the Wnt/β-catenin pathway is involved in PC development and progression. Aberrant cytoplasmic and nuclear expression of β-catenin was frequently found in PC and PanIN samples but not in normal pancreatic tissues [68-71]. High transcriptional activity of Wnt/β-catenin in PC was associated with poor disease-specific survival [72]. Recent reports have also demonstrated the contribution of the Wnt/β-catenin signaling pathway in CSC biology. The Wnt pathway was found to be altered in the side population cells with CSC characteristics isolated from the highly aggressive and metastatic human PC cell line L3.6pl [47]. Another study identifying a tubulogenesis-specific gene signature of pancreatic ductal adenocarcinoma reported that ASPM (abnormal spindle-like microcephaly associated) maintained PC stemness by regulating the activity of the Wnt/β-catenin pathway [71].

In addition to the aforementioned embryonic signaling pathways, other signaling pathways, such as the autophagy pathway, the FOXM1 signaling pathway, the IL-8/CXCR1 signaling pathway, the KRAS/ c-Jun-NH2-kinase (JNK) axis, the mTOR pathway, and the Nodal pathway, have been described in regulating pancreatic CSC activity [73-79]. However, the significance of these signaling pathways remains to be clarified.

2. TAMs in PC

2.1 The tumor microenvironment in PC

Over a decade ago, Hanahan and Weinberg proposed six hallmark capabilities that cells must acquire along the multistep process of tumor pathogenesis to become malignant [80]. With the remarkable progress in the field of cancer research, it is now known that tumor behavior is not completely determined by cancer cells alone but is also modulated by stromal cells that reside in the tumor microenvironment. The extensive stroma surrounding cancer cells referred to as the desmoplastic reaction is a prominent pathological characteristic of PC. The dense desmoplastic reaction may occupy more than 80% of the pancreatic tumor volume and is populated by many cells, including fibroblasts, stellate cells, endothelial cells, and numerous inflammatory cells [81]. The desmoplastic reaction in PC is believed to contribute to the aggressiveness of the disease by promoting tumor growth, metastatic spread, and drug resistance [82]. Macrophages are a major component of inflammatory cells infiltrated in the tumor microenvironment [7]. In the following sections, we discuss recent findings regarding the role of macrophages in PC progression.

2.2. Identification of TAMs

It was initially discovered in the late 1970s that a major leukocyte population infiltrating tumors, the so-called TAMs, could promote tumor growth and progression [83, 84]. TAMs express the M2 macrophage phenotype and exhibit
protumoral activities depending on the microenvironment [85]. Macrophages are derived from circulating monocytes and circulating monocytic precursors that are located in the bone marrow. Two distinct states of polarized activation have been proposed for macrophages: classically activated (M1) macrophage and alternatively activated (M2) macrophages [86, 87]. Classical activation of macrophages occurs in response to microbial stimuli such as lipopolysaccharide (LPS) and the T-helper (Th) 1 cytokine interferon-γ. M1 macrophages show increased expression of inflammatory cytokines, chemokines, and reactive nitrogen and oxygen intermediates, and thus, they promote Th1 responses, possess anti-microbial ability, protect against various types of bacteria and viruses, and display tumoricidal functions. By contrast, Th2 cytokines such as interleukin (IL)-4, IL-10, IL-13, or glucocorticoid hormones drive macrophages into the alternatively activated state. M2 macrophages show increased expression of scavenging receptors and scavenging activity, reduced expression of inflammatory cytokines, and release cytokines including IL-10 that promote a Th2 immune response. M2 macrophages have been demonstrated to contribute to defense against helminthes, drive allergy pathogenesis, inhibit inflammation, regulate wound healing, and drive fibrosis [88-90].

TAMs can be identified based on a number of cell surface markers, the production of cytokines, the expression of transcriptional factors, and their specified functions. The glycoprotein CD68, the hemoglobin scavenger receptor CD163, and the macrophage scavenger receptor CD204 are frequently used to detect TAMs in different types of human tumors [91]. However, several studies showed that the density of CD68+ cells did not correlate with cancer patient survival [13, 92, 93], supporting the heterogeneity (such as M1 and M2) of the macrophages infiltrating tumors. Given that CD68 is expressed by multiple subpopulations of macrophages, including M1 and M2 macrophages, the use of CD68 as a prognostic marker may be unreliable. Instead, CD163 and CD204 are expressed specifically in TAMs and thus are now widely recognized as TAM markers. Antibodies against the LPS coreceptor CD14, CD11b, or mannose receptor CD206 have also been used for the flow cytometric analysis of monocytes or macrophages; however, only CD206 can specifically label M2 macrophages [94, 95]. In addition, TAMs can be functionally characterized by the production of matrix metalloproteinases, inducible nitric oxide synthase, and IL-10 [87, 96, 97]. Activation of signal transducer and activator of transcription (STAT) 3, a transcription factor that acts downstream of the IL-6 and IL-10 signaling pathways, has also been reported to identify TAMs in a previous study [98].

