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Stem Cell Directed Therapies in Pancreatic Cancer

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Abstract

Cancer stem cells (CSCs) are phenotypically distinct cells that are functionally characterized by their ability to initiate tumor formation. CSCs have been identified in many malignancies, including pancreatic ductal adenocarcinoma (PDAC). PDAC continues to have one of the worst prognoses of any malignancy largely due to its propensity for early metastasis and resistance to current therapies. A growing number of studies have implicated a role for CSCs in these processes. In this review, we discuss studies showing an association between the detection of CSCs and worse clinical outcomes, unique molecular signaling pathways that are important for CSC function, and early preclinical and clinical studies aimed at abrogating PDAC CSCs.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and continues to have one of the poorest prognoses of any malignancy [1, 2]. Despite recent advances in treatment, PDAC is still largely resistant to chemotherapy and radiation therapy and novel treatments are desperately needed [3–5]. The genetic and cellular heterogeneity within pancreatic tumors may account for its aggressiveness. Modern sequencing techniques have revealed genetically heterogeneous clones of malignant cells in any given primary tumor and metastatic lesion from patients with PDAC[6, 7]. There is also emerging evidence that the aggressiveness of PDAC may be partly driven by phenotypically distinct cell populations, such as cancer stem cells (CSCs) [8–10].

Originally identified in hematopoietic malignancies [11, 12], CSCs have now been identified in a number of solid tumors [9, 13–15]. CSCs are phenotypically distinct cells that are functionally defined by their ability to initiate tumor formation when implanted into

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immunocompromised mice; thus, they possess the capacity for self-renewal and differentiation [16]. PDAC CSCs have been identified and isolated based on the expression of CD44/CD24/epithelial specific antigen (ESA), CD133, and aldehyde dehydrogenase (ALDH) [8–10]. All three CSC populations are relatively rare and largely non-overlapping, yet they are similarly tumorigenic when as few as 100 cells are injected into immunocompromised mice.

CSCs have been implicated in fueling tumor growth, metastasis, and resistance to chemo- and radiotherapy. In this review, we will discuss recent advances in PDAC CSC biology and emerging strategies to target them.

Clinical Significance of CSCs

CSCs are associated with worse clinical outcomes

The expression of stem-like gene expression profiles and the frequency of phenotypic CSCs have been associated with worse clinicopathological outcomes for patients with PDAC [10, 17], and other malignancies [6, 7, 18–22]. Madea et al. found that CD133 expression in resected specimens from patients with PDAC was associated with worse histologic tumor grade ($p=0.0215$), lymphatic invasion ($p=0.0023$), and lymph node metastasis ($p=0.0024$) [17]. In addition, the 5-year survival of patients with CD133-positive tumors was significantly lower than that of patients with CD133-negative tumors ($p=0.0002$). In another study, Rasheed et al. found that the presence of ALDH-positive PDAC cells in resected surgical specimens was associated with worse survival compared to patients with ALDH-negative tumors [10]. In that study, they also found that ALDH expression in metastatic lesions was greater than that in primary tumors, suggesting a link between ALDH expression and disease progression.

Tumors expressing markers corresponding to a CSC phenotype are also associated with inferior clinical outcomes in other malignancies, including breast cancer [18, 19] and leukemia [20]. ALDH-positive breast cancer specimens were associated with worse histologic grade, ERB2 over-expression, absence of estrogen and progesterone receptor expression, and inferior overall survival [18]. In another study, a gene signature derived from phenotypic breast CSCs was associated with an invasive phenotype and with increased risk of metastases and death [19]. Unique stem cell-like gene signatures in leukemia are also associated with inferior clinical outcomes, including a lower complete remission rate and shorter disease-free and overall survival [20–22].

CSCs are resistant to chemotherapy and radiation therapy

There is increasing evidence that CSCs are resistant to chemotherapy and radiation therapy. Clinically, when chemotherapy is administered, non-CSCs susceptible to the agent may be depleted, but remaining CSCs are able to divide and repopulate the tumor with resistant cells. The mechanisms of resistance in CSCs have been attributed to a number of factors, including high level of anti-apoptosis gene expression, DNA repair, and drug efflux proteins [23–27].

Drug efflux mechanisms have been implicated in PDAC CSC drug resistance in several studies. Zhou et al. identified a “side-population” of pancreatic CSCs that is characteristically identified by their ability to efflux Hoechst 33342 dye [28]. Following gemcitabine administration, the proportion of side-population cells increased, indicating a role for this unique population of cells in conferring drug resistance. In another study, Hong et al. demonstrated that following high-dose gemcitabine treatment, most cells were eliminated; however, a population of CD44⁺CD24⁺ESA⁺ cells proliferated and constituted a population of resistant cells [29]. Verapamil, an inhibitor of ABCB1 (MDR1), re-sensitized

these cells to gemcitabine indicating that the mechanism of resistance was mediated by expression of ABC transporters.

