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Pancreatic Ductal Adenocarcinoma Stem Cells

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Abstract

Background/Objectives: The present article summarizes and analyzes the current knowledge about the role of markers and dysregulated signaling pathways in pancreatic cancer stem cells (CSCs) and their value for possible therapeutic approaches.

Method: An electronic search of PubMed/MEDLINE was used to identify relevant original articles and reviews.

Results: Despite significant effort and research funds, pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest diseases. Because of the lack of symptoms, the majority of patients presents with advanced stage. Patients with advanced disease will receive systemic therapy. However, such therapy only eradicates tumor bulk, but does not eliminate so-called cancer stem cells. These CSCs are thought to be the cause of resistance, metastasis, and recurrence. This review will focus on recent insights into the biology of pancreatic CSCs. It further highlights the importance of PDAC stem cell markers as prognostic indicators and targets for therapies specifically eliminating PDAC stem cells.

Conclusions: Pancreatic CSCs appear to be crucial for the processes of cancer cell invasion and metastasis. Therefore, the understanding of the molecular mechanisms implicated in the biology of CSCs as well as the identification of specific markers may generate novel therapeutic strategies and contribute in the reduction of metastasis.

Keywords: Pancreatic cancer; Treatment; Cancer stem cells; Signaling pathways

Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is the fourth leading cause of cancer related death in the USA and Europe. Despite substantial progress of understanding the molecular biology of PDAC, the prognosis remains still poor with a combined overall 5-year-survival rate of less than 7% [1]. More than 50% of the patients are diagnosed at an advanced stage with a median survival time less than 12 months. This high death rate is due to late diagnosis with no effective screening tools for detection of early tumors and the lack of curative treatment methods [2]. Findings suggest that highly resistant cancer stem cells (CSC) are responsible for metastasis and resistance towards effective therapy [3]. Hence, a better understanding of these pancreatic CSC seems to be a promising way to establish new diagnostic and therapeutic options. In this article, we review the current role of CSCs in pancreatic cancer and its biologic features regarding its role in the tumor micro-environment.

Method

An electronic search of the U.S. National Library of Medicine – National Institutes of Health PubMed/MEDLINE was undertaken in order to identify original articles and reviews about this subject. The terms “cancer,” “stem,” “cell,” “pancreas”, “marker,” “signaling,” and “pathway” were used in various combinations. The key words were identified as truncated words in the title and abstract, or in the medical subject headings (Mesh). Only English language literature was selected for further analysis. Electronic and manual cross-referencing was used to find additional relevant sources. For the purpose of our article we only considered publications after the year 2000.

What are PDAC Stem cells?

CSCs are a rare population of cancer cells that possess the ability of self-renewal and differentiation into multiple cells [4]. The first existence of CSC was reported in 1997 [8]. It was observed that markers that can identify stem cells in normal tissue are also expressed in tumor tissue. These markers are CD34+CD38-. In SCID leukemia-initiating cells (SL-IC), CD34+CD38 can define stem cells: after transplantation into NOD/SCID mice, these cells were able to proliferate and to differentiate, producing a disease in mice that was identical to that in the donor. Because these cells were capable of self-renewal, acute myeloid leukemia (AML) could also subsequently re-establishment of in recipient mice.

Following these results, CSC was also identified in solid tumors including brain [9], breast [10], and liver [11], and colon cancers [12]. It was exciting news that pancreatic cancer (PDAC) also displays subpopulations of stem cells [13]. Li et al. reported that CSCs with ESA +/CD44+/CD24+ cell surface markers were able to form PDAC in NOD/SCID mice with a microarchitecture and pathophysiology similar to the primary tumor [14].

In recent years it has been demonstrated that CSCs might have a crucial role in clinicopathological characteristics of tumors, e.g. in metastasis and drug resistance [5]. It could be shown in PDAC that stem-like cancer cells had higher resistance to Gemcitabine and were more invasive [6]. Additionally, recent investigations demonstrated that tumorigenicity and metastatic potential was increased in pancreatic cancer CSCs expressing CD133 [15], c-Met [16] and ALDH [17]. Moreover, it was repeatedly shown that patients who highly expressed cancer stem cell marker CD133 in pancreatic cancer had poor prognosis [7]. Therefore, CSCs might have an important part in the prognosis of PDAC. Below, we give an overview on the most important markers for CSCs in pancreatic cancer.

