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MINIREVIEWS

Pancreatic cancer stem cells: Perspectives on potential therapeutic approaches of pancreatic ductal adenocarcinoma

Claudia Di Carlo, Jessica Brandi, Daniela Cecconi

Claudia Di Carlo, Jessica Brandi, Daniela Cecconi, Department of Biotechnology, Proteomics and Mass Spectrometry Laboratory, University of Verona, Verona 37134, Italy

ORCID number: Claudia Di Carlo (0000-0001-9728-3032); Jessica Brandi (0000-0003-1643-7023); Daniela Cecconi (0000-0002-7314-8941).

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Correspondence to: Jessica Brandi, PhD, Doctor, Post-doctoral Fellow, Department of Biotechnology, Proteomics and Mass Spectrometry Laboratory, University of Verona, Strada Le Grazie 15, Verona 37134, Italy. jessica.brandi@univr.it
Telephone: +39-45-8027954
Fax: +39-45-8027929

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Abstract

Pancreatic ductal adenocarcinoma is one of the most aggressive solid tumours of the pancreas, characterised by a five-year survival rate less than 8%. Recent reports that pancreatic cancer stem cells (PCSCs) contribute to the tumorigenesis, progression, and chemoresistance of pancreatic cancer have prompted the investigation of new therapeutic approaches able to directly target PCSCs. In the present paper the non-cancer related drugs that have been proposed to target CSCs that could potentially combat pancreatic cancer are reviewed and evaluated. The role of some pathways and deregulated proteins in PCSCs as new therapeutic targets are also discussed with a focus on selected specific inhibitors. Finally, advances in the development of nanoparticles for targeting PCSCs and site-specific drug delivery are highlighted, and their limitations considered.

Key words: Pancreatic cancer stem cells; Pancreatic cancer; Therapeutic approaches; Pancreas; Treatment

Core tip: Pancreatic cancer is characterised by remarkable resistance to treatment conferred by pancreatic cancer stem cells (PCSCs). Unfortunately, most conventional treatments are unable to eradicate tumours. Recent research has focused on characterising PCSCs to accelerate the development of novel therapeutic strategies. In the present paper, we shed light on promising new strategies such as using non-cancer drugs as anticancer therapeutics, targeting of deregulated pathways and proteins of PCSCs, and using nanoparticles for improved drug delivery.

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INTRODUCTION

Pancreatic cancer comprises many types of cancers, of which the most common is an infiltrating neoplasm named pancreatic ductal adenocarcinoma (PDAC)[1], which derives from the pancreatic ductal tree[2]. PDAC is almost always fatal, it is refractory to conventional treatments, and consequently has a documented five-year survival rate as low as 8%. The major driver genes participating in the whole process of disease development include *KRAS, TP53, CDKN2A*, and *SMAD4*. With a near 100% *KRAS* mutation frequency, PDAC is considered the most RAS-addicted of all cancers[3]. PDAC is also characterised by a dense tumour microenvironment, perineural and vascular local growth, and early distant metastases. In particular, it typically has a tendency to metastasise preferentially to the liver where soluble factors and extracellular vesicles deriving from the primary tumour contribute to form a supportive niche[4]. Patients seldom exhibit symptoms. Therefore, early diagnosis of the tumour is very difficult. Indeed, the majority of patients are diagnosed when metastatic events have occurred or during advanced-stage disease. For this reason, primary prevention such as avoiding smoking and having a fat-poor diet is important[5]. Currently, surgery coupled with chemo or radiation therapy is the main treatment approach although it doesn't present satisfactory results[6]. Moreover, disease can persist or recur with local and distant metastases. Most patients subjected to resection of the tumour die from metastasis within five years[7]. Despite its low efficacy, gemcitabine (a pyrimidine analogue) was the first-choice chemotherapeutic strategy in advanced PDAC for many years[8]. It is effective in only 23.8% of PDAC cases[9] due to dense tumour stroma and scarce diffusion of drug and to subsequent development of gemcitabine chemoresistance[10].