While these data support the concept of chemo-resistance in PDAC CSCs, little is known regarding radiation resistance in PDAC CSCs. CSCs in other malignancies are resistant to radiation therapy. Philips et al. showed that CD44⁺CD24^{neg/low} breast CSCs are more resistant to radiation therapy compared to non-CSCs [30]. Similarly, Bao et al. found that CD133⁺ CSCs were enriched following radiation in patient glioblastoma specimens and human glioma xenografts [31]. The authors showed that CD133⁺ cells activated the DNA damage checkpoints and were more effective at DNA damage repair compared to CD133^{neg} cells. Furthermore, an inhibitor of Chk1 and Chk2 reversed the radio-resistance of the CD133⁺ cells. Diehn and colleagues found that human and murine breast CSCs displayed lower reactive oxygen species (ROS) levels than corresponding non-CSCs and were associated with increased expression of free radical scavenging systems [32]. Pharmacologic inhibition of ROS defense mechanisms resulted in re-sensitization of breast CSCs to radiation. Future research will determine if PDAC CSCs are similarly resistant to radiation therapy.

CSCs potentiate metastasis

A number of studies have shown that PDAC CSCs play a role in metastasis formation. In a study using specimens from 80 patients undergoing resection for pancreatic adenocarcinoma, Maeda et al. found that CD133 expression was not present in normal pancreatic ductal epithelium; however, CD133⁺ cells were identified at the periphery of the cytokeratin⁺ cells and its expression correlated with the presence lymph node metastases [17]. Herman et al. identified a distinct subpopulation of CD133⁺ CSCs that are CXCR4⁺ and found that they were more metastatic than CXCR4^{neg} cells [8]. Depletion of CD133⁺CXCR4⁺ cells dramatically diminished the rate of metastases without influencing tumor-initiation. Rasheed et al., found that ALDH⁺ and CD44⁺CD24⁺ PDAC CSCs have a mesenchymal gene expression profile, suggesting a role for these cells in metastasis. Interestingly, ALDH⁺ CSCs were more invasive than CD44⁺CD24⁺ CSCs and non-CSCs in an *in vitro* invasion assay [10]. Li et al. identified a subpopulation of CD44⁺ cells that express c-Met cells that are more metastatic than c-Met^{neg} cells. Therapeutic targeting of c-Met with cabozantinib (XL184) led to decreased CSC function and metastasis [33].

Targeting Pancreatic Cancer Stem Cells

Recent studies have begun to elucidate unique features of the three PDAC CSC populations, and novel therapies are being examined in preclinical and clinical studies (Table 1).

Developmental Pathways

Since CSCs share functional properties with normal stem cells (i.e. self-renewal and differentiation), early focus on CSC-targeting agents has been on developmental pathways such as Notch, Hedgehog, Bmi1, and Nodal/Activin. Notch-1 signaling mediates downstream Kras signaling and has been shown to promote pancreatic intraepithelial neoplasia (a precursor lesion to PDAC) initiation and progression [34]. Given its potential role in tumor progression, angiogenesis, and metastasis, pre-clinical work has focused on potential therapies that down-regulate this pathway [35]. A number of γ -secretase inhibitors (GSIs), which prevent Notch pathway activation by inhibiting γ -secretase dependent cleavage of the Notch receptor and subsequent release of the Notch intracellular domain, have been investigated in PDAC [36–38]. Treatment of PDAC cells with GSI-18 led to a decrease in ALDH⁺ cells, inhibition of colony formation, and reduced xenograft engraftment *in vivo* [36]. Another GSI, GSI IX, decreased CD44⁺EpCAM⁺ CSCs, and suppressed

tumorigenesis in a mouse xenograft model [37]. Yabuuchi et al. showed that treatment with gemcitabine plus PF-03084014, another GSI, not only induced tumor regression in 3 out of 4 human PDAC xenograft models, but also induced apoptosis, inhibition of tumor cell proliferation, and reduced angiogenesis when compared to treatment with gemcitabine alone [38]. Clinical trials utilizing notch targeting have been implemented at multiple institutions, primarily for Stage IV and recurrent PDAC (www.clinicaltrials.gov). Four of these trials, primarily using Notch inhibitors RO4929097 and MK0752, are currently open.

The Hedgehog (Hh) pathway is activated in PDAC and may be responsible for the maintenance of CSCs by regulating cell differentiation, tissue polarity, and cell proliferation via multiple downstream proteins, including Gli transcriptional factors [39, 40]. A number of small molecule antagonists of Smoothened (Smo) have been developed. Cyclopamine has been examined in a number of pre-clinical studies and found to abrogate PDAC CSCs in cell lines and human xenografts [41–43]. Feldmann et al. found that cyclopamine treatment led to a dramatic reduction in metastases in mice with orthotopically implanted tumors [41]; and Jimeno et al. found that treatment of mice with gemcitabine plus cyclopamine induced tumor regression, whereas treatment with either drug alone did not do so [42]. Gu et al. used another small molecule inhibitor of Smo, BMS833923, and found that the combination of radiation and BMS833923 reduced lymph node metastases in mice orthotopically injected with PDAC cells [39]. IPI-269609, another Smo antagonist, demonstrated excellent activity against human xenograft models and a reduction in ALDH⁺ CSCs [41]. Sulforaphane has also been used to block Gli transcriptional activity and resulted in the inhibition of PDAC CSC function [44]. Though early clinical trials with Hh antagonists in PDAC have been disappointing, studies are underway to determine the effect of these compounds against CSCs in clinical specimens (clinicaltrials.gov identifier NCT01088815). In addition, Hh signaling has been shown to be important in the stromal cells of PDAC, which makes interpreting the results of Hh inhibition even more complex [45].

