Biology and Clinical Applications of Pancreatic Cancer Stem Cells

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Pancreatic ductal adenocarcinomas comprise a hierarchy of tumor cells that develop around a population of cancer stem cells. The cancer stem cells promote tumor growth and progression through a number of mechanisms, including differentiation into bulk tumor cells, metastasis, alteration of adjacent stromal cells, and evasion of conventional therapies. As with other cancer stem cells, pancreatic cancer stem cells (PCSCs) can be distinguished from bulk tumor cells based on their expression of unique surface markers, abilities to form spheres under nonadherent conditions and tumors in mice, and self-renewal and differentiation capacities. We review the markers used to identify PCSCs, the signaling pathways that regulate PCSC functions, the complex interactions between PCSCs and stromal cells, and approaches to therapeutically target PCSCs and improve treatment of patients with pancreatic cancer.

Keywords: Pancreatic Cancer Stem Cells; Tumor Microenvironment; Drug Resistance.

Pancreatic ductal adenocarcinoma (PDAC) is by far the most common form of pancreatic cancer, and the deadliest. PDAC has the lowest 5-year survival rate of any cancer, primarily because early-stage tumors metastasize and do not respond to chemotherapy and radiation. Although these features are not unique to pancreatic cancer, they are the most crucial obstacles to overcome in the treatment of the disease. A distinct population of cancer cells that mediate the metastasis and resistance to standard treatments, known as cancer stem cells (CSCs), promote tumor growth and progression, and could be a target for more effective treatment options.

What are CSCs?

Although cancer cells have unrestrained proliferative capabilities and are resistant to apoptotic cues,[1] only a limited number of cancer cells are actually capable of establishing tumors in immunodeficient mice. Built off of these observations, a cellular hierarchy was identified in hematopoietic malignancies, such as acute myelogenous leukemia and chronic myelogenous leukemia.[2,3] In these malignancies, a minority of cells identified based on specific cell-surface molecules (the CSCs), can self-renew and differentiate into all of the other cell types of cancer cells (the bulk cancer or tumor cell population). Bulk cancer cells lack the ability to differentiate into other subpopulations of cancer cells and have limited self-renewal. In limited-dilution experiments, only CSCs can form xenograft tumors in mice, self-renew, and differentiate into all the other tumor cells.

Interestingly, this hierarchy resembles that of normal hematopoietic stem cells—the 2 systems have been shown to have similar molecular patterns.[4] An important feature of leukemia stem cells is their ability to survive exposure to therapeutics; chronic myelogenous leukemia stem cells can survive exposure to imatinib.[3] The ability of CSCs to survive therapies that kill the bulk leukemia cells allows the CSC population to form new tumors after remission. As such, CSCs are an important hurdle to overcome to completely eradicate cancer in patients.

After the discovery of CSCs in leukemias, CSCs were identified in breast tumors,[5] leading to much research to identify similar subpopulations in other types of solid tumors, often with varying degrees of success. CSC-like populations were reported in a variety of tumors, including glioblastoma,[6] pancreas,[7,8] melanoma,[9] prostate,[10] and colon.[11] CSCs were defined by their ability to self-renew, produce differentiated progeny, form tumors in mice, and form nonadherent spheroids (neurospheres for glioblastoma, melanospheres for melanoma, mammospheres for breast cancer, and more generally tumorspheres for other cancers) in vitro.

Abbreviations used in this paper: ALDH1, aldehyde dehydrogenase 1; CSC, cancer stem cells; EMT, epithelial–mesenchymal transition; HGF, hepatocyte growth factor; PDAC, pancreatic ductal adenocarcinoma; PCSC, pancreatic cancer stem cells; SDF-1, stromal-derived factor-1; SHH, Sonic Hedgehog.

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They were identified based on a number of cell surface markers, often distinct from those of leukemia CSCs.

Unfortunately, although many proteins have been proposed to be markers of CSCs for various types of cancer, there has been great variation among the markers reported for each tumor type, leading to dissent over which cells are truly CSC or even whether CSC exist.[12] Some of this variation might have resulted from the limited numbers of primary tumors studied, or the use of highly passaged cell lines, which no longer contain the hierarchies observed in primary tumors. Other explanations include the lack of standardization of digestion techniques, flow cytometry analyses, and antibodies used. Furthermore, many of the reported markers were never validated functionally. These issues hold true for PDAC—a number of putative pancreatic cancer stem cells (PCSCs), with distinct markers, have been described.

