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CURRENT CONCEPTS IN CLINICAL SURGERY

Pancreatic cancer stem cells: new understanding of tumorigenesis, clinical implications

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Abstract
Purpose Since the discovery of cancer cells with stem-like characteristics in hematopoietic malignancies and, more recently, in solid tumors, enormous attention has been paid to the stem-cell nature of pancreatic cancer. Among the most important properties of cancer stem cells their high capacity for tumorigenicity as well as their ability to metastasize is under special research interest today.
Methods Here, we give a brief overview of main components used to confirm the stem-cell-like behavior of putative cancer stem cells and discuss markers and methods for identifying them in pancreatic cancer. Finally, the review provides some new suggestions as to how specifically target these cells and improve current therapy regimens.
Results The cancer stem-cell hypothesis is a fundamentally different model of carcinogenesis composed of two separate but dependent on each other characteristics of stem cells—aberrant activation of their tightly regulated processes of self-renewal and differentiation and their resistance towards chemo- and radiotherapy. The cancer stem cells may further be identified based on their expression of cell surface markers or their functional characteristics. The concept of molecular targeting of such highly tumorigenic cancer cells aimed to sensitize tumors toward conventional therapies and effectively abrogate tumor growth and metastasis.
Conclusions The presence of cancer stem cells in pancreatic tumors has prognostic relevance and influences therapeutic response. Evidence suggests that metastatic potential may be conferred to these highly tumorigenic cells as well. A better understanding of the biological behavior of these cells may further improve therapeutic approaches and outcomes in patients with this devastating disease.

Keywords Pancreatic cancer · Tumor metastasis · Cancer stem cells · Side population · Chemotherapy resistance · Self-renewal

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Introduction

Pancreatic cancer is the fourth to fifth leading cause of cancer-related death in Western societies with an average overall 5-year survival of less than 5% and a median survival period of less than 6 months [1]. One of the hallmarks of this devastating sickness is its extensive local tumor invasion and early systemic dissemination. Currently, surgery is the treatment modality of choice in locally limited disease; however, in locally advanced disease, most neoadjuvant or palliative chemotherapeutic approaches failed to significantly improve the outcome. With regard to the patients who undergo potentially curative resection, the 5-year survival rate is less than 24% because of local recurrence and metastasis [2, 3]. Delayed diagnosis, an intrinsic biologic aggressiveness, resistance to chemotherapy, and whether it is intrinsic or acquired are altogether believed to be major causes of treatment failure in pancreatic cancer [4]. Attempts to better understand the molecular basis for these characteristics of pancreatic cancer have focused on studying gene and protein expression profiles of patient samples as well as pancreatic cancer cell lines [5, 6]. These studies, however, have not resulted

in the significant improvement in disease outcome. Clearly, new strategies to handle this disease are needed.

Recently, evidence for the presence of cancer stem cells in different solid tumors was offered. These cells have been termed cancer stem cells because, like their normal stem-cell counterparts, they possess the ability to self-renew and produce more differentiated cells without stem-cell properties. If correct, the cancer stem-cell hypothesis provides a smart explanation for the limitation of many current pancreatic cancer models and suggests a new understanding of pancreatic cancer carcinogenesis and new strategies for pancreatic cancer prevention and therapy.

Classical model of carcinogenesis can be described as "stochastic," in which any cell in an organ, such as pancreas, can be transformed by mutations [5, 7]. The model argues that tumors are biologically homogeneous. As a result, all or most of the cells in a fully developed pancreatic cancer are equally malignant, and the model concludes that strategies aimed to cure pancreatic cancer require the killing of all these malignant cells. The cancer stem-cell hypothesis is a fundamentally different model of carcinogenesis composed of two separate, but dependent on each other, components. The first is that tumors originate from tissue stem and/or progenitor cells through the dysregulation of the normally tightly regulated process of self-renewal [8]. Consequently, cancer stem cells are defined as cancer cells that have the ability to divide into new malignant stem cells and daughter cells that differentiate and give rise to the heterogeneous tumor cell mass. As a result, tumors might bear a cellular component that retains key stem-cell properties such as self-renewal and differentiation, which initiates and drives tumorigenesis. In the biology of pancreas, increasing evidence suggests that the self-renewal pathways important in vertebrate pancreas development, including Notch [9, 10], Wnt/β-Catenin [11, 12], Hedgehog [13–16], and BMI-1 [17], remain active in a subset of cells within adult organs and that deregulation (i.e., mutation) in their activity contributes to the development and progression of pancreatic tumors [9–17]. This indicates that pancreatic cancer can be considered a disease of unregulated self-renewal in which mutations convert normal stem-cell self-renewal pathways into engines for neoplastic proliferation.

