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Rapid communication

Stem cells & pancreatic cancer

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ABSTRACT

It is now well established that human pancreatic ductal adenocarcinoma (PDAC) contains a subset of cells with self-renewal capabilities and subsequent exclusive *in vivo* tumorigenic capacity as assessed by limiting dilution tumorigenic transplantation assays into immunodeficient mice. These cells are considered pancreatic cancer stem cells (CSCs) and are able to form tumors indistinguishable from parental ones. Furthermore they display strong chemotherapy resistance and are implicated in tumor relapses and metastatic spread. Important next steps for advancing the field of pancreatic CSC research include the identification and characterization of CSCs in the unperturbed *in vivo* setting. This has been achieved just recently for other solid tumors such as glioblastoma using clonal analysis after lineage tracing in mice [1]. *In vivo* imaging of CSCs during tumor development should not only provide new insights into the *in vivo* features of CSCs, but also help to further unravel the influence of the stroma on CSC biology. Comprehensive studies of the tumor heterogeneity with respect to the coexistence of different clones potentially generated by distinct population of CSCs that are evolving by stochastic cell fate decisions may actually unite the CSC concept and the model of clonal evolution for pancreatic cancer. Eventually, the design of specific therapies against CSCs should open new alleys to improve survival of patients with PDAC. Combined therapies targeting CSCs and their progenies as well as the supportive stroma may represent the most promising approach for the future treatment of patients with PDAC.

1. Cancer stem cells in pancreatic ductal adenocarcinoma

In line with findings from other solid tumors, cancer stem cells have been identified in human PDAC. These cells are able to self-renew and propagate the parental tumor in transplantation assays using immunodeficient mice (Fig. 1). CSCs have been identified by a variety of biomarkers, some of which were previously associated with normal stem cells in the pancreas.

1.1. CD44+CD24+EpCAM+ cancer cells

A subpopulation of CD44+CD24+ESA+ cells injected into immunodeficient mice gives rise to tumors, while their triple negative counterparts did not show tumorigenic capacity [2]. This population represented 0.2–0.8% of the whole tumor cellularity and as few as 10^2 CD44+CD24+EpCAM+ cells were sufficient to initiate tumors in 50% of transplanted mice. In contrast, as many as 10^4 CD44−CD24−EpCAM− cells were required to initiate tumors. In addition, the receptor for hepatocyte growth factor, namely c-Met has recently being added to the panel of CSC markers and was particularly informative when combined with CD133 or CD44 [3]. Importantly, the combination c-Met and CD44 was able to identify a population of cells with strongest tumorigenic potential as 50 c-Met+CD44+cells were able to generate subcutaneous tumors and were highly metastatic.

1.2. CD133+ cancer cells

CD133+ cells isolated from freshly resected human tumor samples were highly tumorigenic and represented 1–3% of the tumor cells [4]. Tumor formation in immunodeficient mice was detectable after injecting 5×10^2 CD133+ cells, but no tumor growth was detected after inoculation of up to 10^6 CD133− cells. The formed tumors morphologically and histologically resembled the parental tumors. In addition, CD133+ cells were able to form spheres in serum-free non-adherent conditions and generate tumors in serial transplantation assays demonstrating unrestricted *in vitro* and *in vivo* self-renewal ability. Moreover, a study involving 80 patients with surgical resection of PDAC showed that cytoplasmic CD133

Abbreviations: ALDH, aldehyde dehydrogenase; CSC, cancer stem cell; EMT, epithelial mesenchymal transition; EpCAM, epithelial cell adhesion molecule; PanIN, pancreatic intraepithelial lesion; PDAC, pancreatic ductal adenocarcinoma.