Recently, understanding of pancreatic carcinogenesis has improved and some new therapeutic options have been suggested. For example, it has been demonstrated that FOLFIRINOX, a chemotherapy regimen made up of four drugs (folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin), or nab-paclitaxel plus gemcitabine provide a survival benefit over gemcitabine alone[11]. However, we are still far from a substantially better life expectancy for patients since these new therapeutic options increase the median survival by only a few months.

A growing body of evidence suggests that the drug resistance and metastasis of PDAC are mainly influenced by the presence of cancer stem cells (CSCs). In the present paper, we aim to summarise the current understanding of pancreatic cancer stem cells (PCSCs) and analyse and discuss therapeutic options for targeting PCSCs.

PCSCs

PCSCs characteristics

It has recently been demonstrated that CSCs play critical roles in resistance to anticancer treatment and are responsible for metastasis in several human malignancies, including PDAC[12]. CSCs are rare immortal tumour cells, which have the ability to self-renew, produce differentiated progeny, form tumours in mice, and form non-adherent spheroids called tumour-spheres *in vitro*[13,14]. CSCs are more resistant than non-CSCs to chemotherapy and radiotherapy treatments because they have higher expression levels of anti-apoptotic proteins, ABC transporters, and multidrug resistance genes[15]. These cells reside in a niche, a specific hypoxic/necrotic microenvironment that includes different cell types (each one possessing distinct metabolic properties), such as fibroblastic, immune, endothelial, and perivascular cells, as well as extracellular matrix components, cytokines, and growth factors. In this environment, CSCs protect and reprogramme their metabolism and respond to the metabolism of surrounding cells, increasing tumour growth and preserving phenotypic plasticity[14,16]. Induction and maintenance of CSC phenotypes are related to more than 20 different transcription factors, including NF-κB and the hypoxia inducible factors[13,17]. Moreover, CSCs adjust their metabolism to their microenvironment by acquiring intermediate metabolic phenotypes or shifting from oxidative phosphorylation (OXPHOS) to glycolysis/Warburg effect. CSCs are also characterised by a high autophagic flux, which is involved in resistance to microenvironment stresses, such as hypoxia, starvation, or anticancer treatment[18]. Thus, it has been supposed that autophagy plays a significant role in the resistance to CSCs related anticancer therapy[19].

Pancreatic CSCs, first described in 2007[20], represent less than 1% of all pancreatic cancer cells[21] and are responsible for PDAC tumour growth (initiation, progression, and recurrence), maintenance, metastasis, and chemoresistance. The origin of PCSCs remains unknown. The hypothesized sources are: Tissue stem cells or progenitor cells, stem cells derived from bone marrow, or dedifferentiated cells that result from genetic mutation[22]. PCSCs can be identified by markers, such as CD133, CD24, CD44, ESA/EpCAM (epithelial-specific antigen), c-Met, ALDH1, DclK1, CXCR4, and Lgr5. However, a universal signature is still lacking[13,23,24]. The main signalling pathways of PCSCs, which are essential for self-renewal, are the epithelial to mesenchymal transition (EMT) process, and resistance to conventional therapies include Wnt/β-catenin, Sonic Hedgehog (SHH), and Notch. In addition, other biological aspects, such as autophagy, forkhead box protein M1 (FoxM1), mammalian target of rapamycin (mTOR), Bmi-1, NODAL/ACTIVIN, NF-κB and PTEN pathways, have been shown

to be implicated in PCSC activity.

Importantly, PCSCs co-exist with other cellular and non-cellular components that constitute the tumour microenvironment (including cancer-associated fibroblasts, pancreatic stellate cells, and tumour-associated macrophages). Understanding the relationship between PCSCs and all these components is extremely important to improve the knowledge of the PCSC biology[12]. Recently, it has been demonstrated that PCSCs are involved in highly dynamic cross-talk with the PDAC parenchymal cells[25] by a symbiotic relationship that underlies the initiation and maintenance of early PDAC infiltration and metastasis. In particular, the secretome of PCSCs paracrinically inhibits parental cell growth and autocrinically stimulates their own growth and vascularity, while the secretome of parental cells both paracrinically inhibits PCSC growth and autocrinically inhibits their own growth. It is clear that to make a substantial impact on pancreatic cancer, it is necessary to eradicate PCSCs with targeted therapeutics[26]. For this reason, a complete molecular characterisation of PCSC biology is fundamental. Recently, we have characterised the proteome[7] and the secretome[27] of Panc1 CSCs, demonstrating the functional role of fatty acid synthesis and mevalonate pathways in PCSC viability and identifying secreted proteins involved in cancer differentiation, invasion, and metastasis. Through a combined proteomics and metabolomics approach we also found that Panc1 CSCs, as compared to the parental Panc1 cells, have induced expression of proteins and metabolites involved in glycolysis, pyruvate-malate cycle, folate cycle, pentose phosphate pathway, and lipid metabolism, and reduced expression of proteins and metabolites involved in the Krebs cycle, spliceosome, and non-homologous end joining pathway[7].