CD44

CD44 is a cell surface glycoprotein [18]. CD44 mainly acts as a specific receptor for hyaluronic acid. It has a role in mediation of cell-cell adhesions and has been identified in most solid tumors including colorectal cancer [19], hepatocellular carcinoma [20], ovarian [21], and pancreatic carcinoma [22]. CD44 is an important marker used in combination with CD24 and ESA to enrich PDAC stem cells. Li et al. Found that CD44+/ CD24+ /ESA+ cells were more tumorigenic than other populations in NOD/SCID mice [14]. Another study reported that CD44+/CD24+ cells were 20-fold more tumorigenic than CD44-/ CD24- cells and generated tumors with the same histological features as PANC-1 cells [22]. There is also clinical relevance of CD44: CD44 expression seems to correlate with poor survival [23-25]. Experimental and clinical data therefore suggests importance of this marker. Unfortunately, literature search yielded no plans to use this marker as a candidate for new therapeutic strategies.

CD24

CD24 is a mucin-like cell surface glycoprotein that has been identified in a variety of cancers including breast [26], liver [27], ovarian [28] and PDAC [14]. It is involved in cell adhesion and metastasis [29]. Lee et al. demonstrated that CD24+ cells had capability of self-renewal and initiation of hepatocellular carcinomas [30]. A recent study demonstrated that CD24 expression is observed during EMT and might have a significant role in chemotherapy resistance [31,32]. Chemotherapy resistant cells expressed more CSC markers including CD24 and were more tumorigenic in vitro and in vivo [31].

A recent study reported that targeting CD24+ cells with T-cells generated significant reduction in tumor size and prolonged survival rate in treated mice [33]. In another study Salnikov et al. observed that targeting CD24 significantly reduced tumor proliferation and angiogenesis in a mouse model [34]. Taken together, these findings suggest that CD24 could be promising therapeutic and prognostic marker for patients with pancreatic cancer.

EpCAM

EpCAM is a 39-42 kDa type I transmembrane glycoprotein [35]. It was first described in 1979 [36]. It functions as a homophilic epithelial-specific cell-cell-adhesion molecule [37]. Silencing of EpCAM led to a reduction in proliferation, migratory and invasive capacity in breast cancer cell lines [38]. A potential role of EpCAM in pancreatic cancer was first reported by Li et al. [14]. ESA+/CD44+/ CD24+ cells were associated with stemness properties. Using in vitro cytotoxicity assays, the recent study suggested that targeting CD24 positive and EpCAM positive cells with catumaxomab, a clinical-grade bi-specific antibody that binds to both EpCAM on tumor cells and CD3 on T-cells, combined with activated T-cells might be of benefit in PDAC treatment [39].

CD133

CD133 is an approximately 120-kDa pentaspan transmembrane glycoprotein, first described in hematopoietic stem and progenitor cells [40]. It was identified in many solid tumors including liver [41], colon [42], kidney [43], lung [44], ovarian [45] and PDAC [15]. Hermann et al. reported that CD133+ cells were dramatically more resistant to gemcitabine and had highly tumorigenic and metastatic features [13]. In addition, it was shown that CD133 had significant role in prognosis of PDAC [46]. It was observed that the 5-year survival rate of patients with CD133-negative tumors was almost 5-fold higher than patients with less than 5% CD133-positive cells. Another study suggested an important correlation between CD133 expression and EMT [51]. Regarding novel therapeutic strategies targeting CD133, it is interesting to notice that low concentrations of metformin was reported to selectively target CD133+ cells- [47]. After treatment with metformin proliferation was selectively inhibited in CD133+ cells. CD133+ cells showed a G1/S arrest. However, the exact molecular mechanism remains unclear [47].

C-Met

C-Met is a receptor of the tyrosine kinase family that is stimulated by hepatocyte growth factor [HGF] [48]. These receptors can also promote tumorigenicity and chemotherapy resistance in solid tumors. Li et al. demonstrated that c-Met high cells were as tumorigenic as CD44+ /CD24+/ ESA+ cells [16]. In this study it was shown that c-Met high cells were able to form tumors in mice identical to the original tumor from patients. Moreover, it was observed that targeting c-Met using small hairpin RNAs significantly reduced tumorosphere formation and inhibited growth of tumors in xenografts in a mouse model [16]. Similarly, c-Met inhibitor XL184 reduced sphere formation and inhibited tumor growth in mice. Inhibiting of c-Met with XL184 completely blocked the development of metastasis, while treatment with gemicitabine did not prevent the development of metastasis [16].

ALDH1

Aldehyde dehydrogenase 1 [ALDH1] has been demonstrated as a potential CSC marker in hepatocellular carcinoma [49], lung cancer [50] and colorectal cancer [51]. The data on ALDH1 in PDAC is limited. In PDAC it was reported that ALDHhigh/CD133− cells were more tumorigenic than ALDHlow/CD133+ cells [52]. Interestingly, ALDHhigh /CD133− cells demonstrated higher incidence of tumor formation than ALDHhigh/CD133+ cells in a pancreatic cancer mouse model [52].

