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Mini-review

Pancreatic cancer stem cells: New insight into a stubborn disease

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ABSTRACT

Resistance to conventional therapy and early distant metastasis contribute to the unsatisfactory prognosis of patients with pancreatic cancer. The concept of cancer stem cells (CSCs) brings new insights into cancer biology and therapy. Many studies have confirmed the important role of these stem cells in carcinogenesis and the development of hematopoietic and solid cancers. Recent studies have shown that CSCs regulate aggressive behavior, recurrence, and drug resistance in pancreatic cancer. Here, we review recent advances in pancreatic cancer stem cells (PCSCs) research. Particular attention is paid to the regulation mechanisms of pancreatic cancer stem cell functions, such as stemness-related signaling pathways, microRNAs, the epithelial-mesenchymal transition (EMT), and the tumor microenvironment, and the development of novel PCSCs targeted therapy. We seek to further understand PCSCs and explore potential therapeutic targets for pancreatic cancer.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the United States and has low overall survival [1,2]. Resection is the only radical treatment for this aggressive malignancy. Unfortunately, only 15–20% of patients can be resected, due to the low rate of early diagnosis. Resistance to chemotherapy and radiation therapy also contributes to the dismal prognosis [3–5]. Only 9.4% of patients with metastatic pancreatic cancers acquired partial response, and 34.5% experienced disease progression during chemotherapy with gemcitabine [3].

During the past decades, the carcinogenesis and pathogenesis of pancreatic cancer (PC) have been thoroughly described. However, the overall survival of patients with pancreatic cancer has not significantly improved. An in-depth investigation of the mechanisms of carcinogenesis, progression, and drug resistance that is based on the theory of cancer stem cells (CSCs) might help to improve pancreatic cancer prognosis.

CSCs have been confirmed in hematopoietic and solid tumors, which have the capacity of self-renewal and can differentiate into other cell types of cancer cells [6]. These types of cells promote tumor growth, invasion, metastasis, and therapeutic resistance [7–9]. Pancreatic cancer stem cells (PCSCs) were first identified by Li et al. in 2007 [10]. Later studies revealed that PCSCs could self-renew, differentiate, and divide asymmetrically, like normal stem cells; the critical role of PCSCs in regulating pancreatic cancer progression, metastasis, and drug resistance has since been confirmed [11,12]. However, some critical questions still remain to be answered, including the identification of specific PCSCs, the signaling pathways involved in regulating PCSCs, and the regulatory roles of PCSCs in pancreatic cancer. In this article, we reviewed the recent advances regarding the regulating roles and mechanisms of CSCs in pancreatic cancer, paying particular attention to the metastasis, drug resistance, and prognostic functions of these cells.

Cancer stem cells

Human cancer cells are composed of a heterogeneous population of cells and are characterized by unlimited proliferation and resistance to conventional therapy. However, only a small population of cancer cells survives and proliferates under high doses of chemotherapy drugs or radiation. In immunodeficient mice, few cancer cells can form xenografts, which have the capacity to self-renew, have tumor-initiating potential, and can recapitulate the cellular heterogeneity of the original tumor. This small subset of cells was named CSCs, or "tumor-initiating" cells [6,13,14].

The first identification of CSCs was reported by Bonnet (1997) in acute myeloid leukemia [15]. The authors found that leukemic stem cells ($CD34^+CD38^-$) possessed differentiative and proliferative capacities and the potential for self-renewal. However, bulk cancer cells could not differentiate into other subpopulations of cancer cells and had limited self-renewal. In chronic myeloid

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leukemia patients, STI571 (imatinib) killed almost all dividing cells, but $Lin^{-}CD34^{+}$ cells remained viable even under treatment with growth factors and imatinib [16]. Then, CSCs were identified in a variety of other cancers, including breast cancer [17], melanoma [18], brain tumor [19], pancreatic cancer [10], colon cancer [20], ovarian cancer [21], and prostate cancer [22]. These CSCs have been defined by their ability to self-renew and differentiate. Self-renewal can maintain the cells' ability to survive long-term, whereas the differentiation of CSCs results in the heterogeneity of all cancer cells and histological recapitulation of the original tumor.

CSCs are usually isolated and identified by flow cytometry using cell surface markers, such as CD34, CD24, CD44, CD133, ESA, ALDH, c-Met, and EpCAM [14,23]. These cell surface markers vary in each type of cancer. There is no universal cell surface marker that can identify CSCs from different types of cancer cells, causing confusion regarding which subpopulation cells are truly CSCs. Abel et al. [24] postulated that this confusion may stem from several sources. Highly passaged cell lines do not have the hierarchies observed in primary tumors. Limited studies, lack of standardization of digestion techniques, flow cytometry analyses, and the antibodies used may contribute to the varied findings regarding cell surface markers in CSCs. A combination of different markers may purify the CSC phenotype. $CD44^{+}$c-Met^{+} provided robust selection of pancreatic CSCs [25], and a further enrichment of ovarian CSCs was observed in $ALDH^{+}CD133^{+}$ ovarian cancer cells. As few as 11 $ALDH^{+}CD133^{+}$ cells isolated from human ovarian cancer can form xenografts in immunodeficient mice [26].

CSCs may have heterogeneous and biologically distinct subpopulations. This phenomenon was confirmed in both hematopoietic and solid tumors. These subsets regenerated the phenotypic and functional heterogeneity of the parental tumor. Moreover, different CSC subgroups are related to one another, and can also interconvert each other [27]. However, the molecular mechanisms of these subgroups and interactions are poorly understood.

Because of their capacity to self-renew and differentiate, CSCs are confirmed to be involved in invasion, metastasis, and drug resistance in malignant tumors. CSCs are more resistant to conventional chemotherapy and radiation, and are important drivers of distant metastasis and recurrence. CSCs targeted therapy has been shown to improve chemosensitivity and prevent invasion and metastasis in cancer cell lines and preclinical *in vivo* studies [12,28].