2.3. Role of TAMs in PC progression

It is now generally accepted that TAMs are capable of secreting a series of cytokines, chemokines, and proteases to promote tumor growth, angiogenesis, metastasis, immunosuppression, and matrix deposition and remodeling [99, 100] (Figure 1). Studies have indicated that the tumor microenvironment is rich in a number of chemoattractants, such as colony-stimulating factor (CSF)-1, chemokine (C-C motif) ligand (CCL) 2, and IL-10, all of which mediate the trafficking of circulating monocytes towards target tissues [101, 102]. In PC, the inhibition of macrophage recruitment to the tumor microenvironment by genetic and pharmacologic inhibition of CSF-1 and CCL2 resulted in increased antitumor T-cell responses, improved chemotherapeutic efficacy, and counteracted tumor cell invasion and metastasis [103, 104]. SDF-1α, which is released by murine PC cells, or IL-1β, which is secreted by tumor-derived macrophages, could directly recruit proangiogenic macrophages to tumor tissues and subsequently collaborate with integrin αβ2 of myeloid cells to promote tumor inflammation, angiogenesis, and growth. Combined blockade of SDF-1α and IL-1β attenuated these effects and acted synergistically with the chemotherapeutic agents [105]. Furthermore, inhibition of macrophage invasion into the tumor by targeting adhesion molecule integrin αβ2 in macrophages or myeloid PI3Kγ led to a marked decrease of blood vessel formation and suppressed tumor inflammation, growth, and metastasis in PC mouse models [106].

In addition to the recruitment of TAMs by tumor cells, macrophage conversion also plays an important role in regulating cancer progression. Histidine-rich glycoprotein (HRG), a 75-kDa heparin-binding plasma protein, has been implicated in the regulation of tumor growth and vascularization. By skewing the polarization of TAMs away from M2- to a tumor-inhibiting M1-like phenotype, HRG promoted antitumor immune responses and vascular normalization, effects known to decrease tumor growth and metastasis and to augment chemotherapeutic efficacy [107]. Furthermore, HRG deficiency in mice challenged with pancreatic carcinoma influenced macrophage gene regulation, resulting in excessive stimulation of tumor angiogenesis, suppression of host antitumor immune responses, and increased tumor growth and metastatic spread [108]. The conversion of M2 macrophages to M1 macrophages by an agonist CD40 monoclonal antibody was reported to circumvent tumor-induced immune suppression and invoke productive T cell-dependent antitumor immunity. The authors evaluated the effects of the combination of an agonist CD40 antibody with gemcitabine chemotherapy in human and mouse PC and found that M1 macrophages impeded pancreatic tumor growth by depleting the tumor stroma to improve the therapeutic outcome; however, T cells or gemcitabine did not [109]. By contrast, secreted protein acidic and rich in
cysteine (SPARC), which is overexpressed by tumor-associated fibroblasts in human and mouse PC, has been reported to affect the conversion of macrophages in PC. Macrophages isolated from pancreatic tumors that were orthotopically grown in SPARC-deficient mice displayed an increased M2 immunosuppressive phenotype, and the tumor cells were more invasive and metastatic [110].

Recently, several studies have found that paracrine interactions between TAMs and PC cells orchestrate tumorigenesis. In the endocrine pancreatic tumor mouse model, blocking angiopoitin-2 (ANG2), a Tie2 ligand, and angiogenic factors expressed by activated endothelial cells and TAMs repressed the tumor vasculature and inhibited progression of RIP1-Tag2 pancreatic insulinosomas. While inhibition of ANG2 did not prevent the recruitment of TAMs, it impeded the upregulation of Tie2, which is required for the association with tumor blood vessels and the proangiogenic activity of TAMs, and enhanced the anti-VEGF/VEGFR therapeutic effects [111]. The expression of Vasohibin-1, a negative feedback regulator of angiogenesis, in PC cells cocultured with TAMs was found to be significantly upregulated in response to the inhibition of TGF-β receptors or bone morphogenetic protein (BMP) receptors, which suggested that targeting the TGF-β or BMP pathway might shed new light on strategies for antiangiogenesis therapy [112]. Perineural invasion of cancer cells (CPNI) is found in most PC patients. Specimens from patients with CPNI revealed an increased number of endoneurial macrophages that surround nerves invaded by cancer cells. These macrophages secreted high levels of glial-derived neurotrophic factor (GDNF) to trigger activation of the GDNFR receptor GFRA1 and its coreceptor RET. Phosphorylation of RET induced ERK activation, leading to cancer cell migration [104]. IL-10 is commonly regarded as an immunosuppressive cytokine. Recent reports showed that activation of Toll-like receptor 4 (TLR4) signaling on M2-polarized TAMs stimulated an increased release of IL-10. Activation of TLR4/IL-10 signaling was able to promote EMT in PC cells by TAMs [97, 113]. Collectively, these findings expand our understanding of the protumoral role of TAMs in PC and reveal attractive novel therapeutic strategies.

