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Biological and clinical relevance of stem cells in pancreatic adenocarcinoma

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Abstract

Cancer stem cells (CSC) have been identified in a growing number of human malignancies. CSC are functionally defined by their ability to self-renew and recapitulate tumors in the ectopic setting, and a growing number of studies have shown that they display other functional characteristics, such as invasion and drug resistance. These unique functional properties implicate a role for CSC in clinical consequences, such as initial tumor formation, relapse following treatment, metastasis, and resistance, suggesting they are a major factor in directing clinical outcomes. Pancreatic adenocarcinoma is a highly-aggressive disease with a propensity for early metastasis and drug resistance. Tumorigenic pancreatic cancer cells have been identified using the cell surface antigens CD44, CD24, and CD133, as well as the high expression of aldehyde dehydrogenase (ALDH). *In vitro* and *in vivo* studies have shown that ALDH- and CD133-expressing pancreatic CSC have a greater propensity for metastasis, and ALDH-expressing CSC have been shown to be resistant to conventional chemotherapy. In clinical samples from patients with resected pancreatic adenocarcinoma, the presence of ALDH-expressing CSC was associated with worse overall survival. The development of CSC-targeting therapies might be important in changing the clinical outcomes of patients with this disease, and others and we have begun to identify novel compounds that block CSC function. This review will discuss the biological and clinical relevance of CSC in pancreatic cancer, and will discuss novel therapeutic strategies to target them.

Keywords

cancer stem cell; drug resistance; metastasis; pancreatic cancer

Introduction

Pancreatic adenocarcinoma is a highly lethal disease, with more than 250 000 estimated worldwide deaths in 2011.¹ This disease is characterized by early spread to local and distant organs, and most patients initially present with unresectable disease at the time of diagnosis. Even for patients who initially present with localized disease and undergo curative surgery, the median survival is only 18 months.² Unfortunately, significant advances in treatment have not been realized in more than 10 years, despite better understanding of pancreatic cancer biology and genetics. One area of research focus in recent years has been on cancer stem cells (CSC; also known as “tumor-initiating cells”) in both hematologic and solid

tumor malignancies. CSC have been identified and characterized in a growing number of malignancies, and their role in determining clinical outcomes is becoming better understood.

CSC are a phenotypically-distinct population of cells that are functionally defined by their ability to form tumors, self-renew, and differentiate.³ Prospective isolation of CSC and their role in leukemogenesis were first described in the early 1990s.^{4,5} Since then, CSC have been identified in a growing number of malignancies, including pancreatic adenocarcinoma.⁶⁻⁸ The unique functional properties of CSC might be important in clinical processes, such as disease relapse, formation of metastases, and drug resistance, and are discussed in this review.

Identification of pancreatic CSC

A number of CSC markers, including CD44, CD24, CD133, aldehyde dehydrogenase (ALDH), and Hoechst dye exclusion (side population) have been used to identify normal stem cells and CSC from unrelated organs. However, a universal marker of CSC has not been identified. The first reports to describe CSC in pancreatic adenocarcinoma focused on a set of cell surface antigens, CD44, CD24, and CD133 that have been commonly used for the identification of CSC in unrelated tissues. Two independent reports demonstrated that, compared to unfractionated cells, CD44⁺CD24⁺ and CD133⁺ cells are highly enriched in tumor-initiating capacity.^{6,7}

Another commonly-used marker of CSC and normal stem cells is ALDH.⁹ The functional role of this enzyme in regulating CSC function is not known; however, in normal tissues, it is important for retinoic acid biosynthesis, ethanol metabolism, and metabolism of the alkylating agent, cyclophosphamide. Studies in mice indicate that retinoic acid signaling is important during embryonic pancreas development, suggesting a role for this enzyme in stem cell function or maintenance.¹⁰ Therefore, we studied ALDH as a marker of CSC in pancreatic cancer cell lines and tumors from patients. We found that the tumor-initiating cell frequency in the ALDH⁺ cell population was much greater than unfractionated cells (1 in 300 cells, compared to 1 in 5000 cells).^{8,11} Furthermore, studies in mice have identified pancreatic centroacinar/terminal ductal progenitor cells that are high in ALDH activity and are capable of giving rise to embryonic endocrine and exocrine cell lineages.¹²

Other markers of pancreatic CSC are still being identified, a more recent one being c-Met.¹³ The identification of multiple phenotypic CSC, some of which are non-overlapping, has complicated the understanding of pancreatic CSC biology and their clinical significance. However, this issue is not unique to pancreatic cancer, and multiple CSC phenotypes have been identified in other malignancies, such as breast cancer, colon cancer, glioblastoma, and melanoma.¹⁴⁻²² Current studies are ongoing to determine how phenotypically unique CSC populations in each of these malignancies are related to one another.^{8,23}

Pancreatic CSC are prognostic of clinical outcomes

The unique functional properties of CSC provide a potential explanation for clinical observations, such as disease relapse, metastasis, and resistance to chemotherapy. If CSC are important in these clinical problems or drive the natural history of disease progression, then their frequency or functional potential should correlate with clinical outcomes, such as overall survival, time to relapse, or time to metastasis formation. Indeed, a growing number of studies have examined whether CSC can serve as predictive biomarkers, and have included studies examining the association between clinical outcomes and the frequency of phenotypic CSC.