The second component of cancer stem-cell model might explain why standard chemo- or radiotherapy regimens against pancreatic cancer are usually ineffective and result in further tumor recurrence and spreading. Currently, the conventional treatments indiscriminately kill proliferating nontumorigenic cancer cells; however, therapy can fail due to the survival of quiescent tumor stem cells [18, 19]. Tumor stem cells like their normal stem-cell counterparts are more likely to express drug resistance and antiapoptotic genes, which might serve as a chemo- or radioprotective mechanism of survival. Indeed, some recently published data as well as our own nonpublished observations revealed that the cancer stem cells from pancreatic tumors and in vitro established cell lines are intrinsically resistant to conventional chemo- and radiotherapy [20–22]. Furthermore, other studies suggest that aberrant activation of stem cell self-renewal pathways within tumors may also contribute to cancer stem-cell resistance towards therapy [23–26]. Thus, cancer stem cells would seem the most probable candidates responsible for tumor chemoresistance and recurrence.

So, do the cancer stem cells contribute to pancreatic cancer tumorigenesis and metastasis? Does the initial resistance of pancreatic cancer stem cells to radiation and toxins cause the failure of therapies? How can we capitalize on our knowledge of current stem-cell biology to specifically target these cells and improve current therapy?

Identification of cancer cells with stem-like characteristics in human pancreatic cancer

The existence of cancer stem cells was first observed in acute myelogenous leukemia. Dick and colleagues isolated and identified CD34+ CD38− leukemic stem cells from human acute myelogenous leukemia by FACS and demonstrated that these cells initiated leukemia in NOD-SCID mice compared to the CD34+ CD38+ and CD34− subpopulations [27]. In addition, the engrafted leukemia could be serially transplanted into secondary recipients, providing functional evidence of self-renewal. Such cells have been termed cancer stem cells because, like normal adult stem cells, they can both self-renew and produce differentiated progeny. Recently, cancer stem cells have also been isolated in a number of solid tumors, including pancreatic cancer.

Initially, the relationship between cancer stem cells and pancreatic cancer progression was investigated by Li et al. [16]. To characterize pancreatic cancer stem cells, the authors primarily identified a subpopulation of highly tumorigenic cancer cells by expression of the cell surface markers CD44, CD24, and epithelial-specific antigen (ESA). These markers were chosen as a starting point based on prior work on breast cancer stem cells, in which ESA+ CD24− CD44+ cells generated tumors histologically similar to primary breast cancer when as few as 100 sorted cells were implanted into immunocompromised mice [28]. Similarly, CD44+ CD24+ ESA+ pancreatic cancer stem cells demonstrated typical features seen in adult stem cells, including the ability to self-renew, to generate differentiated progeny, and to activate developmental signaling pathways, such as sonic hedgehog [16]. To further test the hypothesis that pancreatic cancer stem cells would be able to

recapitulate the phenotype of the tumor from which they were derived in vivo, FACS-sorted cells from human pancreatic adenocarcinoma xenografts were suspended in a Matrigel mixture and subcutaneously injected into immunocompromised NOD/SCID mice. Interestingly, pancreatic cancer cells with the CD44+ CD24+ ESA+ phenotype (0.2–0.8% of pancreatic cancer cells in the ten pancreatic cancers assessed) had a 100-fold increased tumorigenic potential compared to nontumorigenic cancer cells with the CD44− D24− ESA− phenotype. As few as 100 cells injected in NOD/SCID mice were able to generate tumors in 50% of mice. In addition, the highly tumorigenic CD44+ CD24+ ESA+ cells produced on the one hand CD44+ CD24+ ESA+ cells, on the other hand phenotypically diverse nontumorigenic cells, demonstrating the same phenotypic complicacy as the original primary tumors from which those cancer stem cells were derived [16]. Histologically, the tumors derived from the highly tumorigenic pancreatic cancer cells appeared remarkably similar to histologic sections of the patient's samples and also had similar patterns of expression of the differentiation markers S100P and stratifin, two proteins expressed in the majority of human pancreatic adenocarcinomas. Furthermore, the group also observed that treatment with ionizing radiation and the chemotherapeutic agent gemcitabine resulted in enrichment of the CD44+ CD24+ ESA+ cell population in pancreatic tumor xenografts [17]. Altogether, despite some potential limitations observed in the study (the authors noted that increased numbers of CD44+ CD24+ ESA+ cells were needed to generate orthotopic tumors compared to subcutaneous tumors), those encouraging innovative results pointed out potentially new therapeutic implications and supported further investigation of pancreatic cancer stem-cell biology.