PCSCs chemoresistance

Chemoresistance is the major obstacle to successful cancer treatment. Many drugs are not able to eliminate PDAC, which represents the primary reason for tumour recurrence and metastasis. PCSCs are very resistant and can survive conventional treatments interfering with the total eradication of a tumour[16,23]. The mechanisms involved in the chemoresistance of CSCs include the metabolic inactivation of the drug and efflux of the drug from the cells, as well as mutation or deregulation of the drug targets[28]. In particular, an altered drug transport activity, as an over-expression of aldehyde dehydrogenase and proteasome, and a decreased expression of the human equilibrative nucleoside transporters (ENTs) and human concentrative nucleoside transporters (CNTs), play a key role in the chemoresistance of PCSCs[23].

As previously reported, PCSCs reside in niches that are responsible for the protection of cancer cells, tumour growth, and phenotypic plasticity. Critical components for the ever-changing tumour microenvironment and for construction of CSCs niche are Wnt/RSPO (R-spondin), c-Jun N-terminal protein kinase (JNK), Nodal/Activin, Notch, or Hedgehog proteins[23]. This specific CSCs microenvironment has also been proposed to contribute to drug resistance.

Chemoresistance is also related to the EMT process, which has a fundamental role in invasive and metastatic behaviour in PDAC. EMT, in pancreatic cancer cells, is controlled by several transcription factors, such as Zeb1, which suppresses the adhesion molecule E-cadherin by repressing the miR-203 (an inhibitor of stemness) and the miR-200 family members (which regulate expression of stem cell factors)[29]. Accordingly, it has been demonstrated that the class I HDAC inhibitor mocetinostat interferes with Zeb1 function, represses EMT, and restores the drug sensitivity of PDAC cells[30]. In particular, EMT contributes to enhanced resistance to gemcitabine because it leads to an increase in cancer cells with reduced expression of nucleoside transporters (ENT and CNT) that are involved in drug uptake[31]. Finally, it has been hypothesised that quiescence protects PCSCs from chemotherapeutic treatment, which usually targets rapidly proliferating cells.

POTENTIAL THERAPIES TARGETING PCSCs

It is broadly accepted that development of anti-cancer drugs to target determinant pathways and proteins of PCSCs will improve chemotherapeutic outcomes[15]. Eradication of these CSCs should be able to stop tumour progression and reduce future tumour insurgences[26,32]. Potential strategies to target PCSCs are discussed in the next section.

Non-cancer related drugs

Some non-cancer related drugs that show anticancer effects against different human CSCs could also represent an option in PCSCs (Table 1). They act through different mechanisms of action including the inhibition of some important PCSCs pathways (Figure 1).

Antibiotics are among the molecules that exhibit extraordinarily diverse biological activities. For example, salinomycin, an antibacterial and coccidiostat ionophore drug, interferes with the activity of KRAS-4B, Wnt, and EMT pathways reducing the viability of breast CSCs[33]. Interestingly, it has also been demonstrated that salinomycin blocks tumour growth and the metastatic spread of PDAC in a genetically engineered mouse model[34]. In addition, the FDA-approved antibiotic azithromycin, which binds to the 50S subunit of the bacterial ribosome, inhibits tumoursphere formation in PDAC and other cancers[35]. Also, the antibiotic tigecycline, developed in response to the antibiotic resistance of some bacteria, reduces the sphere formation of CSCs in pancreatic, breast, lung, and prostate cancers[29]. In particular, it eliminates the therapy-resistant chronic myeloid leukaemia CSCs[36], and a phase I clinical trial demonstrated the safety of its intravenous infusions in patients with acute myeloid