The relationship between clinical outcomes and phenotypic CSC has been examined for a number of malignancies and is of significant interest. We found that the increased expression of ALDH based on immunohistochemistry in primary pancreatic tumors from patients who underwent resection for localized disease was associated with worse median survival, 18 months for patients with ALDH⁻ tumors, and 14 months for patients with ALDH⁺ tumors.⁸ This association between ALDH expression and poorer survival has also been observed in patients with breast, prostate, and ovarian cancers, suggesting an important role for ALDH-expressing CSC in disease progression for a number of malignancies.^{18,24–26}

A growing number of studies have implicated CSC in metastasis formation in several unrelated malignancies. Hermann *et al.* found that a subset of CD133⁺ pancreatic CSC that express chemokine receptor 4 are more invasive and mediate the formation of liver metastases in an animal model.⁶ We found that ALDH expression was increased in metastatic lesions compared to the primary tumors from the same patient, suggesting a role for these cells in mediating distant spread.⁸ Similarly, purified ALDH⁺ CSC from patients' tumors were found to have a mesenchymal phenotype, and were more invasive compared to the bulk tumor cell population.⁸ Recent work by Li *et al.* found that c-Met⁺ pancreatic CSC are also able to mediate metastasis formation in an animal model.¹³

A growing number of studies have implicated pancreatic CSC in drug resistance. Using a mouse model of xenografted human pancreatic tumors, we found that the ALDH⁺ and CD44⁺CD24⁺ cell populations are significantly increased after treatment of the animals with gemcitabine, suggesting that these populations are resistant to this chemotherapy.²⁷ Similarly, Li *et al.* recently demonstrated that CD44⁺c-Met⁺ pancreatic CSC are resistant to gemcitabine. Finally, a gemcitabine-resistant cell line developed by Shah *et al.* was found to have mesenchymal features, as well as higher expression of the pancreatic CSC markers, CD44, CD24, and c-Met.²⁸ Studies are in progress to determine the intrinsic molecular mechanisms by which pancreatic CSC are resistant to conventional chemotherapy, and our understanding of CSC drug resistance in other malignancies, such as myeloma, breast cancer, and glioblastoma, might also shed light.^{29–32}

Therapeutically targeting CSC

A number of studies have begun to identify and target cellular pathways necessary for CSC function. As CSC might share cellular pathways that are required for the regulation of normal stem cells, a major focus has been on developmental signaling pathways. In pancreatic cancer, a number of studies have shown that Hh pathway inhibition leads to the abrogation of pancreatic CSC and a decrease in their function, as measured by tumorigenicity and metastasis formation.^{27,33,34} Notch signaling has also been implicated in pancreatic and breast CSC regulation under the regulatory control of zinc finger E-box binding homeobox 1 (ZEB1) and microRNA-200, and therefore, might be a therapeutic target against CSC.^{35,36} Studies in our laboratory have also found that pancreatic CSC are enriched in the expression of death receptor 5 (DR5), and that treatment of mice harboring pancreatic cancer xenografts with a DR5 agonist leads to a reduction in pancreatic CSC and enhanced antitumor activity when combined with gemcitabine.³⁷ Recent work has also identified that inhibitors of c-Met abrogate pancreatic CSC, and leads to the reduction of metastases in an animal model.¹³ More recent studies from our laboratory have demonstrated that ALDH⁺ cells interact with the tumor microenvironment via specific integrins, and that abrogation of focal adhesion kinase activity leads to a reduction in the percentage of ALDH⁺ CSC and CSC function *in vitro*.³⁸

Strategies to target CSC in other malignancies have also focused on developmental signaling pathways, including Hh, Notch, and Wnt. Clinical trials utilizing inhibitors of the Hh

(GDC-0449, LDE-225, PF-04449913, BMS-833923, IPI-926, TAK-441), Notch (RO4929097, BMS-906024, MK0752), and Wnt pathways (PRI-724), and telomerase (GRN163L) have begun to emerge^{39,40} (<http://clinicaltrials.gov>), but their efficacy against CSC function remains to be determined.

Conclusions

The increasing number of reports regarding CSC in pancreatic cancer and other malignancies has increased our understanding of the biological and clinical importance of these distinct cell populations. In addition to their defining feature of tumorigenicity, an increasing number of properties, such as metastasis and drug resistance, have been attributed to them. These important functional features suggest that CSC play an important role in dictating clinical outcomes, and indeed, a growing number of studies have demonstrated an association between the presence of CSC and worse clinical outcomes for patients with pancreatic cancer and other malignancies. Improved understanding of CSC biology has led to the development of novel therapeutic targets and drugs that are in the process of being tested preclinically and in the clinic. Finally, perhaps evidence from clinical trials and their laboratory correlative studies will provide the most definitive proof that that inhibition of CSC leads to improvements in long-term clinical outcomes.

Biography



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