The recently discovered list of potential markers of cancer stem cells is, however, long. Thus far, CD133 has also been used to identify putative cancer stem cells in breast, brain, liver, colon, prostate, and pancreatic tumors (Table 1). CD133, also known as prominin-1, was first discovered as a marker of normal hematopoietic stem cells and later was found to mark stem/progenitor cells from a variety of tissues [29]. Evidence for existence of CD133+ pancreatic cancer stem cells was first reported by Hermann et al. [20]. The authors also utilized a similar to Li et al. FACS approach to prospectively isolate and characterize CD133+ cells from primary pancreatic cancer patient tissue samples and tumor cell lines. They found that in the majority of patient's samples, the capacity of cells to form primary tumors following orthotopic implantation in nude mice was limited to a subpopulation of CD133+ cells that were exclusively tumorigenic and highly resistant to standard chemotherapy (gemcitabine). The CD133+ cells were serially passaged, demonstrating self-renewal capacity and were able to generate tumor heterogeneity, producing differentiated nontumorigenic progeny. The authors then used a highly metastatic human pancreatic cancer cell line L3.6pl [30] and found that the CD133+ subpopulation of this cell line could be further subdivided into two subsets based on the expression of the CXCR4 receptor, a marker for stem-cell migration [31]. Further comparing the tumorigenic capacity of all L3.6pl CD133+ cells (containing a mixture of CD133+/CXCR4− and CD133+/CXCR4+ cells) with that of CD133+ cells depleted of the CXCR4 subset demonstrated that both populations were equally capable to sustain tumor growth. However, depletion of the CXCR4 subset of all CD133+ cells completely inhibited the formation of spontaneous liver metastases. A similar effect was obtained using the pharmacological targeting of CXCR4 by AMD-3100 [20]. Comparable results were obtained for several other pancreatic cancer cell lines as well as human pancreatic cancer specimens. Other cell lines such as MiaPaCa, a cell line with a rather invasive growth pattern, also contained a distinct subpopulation of those so-called migrating cancer stem cells, and the migratory activity of these cells was clearly dependent on their SDF-

Table 1 Cell surface markers associated with solid tumor stem cells

Tumor Type	Cell Surface Markers	References
Breast	CD44, CD24	[28]
	CD29	[68]
	CD44	[69]
	CD133	[70]
	CD200	[71]
Brain (glioma)	CD133	[72–74]
	CD200	[71]
Bone (sarcoma)	Stro-1, CD105, CD44	[75]
Colorectal	EpCAM, CD44, CD166	[76]
	CD133	[77, 78]
Head and neck	CD44, BMI-1	[79]
Liver	CD90	[80]
	CD133	[47, 81, 82]
Lung	Sca-1, CD45, PECAM, CD34	[83]
	PODXL-1, BMI-1	[84]
Melanoma	CD20	[85]
	ABCB5	[86, 87]
Pancreatic	CD44, CD24, ESA	[16, 17]
	CD133	[20, 88]
	CD133, CXCR4	[20]
Prostate	CD133	[89, 90]
	CD44	[91, 92]
	CD44, CD24	[93]
	CD44, integrin a2b1, CD133	[94]
	CD200	[71]