Table 1 Non-cancer related drugs and their potential effects on pancreatic cancer stem cells

Drug	Function	Relative pathway/process	Ref.
Salinomycin	Anti-bacterial antibiotic	Wnt, EMT	[33]
Azithromycin		Mitochondria	[35]
Nigericin		EMT	[38]
Tigecycline		OXPHOS	[29]
Chloroquine	Anti-malaria	OXPHOS	[39]
Atovaquone		OXPHOS	[36,40]
Aprepitant	Anti-emetic	Wnt	[41]
Ketamine	Anti-depressant	Wnt	[42,43]
Aspirin	Anti-pyretic Anti-inflammatory	ALDH1, NF-κB	[44,45]
Metformin	Anti-diabetic	mTOR, PI3K/Akt	[46-48]
Disulfiram	Anti-alcoholism	NF-κB	[49-53]
Atorvastatin	Anti-cholesterol	Mevalonate	[7,54]

Wnt: Wingless-type MMTV integration site family; EMT: Epithelial mesenchymal transition; OXPHOS: Oxidative phosphorylation; ALDH1: Aldehyde dehydrogenase 1; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; mTOR: Mammalian target of rapamycin; PI3K: Phosphatidylinositol 3-kinase; Akt: Protein kinase B.

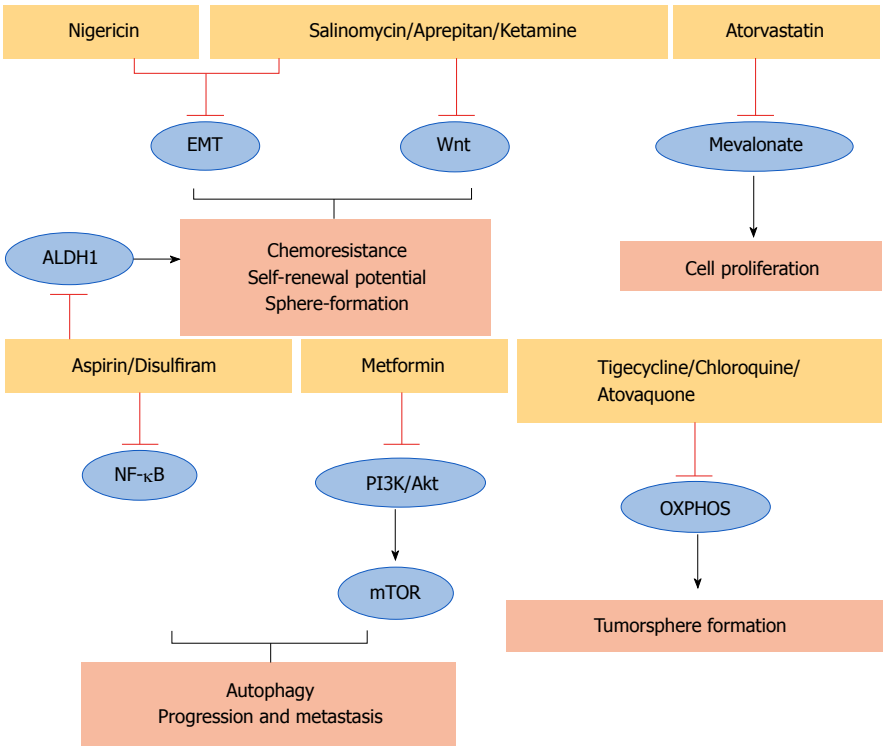

Figure 1 Mechanism of action of different non-cancer related drugs against pancreatic cancer stem cells. EMT: Epithelial mesenchymal transition; ALDH1: Aldehyde dehydrogenase 1; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; mTOR: Mammalian target of rapamycin; PI3K: Phosphatidylinositol 3-kinase; Akt: Protein kinase B; OXPHOS: Oxidative phosphorylation.

leukaemia[37], supporting its transfer to clinical use. Moreover, it has been demonstrated that the antibiotic nigericin increases E-cadherin expression and inhibits the EMT process of CSCs leading to a reduction of invasion and metastasis of colorectal cancer[38]. This observation suggests that it should be further investigated to determine whether if it is also effective for targeting PCSCs.