Molecular signaling pathways in regulating pancreatic CSCs

A very important question for the development of possible therapies is which signaling pathways in CSC might mediate the pathophysiological characteristics and therefore mediate properties like “metastasis” and “drug resistance” [53, 54]. Recently, several signaling pathways such as Notch [55], Hedgehog [56], Wnt [57],

CXCR4 [13] and Nodal/Activin [58] signaling pathways were reported to be deregulated in pancreatic CSC.

The Notch signaling pathway is known for its fundamental role in embryogenesis, cellular homeostasis, and stem cell renewal [59]. In addition, Notch signaling pathway plays an important role in oncogenesis [60]. Wang et al. reported that down-regulation of Notch-1 induced apoptosis, decreased cell migration and invasive properties of PDAC cells [61, 62]. Recently, it was reported that Notch pathway components were upregulation of in CSCs compared to non-CSCs, indicating that Notch signaling pathway might contribute to CSC maintenance [54]. However, mechanisms underlying this regulation need to be further exploited.

Another important signaling pathway in PDAC is the Hedgehog [Hh] pathway. This is a ligand-dependent signaling pathway which plays critical role not only in embryogenesis, but also in maintenance of CSC [63]. Recently, inhibition of Hh signaling was reported to inhibit the self-renewal of pancreatic CSCs and reverse chemoresistance [64]. Moreover, it was found that expression of the Sonic Hedgehog [SHH] transcript was increased 46-fold in CD44+CD24+ESA+ cells when compared to normal pancreatic epithelial cells [65].

The Wnt signaling pathway is another developmental pathway that is commonly activated in a variety of solid tumors [66]. Wnt can bind to cell membrane receptor to initiate expression of different important target genes [67]. Although, correlation between Wnt signaling activity and CSC has been reported in some solid tumors [68], its role in pancreatic CSC need to be further investigated. Most recently, two different genetic mouse models, low tumorigenic KC-428 and high tumorigenic KPC-1050 were used to determine the significance of Wnt enhancer genes in primary tumor cells. Both lines were sorted by FACS differing in their CSC marker profile into CD24/CD44high and CD24/CD44low tumor cells. Significantly higher levels of Rspo2, Lgr5, Axin2, and Snail was found in the highly tumorigenic KPC-1050, suggesting an active role of RSPO2-enhanced Wnt signaling in KPC-1050–derived CSCs [69].

Taken together, these findings indicate that the above mentioned pathways can be effective targets to eradicate pancreatic CSCs.

Therapeutic Relevance of PDAC Stem Cells

Conventional therapy and radiation for patients with PDAC mainly eradicate differentiated tumor cells but not cancer stem cells which are resistant to various drugs [70]. Thus, they can re-establish therapy-resistant and more aggressive tumors [71]. Therefore, the therapeutic agents that target CSC might be promising future therapeutic drugs for PDAC.

Recently, an antibody against CD44 has been shown to reduce tumorigenicity in a mouse model of pancreatic cancer and down-regulated genes associated with stem cell self-renewal such as Nanog, Sox-2 and Rex-1 in cultured PDAC cell lines [23]. Moreover, inhibition of c-Met, another important CSC marker for PDAC, with XL184 reduced sphere formation and inhibited tumor growth in mice [16]. In addition to targeting PDAC stem cell markers, inhibition of the signaling pathways deregulated in PDAC might reduce CSC activity. For instance, inhibition of Notch signaling pathway inhibited tumor growth [72], decreased invasion and reduced the percentage of cells expressing the PDAC stem cell markers [73].

Conclusion

To date, pancreatic CSC can be identified by several markers such as CD44, CD24, ESA, and c-Met. It is noteworthy that combination of CD44/CD24/ESA is more appropriate to identify stem cell markers are than single markers alone. Since CSCs are responsible for chemoresistance, targeting CSCs could generate more effective treatment options. Several experimental studies targeting CSCs with agents against CSCs markers had promising results.

Additionally, signaling pathways deregulated in CSC biology need to be further elucidated. Knowledge on signaling pathways such as Notch and Hedgehog in PDAC is quite good. However other signaling pathways should also be closer investigated in regard to their role in CSC, as they might promise therapeutic targets.

Pancreatic CSCs appear to be crucial for the processes of cancer cell invasion and metastasis. Therefore, the understanding of the molecular mechanisms implicated in the biology of CSCs as well as the identification of specific markers may generate novel therapeutic strategies and contribute in the reduction of metastasis.

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