1/CXCR4 expression. SDF-1, a specific ligand of the CXCR4 receptor, appeared to be the strongest inducer of migration for CD133+ cancer cells in vitro. This also correlated well with metastasis found in patients. Interestingly, when these tumors were stained for the epithelial cell marker, cytokeratin, the CD133+ cells were negative but the CD133− cells stained positive. Coincidentally the loss of cytokeratin may be an indication of epithelial to mesenchymal transition (EMT), which is a hallmark of metastasis. Recently, a report demonstrated that when highly tumorigenic cancer cells undergo EMT, they begin to express tumor-initiator markers [32]. It is possible that these CD133+ cells have undergone EMT and may explain their tumor initiating and highly metastatic phenotype. All together, these observations indicate that migration of CD133+ cells from human pancreatic cancer cell lines is primarily mediated through the SDF-1/CXCR4 system; thus, the potential targeting of the SDF-1/CXCR4 axis in pancreatic cancer might be a new clinical strategy with the aim to preferentially inhibit pancreatic cancer metastasis. However, it should be mentioned that CXCR4 is expressed by a broad range of cells including leucocytes and endothelial precursors (therefore, long-term CXCR4 inhibition may result in unacceptable side effects whereas short-term therapy following surgery may be well tolerated).

Further investigations with regard to CD133 expression and distant lymph node metastasis in pancreatic cancer were performed in a study from Maeda et al. [33]. Pancreatic head carcinoma specimens from 80 patients who underwent surgical resection were immunohistochemically assessed for CD133, CXCR4, VEGF-C, and cytokeratin. In their study, no CD133 immunoreactivity was observed in normal pancreatic ductal epithelium, which was clearly cytokeratin positive. Interestingly, a median amount of CD133+ and cytokeratin negative tumor cells were located at the peripheral site (facing interstitial space) of adenocarcinoma glandular structures. Furthermore, the expression of VEGF-C as well as lymphatic invasion and lymph node metastasis significantly correlated with the expression of CD133 in those tumors. With regard to a possible correlation between prognosis and percentage of CD133+ cells, the authors evaluated a 23.5%, a 3.4%, and a 0% 5-year survival rate in patients with CD133− pancreatic tumors, in patients with less than 5% CD133+ cells and in patients with more than 5% CD133+, respectively. As concluded by authors, the expression of CD133 in human pancreatic cancer represents a new independent prognostic survival factor.

Nevertheless, the identification of the function of CD133 still has to be explored, and the further understanding of biological function of CD133 expression is definitely necessary. Some recent studies on metastatic colon cancer suggested that CD133 expression might not be limited to cancer stem cells. Shmelkov et al. [34], using a knock-in lacZ reporter mouse model (CD133lacZ/+), demonstrated that CD133 expression in normal colon is not restricted to stem cells only. Similarly, CD133 was widely expressed on human primary colon cancer epithelial cells, whereas the CD133− population was composed mostly of stromal and inflammatory cells. Conversely, CD133 expression did not identify the entire population of epithelial and tumor-initiating cells in human colon cancer. Interestingly, the authors demonstrated that both CD133+ and CD133− tumor subpopulations of human colon cancer specimens formed colonospheres in vitro and were capable of long-term tumorigenesis and metastases in a NOD/SCID serial xenotransplantation model. Taken together, the findings presented by Shmelkov could suggest that CD133 should at least not be exclusively considered as marker for cancer stem cells in colon cancer.

Another question that still remains to be answered is how reliably the cancer stem cells are defined by this marker. On the other hand, the CD133+ cell population exhibiting stem-cell properties might be well depend on the conditions under which cancer stem cells are grown and the environmental influences they encounter during separation and culture. As obtaining single-cell suspensions from solid tumors often involves mechanical and enzymatic disaggregation usually lasting hours, the possibility of altering surface marker expression profiles has to be taken into consideration. To clarify this issue for pancreatic cancer, further preclinical and clinical studies are required (probably using in addition to CD133 other markers such as EpCAM and CXCR4). Furthermore, immunohistochemistry or fluorescent labeling of both the original tumor tissue and the isolated cells is necessary to confirm that surface expression patterns are not an artifact of cancer cell isolation. Nevertheless, the above findings might suggest that a more distinguishing expression marker or set of markers to identify pancreatic cancer stem cells may yet to be discovered.