Some anti-malarial agents may have the potential to target PCSCs. For example, it has been demonstrated that chloroquine has significant effects on PCSCs by inhibiting CXCR4 and Hedgehog pathways[39]. The same can also be said for another anti-malarial compound atovaquone, which acts as a potent and selective OXPHOS inhibitor, inhibiting the sphere-formation of CSCs in breast cancer[40].

Also showing promise for targeting PCSCs is aprepitant, an FDA-approved antiemetic drug that inhibits Wnt signalling, sphere formation, growth, and stemness of CSCs in colon cancer[41]. Ketamine, a drug used as an anaesthetic and depression, reduces CSCs traits and tumour growth in a colorectal cancer model. In

particular, it acts by decreasing Wnt activity[42]. Notably, ketamine reportedly inhibits the proliferation of PDAC cells[43].

Salicylic acid, also known as aspirin, is another non-cancer related drug that may be a candidate for eliminating PCSCs in the successful treatment of PDAC. Indeed, aspirin, commonly used as an antipyretic and anti-inflammatory drug, counteracts PCSCs features such as ALDH1 activity, NF-κB signalling, self-renewal potential, and gemcitabine resistance[44]. A phase Ⅲ trial confirmed the beneficial effect of aspirin as an adjuvant treatment to prevent disease recurrence and contribute to survival after primary therapy in breast, colorectal, gastro-oesophageal, and prostate tumours[45].

Metformin, a dimethylbiguanide used as an anti-diabetic drug, is also able to counteract the features of PCSCs. It inhibits the mTOR and PI3K/Akt pathways, reducing the expression of PCSCs markers in pancreatic tissue, as well as the size and number of tumour spheres. Moreover, *in vivo* experiments demonstrated that metformin prevents progression and metastasis in PDAC[46]. Unfortunately, a phase Ⅱ trial showed that metformin does not improve the outcome in patients with advanced metastatic PDAC treated with standard therapy[47,48]. These findings suggest that future research should include studies of more potent biguanides.

Another non-cancer related drug is disulfiram, a drug widely used to control alcoholism, which is involved in the inhibition of NF-κB, ERK and proteasome pathways in PDAC. It has been demonstrated that disulfiram in combination with chemotherapy or chemoradiation, is able to target PCSCs[49-52]. Notably, a phase IIb trial demonstrated that the addition of disulfiram to chemotherapy prolonged survival in patients with newly diagnosed non-small cell lung cancer[53].

Moreover, we have recently demonstrated[7] that atorvastatin, a drug used to lower blood cholesterol, reduces the viability of PCSCs. Accordingly, the anti-cancer effect of cholesterol-reducing agents has been demonstrated against other CSCs. To date, in a clinical setting statin intake was significantly associated with longer recurrence-free survival in hepatocellular carcinoma patients with hepatectomy[54].

Taken together, these findings indicate that repurposing established compounds to target PCSCs could represent a good strategy for combating PDAC. It is also economically advantageous and assures rapid translation into clinical because these compounds often are already approved by the FDA and show minor side effects compared to traditional chemotherapeutic drugs[29].

Compounds focused on deregulated pathways and proteins

In the last ten years, many agents that target specific deranged pathways of pancreatic tumour cells have shown promise in preclinical studies. Accordingly, potential therapies targeting PCSCs could be developed based on their deregulated pathways and/or proteins (Table 2). As stated above, multiple signalling pathways are known to be important for stemness, including the Wnt/β-catenin, SHH, Notch, and mTOR pathways. Some compounds that inhibit the Wnt signalling pathway have been reported in the previous section on non-cancer related drugs (*i.e*., salinomycin, aprepitant, and ketamine). It has been demonstrated that crocetinic acid (a carotenoid obtained from saffron) is able to target PCSCs by inhibiting the expression of both SHH and smoothened proteins, which play a key role in the SHH pathways[55]. SHH and smoothened proteins lead to the activation of the Gli transcription factor and target genes involved in stem cell maintenance. In particular, crocetinic acid decreases the number and size of the spheroids in a dose-dependent manner and supresses the expression of DclK1, a PCSCs surface marker[55]. Another natural compound that inhibits the SHH pathways is sanguinarine (an isoquinoline alkaloid derived from *Sanguinaria canadensis*). It has been recently reported to be an effective agent for the inhibition of PCSCs[56]. It inhibits the self-renewal capacity of PCSCs, as well as their migration, invasion, and EMT by suppressing the SHH pathway. Recently, PCSCs have been efficiently eliminated by targeting the SHH pathway using the Gli inhibitor GANT61 in combination with rapamycin (an mTOR inhibitor)[57].