3. Interplay between CSCs and TAMs

The association between CSCs and TAMs has been demonstrated in several studies (Table 2). In glioma, CSCs were reported to play a leading role in macrophage infiltration and polarization. Glioma CSCs produced macrophage inhibitory cytokine-1, transforming growth factor-β1 (TGF-β1), and soluble CSF, cytokines known to recruit and polarize macrophages to a M2 phenotype, to inhibit macrophage phagocytosis, to induce secretion of the immunosuppressive cytokines IL-10 and TGF-β1 from TAMs, and to reduce T-cell proliferation. The CSCs modulated innate immunity in glioma by inducing the generation of immunosuppressive macrophages, and this effect could be reversed by inhibiting phosphorylated STAT3 [114]. Another paper reported that the production of CSC-derived chemokine, including CCL2, CCL5, CCL7, VEGF-A, and neurotensin, was much higher in migratory glioma cells than in adhesive glioma cells, promoting the infiltration of TAMs. In human glioma tissues, TAMs were frequently found distributed around CSCs and correlated positively with the histological tumor grade and the number of CSCs [115]. Similar results were also observed in hepatocellular carcinoma (HCC). The number of TAMs correlated positively with the density of CSCs in the margin of human HCC and was strongly associated with patient survival. TAMs could promote CSCs properties via TGF-β-induced EMT, which resulted in the acquisition of a higher invasive capability in CSCs [116].

In breast cancer, similar interplays also have been reported between CSCs and TAMs. Okuda et al. found that the expression of hyaluronan synthase (HAS) 2, which correlated with tumorigenicity and tumor progression in several cancers, was upregulated in highly metastatic breast CSCs, leading to enhanced secretion of platelet-derived growth factor-BB (PDGF-BB) by TAMs. Subsequently, PDGF-BB activated stromal cells to secrete fibroblast growth factor (FGF) 7 and FGF9, which could stimulate CSC proliferation, self-renewal and metastasis in the bone [118]. It was also reported using a coculture system that TAMs induced the enrichment of breast CSCs and metastatic potential of breast cancer cells. Interruption of the interaction between TAMs and CSCs by pterostilbene suppressed the generation of CSCs and metastatic potential by modulating EMT-associated signaling pathways, especially the NF-κB/microRNA-488 (miR-488) circuit, which suggested that modulation of this interaction could be an ideal strategy in clinical settings [120]. In murine breast cancer, TAMs have been observed to upregulate several CSC properties, such as increased gene expression of Sox-2, Oct-4, Nanog, and ABCG2, enhanced drug-efflux capacity, resistance to chemotherapy, and tumorigenicity. The novel epidermal growth factor receptor (EGFR)/STAT3/Sox-2 paracrine signaling pathway between TAMs and mouse breast cancer cells was required for TAM-induced Sox-2 upregulation and CSC phenotype formation in tumor cells [121]. Using polyethylene glycol, TAMs could merge with breast cancer cells to form fusion hybrids. The fusion hybrids displayed a CSC phenotype, including increased migration, invasion, and tumorigenicity but reduced proliferative ability, compared with the parental cells. These results suggested that TAMs might promote breast cancer metastasis through cell fusion, and the hybrids could gain a CSC phenotype [119].