Assays that measure functional characteristics of normal stem cells which are complementary to marker analysis may, therefore, prove useful in helping to identify cancer stem cells which avoid the problem of marker expression instability. For example, one such characteristic of stem cells is the capacity to extrude dyes such as Hoechst 33342. This characteristic is mediated by multidrug efflux pumps and cells that exclude dyes are referred to as side population (SP) cells. The existence of SP cells in pancreatic cancer was primarily evident in the study of Olempska et al. [35]. The goal of the study was to determine highly tumorigenic cancer stem cells within five established pancreatic adenocarcinoma cell lines based on the expression of markers such as ABCG2 and CD133

using real-time RT-PCR and flow cytometry analyses. The ABCG family of transporters (MDR1 and MRP1 transporters) are responsible for the SP phenotype observed in some stem-cell-derived organs and the basis for the application of the ABCG family proteins as markers for stem-cell populations comes from the observation that long-lived cell populations (such as stem cells) are under constant fire from genotoxic chemicals; thus, they must be efficient at effluxing these chemicals from the cell [18, 36, 37]. In the study of Olempska, all pancreatic carcinoma cell lines expressed significantly higher levels of the ATP-binding cassette transporter ABCG2 [35]. Interestingly, the expression of CD133 was strongly elevated in two cell lines tested, PancTu1 and A818-6 (representing poorly and moderately differentiated tumors, respectively), whereas it was only slightly increased in Panc1 cells. As declared by the authors, the ABCG2/CD133 positive cells might represent a unique population of cancer stem cells within human pancreatic carcinoma cell lines tested and be a promising target for new drug developments. However, whether the SP cells within pancreatic tumors are enriched for cancer stem cells is still unclear [38–40]. Further characterization of the SP cells should reveal whether they possess features of stem cells such as the capacity for self-renewal and differentiation.

With the aim to investigate the prevalence of SP cells in human pancreatic cancer and their role and mechanism in drug resistance, Zhou et al. [21] stained the human pancreatic cancer cell line Panc1 with Hoechst 33342 dye and identified SP cells based on their characteristic fluorescent profile in dual-wavelength analysis. The study revealed that Panc1 contained SP cells from 2.1% up to 8.7% (median 3.3%) in the total amount of viable cells. Further results suggested that SP cells have an enhanced efflux capacity not only for Hoechst 33342 dyes but also for antineoplastic drugs (gemcitabine). Upon 72 h exposure to gemcitabine, the viability of SP cells markedly increased as compared to that of the non-SP cells. The drug efflux capacity of Panc1 SP cells led to a significant survival advantage. These results might support the hypothesis that SP cells within tumors play an important role in maintaining chemotherapy-resistant cancer foci using—comparable to normal stem cells—their self-renewal capacities. Nevertheless, targeting SP cells may offer an alternative option to diminish drug resistance and improve patient's survival.

Attempts to identify putative cancer stem cells within 5-Fluorouracil (5-FU) chemotherapy-resistant pancreatic cancer cell lines were performed in our laboratory. As detected by western blotting, the vast majority of the cell lines analyzed showed the presence of the cancer stem-cell marker CD133. Interestingly, flow cytometry and western blotting analyses revealed a significantly increased level of CD133+ tumor cells as well as SP cells in AsPC-1- and L3.6pl 5-FU-resistant cell lines when compared to their corresponding sensitive cell lines. Such findings allow us to speculate that cancer stem cells in chemotherapy-resistant cell lines probably differ in their regulation of self-renewal and possibly bear particular mechanisms of acquired chemoresistance which then might be transmitted to their nontumorigenic progeny (data not published). The specific mechanisms involved in the resistance of cancer stem cells and the contribution of developmental pathways to this resistance remains to be elucidated.

In a separate study, Shah et al. [22] from MD Anderson Cancer Center developed two different pancreatic carcinoma cells lines with acquired gemcitabine-resistant properties with the aim to further analyze those cells with regard to alterations in E-cadherin and β-catenin localization (known hallmarks of EMT) as well as expression of the stem-cell markers CD44, CD24, and ESA. Interestingly, in gemcitabine-resistant cells, β-catenin was located almost exclusively in the nucleus promoting the transcription of signals for migration, invasion, and EMT. Furthermore, gemcitabine-resistant cells were increased in vimentin and decreased in E-cadherin expression. Finally, FACS analysis of stem-cell markers demonstrated a marked enhancement in the expression of CD44+ CD24+ ESA+ cells in the chemotherapy resistant cell lines [22]. Thus, selection may have resulted in the enrichment of a minor fraction of pancreatic cancer stem cells in sensitive population. The authors are currently isolating the stem-cell-like population, and its characterization is likely to provide new insights with respect to the association of the stem-cell phenotype and EMT. Further, such findings could have fundamental and profound implications for therapy of pancreatic tumors bearing acquired chemoresistance.