Another deregulated PCSCs pathway that can be targeted is Notch signalling. Its inhibition by γ-secretase inhibitor (RO4929097) as well as by Hes1 shRNA reduces the formation of tumour-spheres and the proportion of PCSCs[58]. Notch signalling can reportedly be inhibited by using quinomycin A (an antibiotic and also classifiable as a non-cancer related drug). Quinomycin A suppresses PCSCs by reducing Notch 1-4 receptors and by decreasing the expression of their ligands (Jagged1, Jagged2, DLL1, DLL3, and DLL4) of the downstream protein Hes1 and the γ-secretase complex[59]. Quinomycin A also decreases the expression of DclK1, CD44, CD24, and EPCAM, retarding the tumour-sphere formation of PCSCs[59]. Clinical trials published several decades ago and not related to pancreatic cancer indicated a modest activity of quinomycin A against some tumours.

Inhibition of mTOR signalling has also been proposed as a novel strategy for targeting CSCs. In particular, it has been shown that greater suppression of PCSCs is obtained by combining gemcitabine with the mTOR inhibitor rapamycin[60] or c-Met/RON inhibitor with the mTOR inhibitor AZD8055[61].

Changes in the expression of PCSC proteins may represent a good starting point to investigate potential therapeutic targets. Recently, we indicated that fatty acid synthase (FASN) might represent a means of eradicating PCSCs[7]. Treatment with cerulenin, a specific FASN inhibitor, led to a reduction of Panc1 CSCs viability and decreased the formation of spheroids. Accordingly, it has been demonstrated that FASN plays a pivotal

Table 2 Deregulated pathways and proteins to target pancreatic cancer stem cells

Deregulated pathways	Compound or strategy	Ref.
Hedgehog	Crocetinic acid	[55]
	Sanguinarine	[56]
	GANT61	[57]
Notch	RO4929097, shRNA	[58]
	Quinomycin A	[59,97,98]
mTOR	Rapamycin	[60]
	AZD8055	[61]
Deregulated proteins	**Compound or strategy**	
FASN	Cerulenin	[7]
AnxA1	siRNA	[64]
MARCKS	MANS peptide	[66]
Galectin-3	Polysaccharide RN1	[70]
PKM2	Lapachol	[72]
	Diallyl disulphide	[73]
ERRγ	GSK5182	[82,83]

shRNA: Short hairpin RNA; siRNA: Small interfering RNA; FASN: Fatty acid synthase; mTOR: Mammalian target of rapamycin; AnxA1: Annexin A1; MARCKS: Myristoylated alanine-rich C-kinase substrate; PKM2: Pyruvate kinase isozyme M2.

role in the maintenance of stemness in other CSCs[62]. Among the potential PCSCs targets we identified annexin A1 (AnxA1)[7], which is an important player in the development and progression of different types of cancer, including pancreatic cancer, and plays a role in the maintenance of stemness and drug resistance in some CSCs[63]. Recent studies have shown that knockdown of AnxA1 decreases cell invasion and metastatic potential in several types of cancer, including PDAC[64].

Another potential therapeutic target that is both overexpressed and oversecreted by Panc1 CSCs and that should be investigated to reduce the viability of PCSCs is myristoylated alanine-rich C-kinase substrate (MARCKS)[7,27], a protein involved in cell motility, cell shape, cell cycle regulation, secretion, and transmembrane transport[65]. It has been demonstrated that a peptide (MANS peptide) that inhibits the function of MARCKS reduces lung cancer metastasis[66].