CSCs from murine colon and lung cancer could
specifically stimulate TAMs to express milk-fat globule epidermal growth factor-VIII (MFG-E8), which has been identified as a growth factor involved in phagocytosis, angiogenesis, and immune tolerance. The release of MFG-E8 and IL-6 by TAMs could induce CSCs to form tumors and develop therapeutic resistance through the STAT3 and Hh pathways. Colon CD44+ CSCs, when cultured or co-infected with macrophages, triggered macrophages to produce and secrete high levels of osteopontin (OPN), which in turn augmented the tumorigenic properties of colon cancer cells. Knockdown of CD44 expression or treatment of CD44 blocking antibodies attenuated OPN secretion. The enhancement of tumorigenicity by OPN was likely through the JNK signaling pathway in a CD44v6-dependent manner. Moreover, patients with high levels of OPN and CD44v6 had poor survival rates. In gastric cancer, macrophage infiltration was found to play a critical role in the activation of Wnt/β-catenin signaling, leading to CSC maintenance and tumor development. Mechanistically, tumor necrosis factor-α derived from activated macrophages could upregulate the Wnt/β-catenin activity in gastric cancer cells through the suppression of glycogen synthase kinase 3β. The results suggested that the suppression of macrophage infiltration and activation might be a good strategy for chemoprevention against gastric cancer. Thus far, little is known about the interplay between pancreatic CSCs and TAMs. A recent study reported that depletion of TAMs or inflammatory monocytes by CSF-1R antagonists, chemokine (C-C motif) receptor (CCR) 2 inhibitors, or knockout of CCR2 in mice bearing orthotopic pancreatic tumors decreased the number of ALDHbright CSCs, reduced metastasis, and enhanced resistance to gemcitabine. By contrast, TAMs appeared to directly enhance the tumor-initiating capacity of PC cells by activating STAT3 and thereby facilitating macrophage-mediated suppression of CD8+ T lymphocytes. The results demonstrated the important role of TAMs in regulating pancreatic CSC properties (Figure 1); however, further studies are required to confirm the significance of the interplay between pancreatic CSCs and TAMs in the development of PC.

TAMs have been identified in situ predominantly by immunolabeling with CD68, CD163, or CD204 antibodies. Most studies suggest that a large number of infiltrating TAMs in tumors is associated with a poor prognosis. TAMs have been observed in tumor front, stroma, and metastatic lesions of patients with glioma, ovarian tumors, gastrointestinal stromal tumors, melanoma, gastric cancer, or PC, and were associated with a shorter survival time. The protein expression of CD68 and CD163, as well as the CSC markers ALDH1, CD44, and Sox-2, increased successively from normal oral mucosa to oral squamous cell carcinoma (OSCC), revealing the clinical significance of the correlation between TAM markers and CSC markers.

The expression levels of CD68 or CD163 correlated significantly with lymph node status. Sox-2 expression correlated significantly with the pathological grade and lymph node status, whereas ALDH1 was only associated with the tumor stage. Furthermore, CD68 and CD163 were positively associated with CSC markers, but only high expression levels of CD163 correlated with poor overall survival of patients with OSCC. Consistent with these findings, our previous study showed that the expression levels of CD44+/CD133+ CSCs and CD204+ were significantly higher in PC lesions than in normal regions of the tissue. There was a positive correlation between CD44/CD133 and CD204 expression, and the elevated expression of both CD44/CD133 and CD204 were significantly associated with a shorter survival time. These results suggest that co-expression of CSC markers and TAM markers could have potential prognostic value in PC. Taken together, these studies strongly support a critical role for TAMs in the induction, maintenance, and expansion of CSCs in the tumor microenvironment. Thus, targeting key factors derived from CSCs or TAMs provides a unique strategy to eradicate tumors that are resistant to therapy by manipulating CSC activities.

Conclusions

The CSC theory has recently emerged as an attractive model for tumor development and progression. The identification of various potential CSC markers and important signaling pathways for CSC functions opens a new door for PC therapy via targeting CSCs. To design optimal therapeutic strategies, further investigations of CSC biology are required to identify reliable markers of pancreatic CSCs, to determine the specific signaling pathways that are critical for the formation of pancreatic CSCs but are not shared by pancreatic normal stem/progenitor cells, and to understand the detailed mechanisms underlying the resistance of pancreatic CSCs to radio/chemotherapy.

As a predominant component of leukocyte infiltrates in the tumor microenvironment, TAMs do not mount an effective host antitumor immune response but engage in the establishment of an immunosuppressive microenvironment and promote tumor growth, progression, and metastasis. Accumulating evidence has indicated that TAMs are able to release growth factors, cytokines, or other factors to promote the survival and self-renewal of CSCs, which suggests that TAMs are a promising target for cancer therapy. It is necessary in the future to identify TAM-specific molecular signatures and dissect the signaling networks responsible for the crosstalk between pancreatic CSCs and TAMs. Therefore, novel therapeutic approaches for targeting pancreatic CSCs in combination with interrupting the interplay between pancreatic CSCs and TAMs may give rise to more effective clinical treatments.
or even a cure for PC.

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Conflict of interest

The authors disclose no conflicts.

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