Several signaling pathways are involved in regulating the functions of CSCs. Different CSCs and normal stem cells share key regulation genes and pathways, such as Sonic Hedgehog, Wnt/β-catenin, Notch, Hippo, c-myc, and Bmi-1 [24,28,29]. The Hedgehog (Hh) signaling pathway controls a number of genes involved in the determination of cell fate and stemness features. Hh signaling is active in various cancer stem cells. The signaling pathway can increase the tumor-initiating population, and contribute to cell migration, growth, and survival. SMO inhibitors can inhibit the CSC-promoting effects. In addition, Hh signaling has been shown to promote tumor metastasis and recurrence by regulating the core genes involved in the epithelial-to-mesenchymal transition (EMT) process [30]. Wnt/β-catenin and the Notch pathway also play key roles in stem cell self-renewal in normal and cancer stem cells. These pathways or genes may provide candidate targets for future cancer therapies.

Pancreatic cancer stem cells (PCSCs)

In pancreatic cancer, a small subset of "tumor-initiating" cancer cells has been identified. This subset has been named PCSCs. Recent evidence has demonstrated that PCSCs are responsible for the progression and relapse of cancer, as well as its resistance to chemotherapy and radiation. A thorough understanding of PCSCs and its regulating mechanisms may provide a potential novel target therapy for pancreatic cancer.

Discovery and identification of PCSCs

PCSCs were first described by Li et al. in 2007 [10]. The authors used a xenograft model originating from cells from a human patient with pancreatic adenocarcinoma. They found that $CD44^{+}CD24^{+}ESA^{+}$ pancreatic cancer cells accounted for only 0.2–0.8% of all pancreatic cancer cells, but had a 100-fold higher tumorigenic potential than nontumorigenic cancer cells. As few as 100 cells were able to form tumors in half of the mice. Only one in twelve mice developed a tumor following injection with 10,000 $CD44^{-}CD24^{-}ESA^{-}$ cells. Meanwhile, the tumors formed by the $CD44^{+}CD24^{+}ESA^{+}$ cells shared the same histological features as the original tumors. These tumor-initiating cells were also observed in pancreatic cancer cell lines. $CD44^{+}CD24^{+}$ cells were 20-fold more tumorigenic than $CD44^{-}CD24^{-}$cells, and could form tumors identical to unsorted PANC-1 cells.

Hermann et al. [31] reported that $CD133^{+}$ pancreatic cancer cells had CSC properties, just a few months after $CD44^{+}CD24^{+}ESA^{+}$ cells were identified. $CD133^{+}$ pancreatic cancer cells are more tumorigenic than $CD133^{-}$ cells. $CD133^{+}$ subpopulation cells are highly resistant to gemcitabine chemotherapy. The authors concluded that a subset of $CD133^{+}$ $CXCR4^{+}$ cancer stem cells was essential for tumor metastasis. Interestingly, the authors showed that $CD44^{+}CD24^{+}ESA^{+}$ subpopulation partially overlapped with the $CD133^{+}$ population. The results from a study aimed at revealing the relationship between CD133 expression and the clinical and pathological features of PCs suggested that CD133 expression in pancreatic cancer was significantly associated with lymphatic metastasis, vascular endothelial growth factor-C (VEGF-C) expression, and poor long-term survival [32].

c-Met is a receptor of the tyrosine kinase family and is stimulated by hepatocyte growth factor (HGF) to mediate normal organ development; it is a marker of normal mouse pancreatic stem and progenitor cells [24,33,34]. Previous studies confirmed that the level of c-Met is increased in pancreatic cancer and is associated with invasion, metastasis, and chemoresistance [35]. Li et al. (2011) confirmed c-Met as a cell surface marker of PCSCs. The researchers reported that c-Met^{High} $CD44^{+}$ had the capability for self-renewal and the highest tumorigenic potential of all cell populations studied. c-Met inhibitor XL184, alone or combined with gemcitabine, inhibited tumor growth and reduced the population of CSCs [25]. Cabozantinib, a c-Met inhibitor, can downregulate CSCs markers CD133, SOX2, and c-Met, which results in improved gemcitabine sensitivity and induces apoptosis [36].

The aldehyde dehydrogenase-1 (ALDH1) activity assay is also used to identify pancreatic CSCs. Kim et al. reported that $ALDH1^{High}$ alone was sufficient for efficient tumor initiation, while $ALDH1^{High}$ $CD133^{+}$ subpopulation cells had enhanced tumorigenic potential [37]. ALDH1 is also a functional marker of PCSCs. Kahlert et al. reported that low expression of ALDH1A1 was a prognostic marker for poor survival in pancreatic cancer [38]. The level of ALDH1A1 was significantly higher in the gemcitabine-resistant MIA PaCa-2 cell line. Knockdown of ALDH1A1 combined with gemcitabine significantly increased apoptosis and decreased cell viability [39].

Although many molecules have been reported to be markers of PCSCs (Table 1), there are no generally accepted markers to define PCSCs. This indicates a significant bottleneck in PCSCs research. Further studies are needed to define and sort the subgroup of stem-like cells in pancreatic cancer.

Signaling pathways involved in regulating PCSCs characteristics

Thoroughly understanding the signaling pathways that regulate the maintenance and epigenetics of PCSCs is important for the progress of further study and development of novel therapy. As mentioned before, PCSCs and normal tissue stem cells seem to share the same key regulatory genes and signaling pathways, such as Notch, Sonic Hedgehog, Wnt/β-catenin, PI3K/AKT, c-Myc, Bim-1, and FOXM1.