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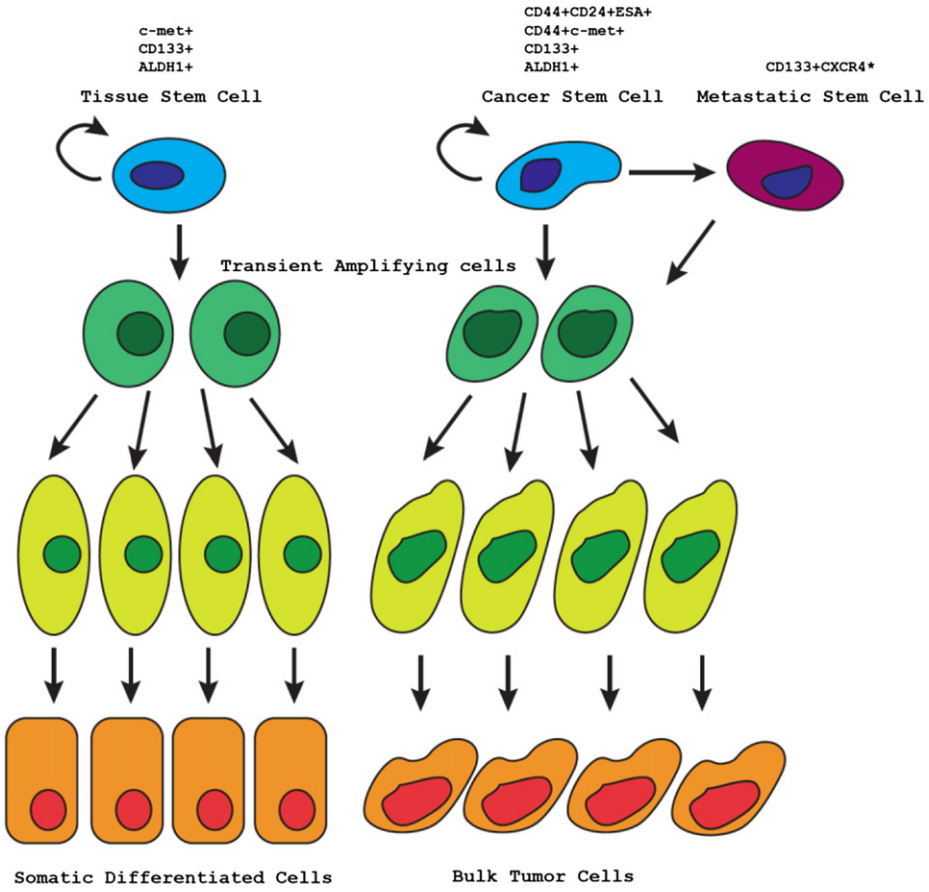

Fig. 1. Model for cancer stem cells in PDAC. Cancer stem cells share several markers with putative tissue stem cells. Consistent with a normal stem cell phenotype, cancer stem cells also give rise to more differentiated progenies. Importantly, the cancer stem cell pool is not homogeneous, but also contains a subpopulation of metastatic cancer stem cells expressing CXCR4 as a characteristic feature. A heterogeneous cancer stem cells pool can be rationalized by the existence of different clones inside the bulk tumor, which arose during tumor evolution.

expression in the specimens was significantly correlated with patients' outcome [5].

There is a certain overlap between CD133+ and CD44+CD24+EpCAM+ (ranging from 10.3 to 37.4%) [4], but none of the proposed enrichment procedures for CSCs is currently capable of identifying single tumorigenic cells. Therefore, a definitive combination of cell surface markers that is capable of identifying a pure CSC population with *in vivo* tumorigenicity at the single-cell level is still missing.

1.3. Other CSC markers

Apart from these more commonly used markers, other approaches have been proposed for the enrichment of CSCs. ALDH1, which has also been proposed for the isolation of murine pancreatic stem cells, has also been used for isolating tumorigenic cells in the human pancreatic line L3.6pl [6]. In a study including 269 tumor samples, ALDH1 expression was also predictive for poor outcome and ALDH1+ cells were 5- to 11-times more clonogenic as well as presented enhanced migratory and invasive potential compared to their negative counterparts [7]. Another study identified CSCs via their functional properties using a fluorescent reporter system to detect proteasome activity. Cells with low activity of 26S proteasome represented a population enriched for CSC properties [8].

1.4. Epithelial-to-mesenchymal transition and cancer stem cells

It is well established that switching from an epithelial phenotype to a mesenchymal phenotype involves a series of changes in the epigenetic and gene expression profile that have been defined as epithelial to mesenchymal transition (EMT). EMT provides the machinery for motility and invasiveness required for abandoning the tumor bulk, extravasate, travel through the blood stream, home to new distant sites, and colonization of the new host tissue. A putative link between metastasis and CSCs has already been proposed several years ago [9]. Later it was demonstrated that EMT confers many of the properties of normal and neoplastic stem cells including sphere formation and self-renewal and that stem cells are enriched for EMT genes as compared to their epithelial progeny [10]. More recently it has been shown that extravasation of murine pancreatic cancer cells into the bloodstream is associated not only with expression of EMT genes, but also with phenotypic and functional characteristics of pancreatic stem cells [11]. The invasive potential was acquired very early during the process of transformation. Therefore, these findings are consistent with the hypothesis that CSCs probably display a high degree of heterogeneity consistent with the clonal evolution model. Indeed, while CD133+ human pancreatic cancer cells bear tumor-propagating capability, only the contained subfraction of CD133+CXCR4+ cells exhibits metastatic potential. CXCR4 is a chemokine receptor specific for stromal derived factor-1 (SDF-1) involved in responses to chemotactic gradients. Depletion of CD133+CXCR4+ cells from the CSC pool did not alter the tumor formation capacity, but abolished metastatic potential [12].

2. Microenvironment of cancer stem cells

Due to the vast abundance of tumor stroma in pancreatic cancer, current research efforts are expanding towards a better