Another protein overexpressed and oversecreted in Panc1 CSCs is galectin-3 (Gal3)[7,27], which activates RAS signalling[67]. Many studies reported that Gal3 is implicated in cancer stemness, in particular by activation of Notch signalling[68], and that it may therefore represent a good therapeutic target[69]. Accordingly, it has been shown that down-regulation of Gal3 by an allosteric inhibitor, *i.e.*, the polysaccharide RN1 (purified from the flower of *Panax notoginseng*), increases metastatic cancer cell apoptosis and decreases pancreatic cancer cell growth[70,71].

Another potential target of PCSCs we identified was pyruvate kinase isozyme M1/M2 (PKM1/PKM2)[7]. Although PKM2 is a key mediator of glycolysis in cancer cells, research focused on exploiting metabolic pathways for cancer therapy is still scarce. To date, anti-tumour effects have been demonstrated in melanoma cells following treatment with lapachol (a specific PKM2 inhibitor)[72], and inhibition of stemness has been reported in breast CSCs treated with diallyl disulphide which targets PKM2 (and also CD44 and AMPK signalling)[73]. Recently a new series of small molecule PKM2 inhibitors able to inhibit the growth of tumour cells has been synthesised[74]. It could be worthwhile to evaluate their efficacy also on PCSCs.

Another protein involved in metabolism that is overexpressed and oversecreted by Panc1 CSCs is lactate dehydrogenase A (LHDA)[7,27]. LDHA is an important supporter of glucose metabolism in cancer cells. It generates adequate extracellular lactate to provide a favourable microenvironment for CSCs growth and invasion[75]. Although inhibition of LDHA activity has been proposed as an approach to cancer therapy[76], a limited number of LDHA inhibitors are reported in the literature[77]. However, LDHA is transcriptionally regulated by the oncogenic transcription factor FoxM1[78], and some FoxM1 inhibitors (such as thiostrepton, troglitazone, and the FDI-6 molecule) have been reported that could indirectly lead to a reduction of LDHA[79].

Among the upstream regulators of deranged PCSCs proteins, we found there is the oestrogen-related receptor gamma (ERRγ)[7], which promotes metabolic reprogramming in CSCs, pluripotency, OXPHOS, and the glycolysis pathway[80]. A novel strategy for targeting PCSCs could be represented by the inverse agonists of ERRγ, which decrease OXPHOS and mitochondrial activity and promote apoptosis[81]. Accordingly, it has been demonstrated that GSK5182 (an inverse agonist of ERRγ) determines the up-regulation of p21 and p27, promotes G1 phase arrest, and leads to ROS accumulation and pluripotency inhibition in iPS cells[82-84].

Nanoparticles for improved drug delivery

Surgery, chemotherapy, and radiotherapy are the most common anti-cancer therapeutic approaches; however, the non-specific targeting of cancer cells has made these approaches often non-effective with the consequence that higher doses of drugs need to be administered to reach the tumour region[85]. In order to