Nodal and Activin are secreted proteins expressed during development and critical for mesoderm formation as well as embryonic stem cell maintenance [46]. Lonardo et al. found that inhibition of Nodal/Activin signaling using an ALK4 receptor antagonist in PDAC cells led to decreased CD133⁺ CSC function as well as reversal of resistance to gemcitabine [46]. These effects were enhanced in engrafted human pancreatic xenografts when Nodal/Activin receptor inhibition was combined with Hh pathway inhibition [46].

Cell Surface Antagonists

Since CSCs can be identified based on the expression of distinct cell surface antigens, some CSC-targeting strategies have focused on targeting those proteins. The proto-oncogene c-Met has been reported as a marker of normal pancreatic ductal progenitor cells and PDAC CSCs [33, 47] and plays an important role in PDAC cell motility, invasion, and metastasis [48]. Two studies have investigated the role of cabozantinib, a small molecule inhibitor of c-Met, against PDAC CSCs [33, 49]. Preclinical data demonstrates reduced tumor sphere formation with cabozantinib, as well as slowed tumor growth and reduced metastatic potential in xenograft models [33]. In addition, Hage et al. found that cabozantinib enhanced the efficacy of gemcitabine in pancreatic cancer cell lines [49].

Other cell surface proteins expressed in PDAC CSCs are Muc1, and death receptor 5 (DR5) [50, 51]. DR5 expression is enriched in PDAC CSCs, and Tigaztuzumab, a DR5 agonist, treatment in combination with gemcitabine, greatly reduced PDAC CSCs, enhanced tumor shrinkage, and lengthened time to tumor progression in a human PDAC xenograft model [51]. MUC1, another cell surface protein that is associated with worse clinical outcomes in patients with PDAC, was recently found to be co-expressed with CD133⁺ and CD44⁺CD24⁺

PDAC CSCs in patients and mouse models [50]. These data provide rationale for future therapeutic targeting of PDAC CSCs via MUC1.

Other CSC Targets

Epithelial-mesenchymal transition (EMT) is a process that has been implicated in metastasis, drug resistance, and generation of CSCs [52, 53]. Using breast cells forced to undergo EMT and acquire a CSC phenotype, Gupta et al. identified salinomycin from a large chemical compound screen that selectively eliminates CSCs [54]. The mechanism by which salinomycin targets CSCs is not yet clear, but it may disrupt EMT. Recent studies have also demonstrated that oxidative metabolism is upregulated in CSCs, and that metformin may play a role in targeting this process [55–57]. Gou et al. recently found that metformin selectively abrogated CD133⁺ PDAC CSCs, and mTOR and Erk activation may play a critical role [39].

Conclusions

The ability to identify and isolate pancreatic CSCs has enabled us to identify important molecular pathways that are essential to the function of these relatively rare populations of cells. These pathways were previously underappreciated because studies have traditionally focused on “bulk” tumors or cell lines. Successfully targeting pancreatic CSCs may have the potential to dramatically change the clinical outcomes for patients with PDAC. Given the number of the pathways that are activated, and the possibility that different pathways are activated in distinct CSC populations, it may be important to use combinations of “targeted therapies” to eliminate all CSC populations. Furthermore, it will likely be important to implement combinations of CSC-targeting and non-CSC targeting therapies together if we are to optimize tumor response, enhance long term control, and ultimately, improved patient survival.

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Table 1
Targeting Pancreatic Cancer Stem Cell Pathways

Known cellular targets of pancreatic cancer stem cells including agents and mechanisms utilized to target these stem cells.

	Target	Drug	CSC population Targeted
<i>Developmental Pathways</i>	Notch-1	GSI 18 GSI IX PF-03084014	ALDH ⁺ CD44 ⁺
	Hedgehog	Cyclopamine BMS833923 IPI-269609	ALDH ⁺ CD44 ⁺ CD24 ⁺ ESA ⁺
	Nodal/Activin	SB431542	CD133 ⁺
<i>Cell Surface Antigens</i>	c-Met	Cabozantinib (XL184)	CD133 ⁺ CD44 ⁺
	MUC1	TAB 004 antibody	CD133 ⁺ CD44 ⁺ CD24 ⁺
	DR5	Tigaztuzumab	CD133 ⁺ CD44 ⁺ 24 ⁺
<i>Other</i>	Glycolysis	Metformin	CD133 ⁺
	EMT	Salinomycin	CD133 ⁺