Interestingly, Stanger et al. [41] observed an expansion of centroacinar cells, increased expression of Pdx1 and HES1 (two markers of pancreatic progenitor cells), and decreased expression of amylase from the surrounding tissue in mouse pancreas of phosphatase and tensin homolog (PTEN) knockout mice, which were used as model of pancreatic ductal adenocarcinoma (PDAC). The expansion of centroacinar cells eventually progressed to PDAC. These findings might support the idea that centroacinar cells are the source of stem cells of the acinar and ductal cells and perhaps the precursor cells that are transformed prior to PDAC. However, other cell-surface markers or dye-effluxing capability was not reported.

Targeting pancreatic cancer cells with stem-like characteristics

One of the most beneficial aspects of applying stem-cell biology to pancreatic cancer research is the resulting

conceptual advance with respect to discovering therapeutic mechanisms to specifically reach the tumorigenic cancer stem cells themselves. Pancreatic adenocarcinoma is almost universally unresponsive to many conventional therapies [4, 42]. In many cases, after attempts to treat the disease, the tumor becomes chemoresistant resulting in the poor prognosis of most pancreatic cancer patients. There have been many studies characterizing the cause of this resistance [43, 44]. The central question, however, is if a small rare subset of stem-like pancreatic cancer cells retains their transporter activity or antiapoptotic genes that will eventually dominate the content of the tumor.

Unfortunately, conventional chemo- or radiation therapy seems to have little to no effect on cancer stem cells in a variety of tumor models [17, 20, 21, 45–47]. Therapies specifically targeting pancreatic cancer stem cells will likely be needed to result in tumor eradication and prevention of metastasis. New screens specifically designed to target cancer stem cells may yield more effective antitumor treatments (for example, DNA and tissue microarray analyses of tumors which are widely used to identify cancer subtypes to improve diagnosis and treatment). The use of sphere formation assays in vitro as well as implantation and serial transplantation assays in vivo might be also useful in the identification of agents that selectively kill cancer stem cells. Because cancer stem cells possess many of the features of normal stem/progenitor cells, it will be important to determine if such strategies may be effective in targeting cancer stem cells without harming normal stem/progenitor cells. This will require identification of realistic drug targets unique to cancer stem cells. Some recent research articles suggest that selective targeting of cancer stem cells may indeed be possible, and we believe that such findings might be also applicable to the pancreatic cancer stem cells [48].

A steroid-like compound cyclopamine [49] binds to and inhibits the SMO protein (G-protein-coupled receptor family protein of hedgehog signaling) and inhibits the growth of cells and tumors with activated hedgehog signaling [50, 51]. In pancreatic cancer cell lines, inhibition of hedgehog with cyclopamine has been shown to result in the downregulation of Snail and upregulation of E-cadherin. This was consistent with the inhibition of epithelial-to-mesenchymal transition and was mirrored by a striking reduction of in vitro invasive capacity of tumor cells. In an orthotopic xenograft model, cyclopamine profoundly inhibited metastatic spread and a combination of cyclopamine and gemcitabine significantly reduced the size of primary tumors [52]. Furthermore, the study of Mimeault et al. [53] revealed that the combination of cyclopamine and EGFR inhibitor led to an increased rate of apoptotic death and decreased invasive abilities of prostate cancer cells in vitro. The same effect was found in human pancreatic cancer cells PANC-1, SUIT-2, and AsPC-1, when cyclopamine and EGFR inhibitor were combined [54]. Furthermore, cyclopamine was shown to increase the cytotoxic effects of paclitaxel and radiation therapy in human pancreatic cancer cells MiaPaCa-2, BxPC-3, and HCT116 [55]. Unpublished data from our research group also suggest that treatment of different pancreatic cancer cell lines with cyclopamine (alone or in combination with 5-FU) reduces the amount of SP cells and, most interestingly, restores the acquired 5-Fluorouracil-induced chemoresistance in AsPC-1 and L3.6pl pancreatic cancer cell lines in vitro. Of particular therapeutic interest, the long-term treatment of glioma-sphere cells with cyclopamine alone killed all cancer stem cells in culture and induced the regression of glioma tumors established from the glioma-sphere cells in nude mice in vivo, without detectable secondary effects [56]. Finally, in multiple myeloma (MM), the hedgehog ligand promoted expansion of MM stem cells without differentiation, whereas the hedgehog pathway blockade, while having little or no effect on malignant plasma cell growth, markedly inhibited clonal expansion accompanied by terminal differentiation of purified MM stem cells [57].