Markers

CD44, CD24, and ESA

The first population of PCSCs was described in 2007 by Li et al.[7] Using tumor xenografts grown from patients' cancer cells, the authors studied the cell-surface markers CD44, CD24, and ESA, based on the CSC properties reported for $CD44^+/ESA^+/CD24^{low}$ cells from breast tumors.[5] Sorting cells by single-, double-, or triple-positive or -negative status, Li et al found that $CD44^+/CD24^+/ESA^+$ cells (which comprised 0.2%–0.8% of the cells in 10 tumors studied) were far more tumorigenic than other populations—as few as 100 cells were able to form tumors in half of the mice.[7] By contrast, only 1 in 12 mice injected with 10,000 triple-negative cells developed a tumor, indicating a 100-fold enrichment of tumor-promoting cells, using triple-positive labeling as a criterion. In addition, the tumors formed by the $CD44^+/CD24^+/ESA^+$ cells had histologic features identical to those of the original tumors from patients. These findings indicated that the cells were able to form tumors with an architecture that resembled human pancreatic tumors.

The $CD44^+/CD24^+/ESA^+$ cells could also re-establish the cell-surface profile of markers of the original tumors, via self-renewal and differentiation into triple-negative or single- and double-positive populations. Finally, the $CD44^+/CD24^+/ESA^+$ cells expressed the developmental factor Sonic Hedgehog (SHH), a ligand in the HH signaling pathway, at much higher levels than in normal pancreatic cells, unsorted pancreatic cancer cells, and triple-negative cells. These findings indicated that this pathway might be important for the tumor-promoting functions of these cells.

$CD44^+/CD24^+$ PANC-1 cells were also found to be 20-fold more tumorigenic than $CD44^-/CD24^-$ cells, and could form tumors identical to unsorted PANC-1 cells.[13] Interestingly, $CD44^+/CD24^+$ cells (2.1%–3.5% of tumor cells) were found to proliferate slowly in culture,[13] in line with reports from other tumor types that CSCs were slow-cycling compared with the nontumorigenic, transient replication of the bulk tumor cells.[14,15]

Although CD44, CD24, and ESA are markers of PCSCs, their functional significance is unclear. As lineage markers, these proteins might be up-regulated by transcriptional networks that control the stem-cell properties of PCSCs—their expression could be a byproduct of the unique signaling events in PCSCs. Alternatively, these proteins could functionally contribute to the PCSC phenotype, perhaps by facilitating cell–cell interactions[16-18] or modulating signaling pathways. CD44 has been shown to promote c-Met activity[19] and inhibit Hippo signaling—a developmental pathway important for development of glioblastoma.[20] Although Hippo signaling has not been described in PCSCs, the pathway has been implicated in the proliferation and clonogenicity of pancreatic cancer cell lines.[21] It would be interesting to learn whether Hippo signaling regulates PCSCs and, if so, whether CD44 controls its activity.

CD133

Only a few months after the identification of $CD44^+/CD24^+/ESA^+$ PCSCs, Hermann et al showed that pancreatic cancer cells that expressed CD133 (Prominin-1/AC133) had CSC properties.[8] A glycoprotein with an unknown function, CD133 is expressed by a number of normal stem and progenitor cell populations[22] and is a marker of CSCs of various origins.[6,9,10] Like $CD44^+/CD24^+/ESA^+$ cells, $CD133^+$ pancreatic cancer cells had increased tumorigenicity compared with $CD133^-$ cells, which failed to form tumors. In addition, $CD133^+$ cells are highly resistant to gemcitabine; administration of gemcitabine to mice with pancreatic xenograft tumors enriches for $CD133^+$ cells.[8] These data indicate that, in addition to promoting tumor growth, $CD133^+$ cells can survive chemotherapy and maintain a foothold for tumors in patients. Interestingly, the authors reported a partial overlap between $CD133^+$ and $CD44^+/CD24^+/ESA^+$ pancreatic cancer cells,[8] although the tumorigenic potential of this quadruple-positive subset was not assessed.

CXCR4

Although CXCR4 is not a determinant of tumorigenic vs nontumorigenic cells, this chemokine receptor was shown to mark a subpopulation of $CD133^+$ PCSCs with a high propensity to metastasize.[8] As the receptor for stromal-derived factor-1 (SDF-1/CXCL12), CXCR4 is important for hematopoietic stem cell homing to the bone marrow and metastasis and proliferation of cancer cells.[23–26] Hermann et al showed that CXCR4 was expressed at the invasive edge of tumors in patients, supporting its role in invasion and metastasis.[8] Importantly, blocking $CD133^+/CXCR4^+$ cells prevented metastasis of tumor xenografts in mice.[8] These data indicate that CXCR4 might serve as target for therapeutics designed to slow or prevent metastasis of PCSCs. Like $CD133^+/CXCR4^-$ cells, $CD133^+/CXCR4^+$ cells were capable of