The results from a recent study from Fan et al. [58] have indicated that blockade of Notch self-renew signaling by use of an inhibitor of γ-secretase (GSI-18) significantly reduced the CD133+ cell population (which overexpressed Notch signaling) and led to eradication of the SP cells detected in medulloblastoma cell mass. Since the medulloblastoma cells treated with GSI-18 did not grow in vivo, it was suggested that the cancer stem cells were effectively eradicated by the treatment. As reported by Murtaugh et al. [59], these findings might be also applicable to the pancreatic cancer stem cells. In another study performed on human glioblastoma, treatment of CD133+ cancer stem cells with bone morphogenic protein 4 (BMP4) resulted in depletion of pool of brain cancer stem cells in vitro and in vivo [60]. As recent studies have demonstrated an important role for BMP4 in the regulatory processes of pancreatic progenitor cell expansion [61], BMP4 might also be suggested as a new interesting target for elimination of cancer stem cells in pancreatic neoplasia.

Another interesting target modality to eliminate cancer stem cells was proposed by Yilmaz and colleagues [62]. The authors investigated the role of PTEN in leukemia. PTEN is a known intracellular modulator of several major signaling pathways and is also a tumor suppressor gene that is commonly deleted or inactivated in different types of cancers [63]. It negatively regulates the PI3K/AKT pathway, which further triggers several downstream targets such as the mammalian target of rapamycin (mTOR) and elicits diverse cellular responses involved in proliferation, survival, and cell growth [64]. Yilmaz et

al. found that conditional deletion of PTEN in adult hematopoietic cells in mice leads to expansion of leukemic cancer stem cells and depletion of normal hematopoietic stem cells. Treatment of the mice with mTOR inhibitor Rapamycin (sirolimus), used to counter the effects of PTEN deletion, inhibited the development of cancer stem cells while preserving normal stem-cell populations [62]. The results from our group were also able to demonstrate the in vitro inhibitory effects of Rapamycin on SP cancer cells with the most emphasizing inhibitory effect of Rapamycin on 5-FU chemoresistance when the former was applied as chemosentisizer (unpublished observations).

Another strategy to eliminate cancer stem cells has been recently described in the study of Moserle et al. [65]. The researchers investigated the presence of SP cells in ovarian cancer and further identified an IFN-α as a potential "killer" of these cells in vitro and in vivo. Treatment with IFN-α demonstrated marked antiproliferative as well as proapoptotic effects on primary cultures of epithelial ovarian cancer cells containing high numbers of SP cells. Furthermore, in vivo gene therapy with human IFN-α resulted in regression of established tumors bearing a large SP fraction, which was not evident when tumors bearing low SP levels were treated. Taken together, these findings could have relevant and promising clinical implications because they imply that tumors with large SP numbers, albeit rare, could be sensitive to IFN-α treatment [66, 67].

Conclusion

Initial studies on pancreatic cancer suggest that the presence of stem-like cancer cells in primary tumors and cell lines have prognostic relevance and influences therapeutic response. Evidence suggests that metastatic potential may be conferred to these highly tumorigenic cells. It is, however, clear that much work is needed to better understand the molecular machinery behind the regulation of self-renewal signaling pathways (e.g., Hedgehog, Wnt, Notch) and chemo- and radiotherapy resistance. Furthermore, the potentially severe side effects of cancer stem-cell-targeted therapy still have to be evaluated in animal models before we can suggest it for clinical trials (an important question, however, is how realistically tumor xenograft models in immunodeficient mice recapitulate what is happening in human patients). Such studies are likely to yield important insight that ultimately may improve therapeutic approaches and outcomes in patients with this devastating disease.

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