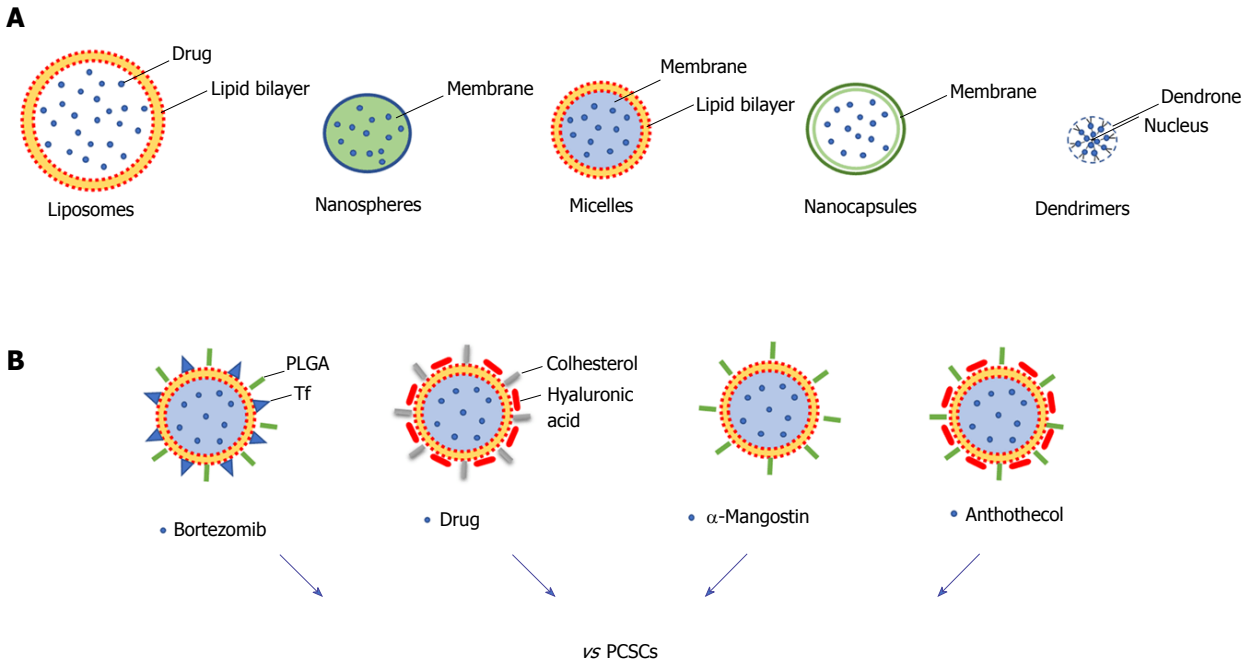

Figure 2 The types of nanoparticles. A: Different types of nanoparticles for targeted drug delivery; B: Specific nanoparticles for targeting pancreatic cancer stem cells. PCSCs: Pancreatic cancer stem cells.

improve the delivery of the drug, nanoparticles (NPs) have been developed to specifically and effectively target CSCs, reducing cytotoxicity and increasing the efficacy of treatments[86]. The different types of NPs include polymeric, magnetic, gold, and mesoporous silica NPs, and they provide a wide range of applications such as cancer therapy, tumour destruction through heating (hyperthermia), and drug/gene delivery[87,88]. In particular, for targeted drug delivery NPs comprise materials such as liposomes (100-400 nm), nanospheres (1-100 nm), micelles (10-100 nm), nanocapsules (10-1000 nm) and dendrimers (3-20 nm) (Figure 2A). These nanocarriers enhance the solubility and formulation of hydrophobic or water-insoluble drugs and control the drug delivery at the cancer tissue.

Some NPs have been developed to target pancreatic cancer, and liposomal formulations have gained regulatory approval[89]. The first clinical trial of NPs conducted in PDAC patients was done using a PEGylated colloidal gold-rhTNF nanomedicin, termed CYT-6091, which demonstrated that NPs greatly reduce the toxicity of chemotherapeutics and may target tumours[88]. In particular, some NPs have been developed to specifically target PCSCs (Figure 2B). PDAC is characterised by dense stroma with a high amount of hyaluronic acid (HA), which reduces drug delivery and interacts with CD44 surface marker regulating the invasion of PDAC cells. HA-based nanogel-drug conjugates with enhanced anticancer activity have been designed for the targeting of CD44-positive and drug-resistant tumours. These conjugates are based on membranotropic cholesteryl-HA (CHA) with various encapsulated drugs, such as the non-cancer related drug salinomycin, etoposide (a chemotherapeutic agent), or curcumin (a natural compound), and all have higher cytotoxicity in CD44-expressing drug-resistant PDAC cells compared to free drugs and to non-modified HA-drug conjugates[90]. Recently, HA-modified poly (dl-lactic-co-glycolic acid)-poly (ethylene glycol) (HA-PLGA-PEG) NPs have been developed for targeted delivery of TTQ (thio-tetrazolyl analogue of a clinical candidate, IC87114) to CD44 over-expressing cancer cells. *In vitro* results showed that cellular uptake led to higher cytotoxicity and enhanced intracellular accumulation of these NPs in high expressing CD44 MiaPaCa2 cells[91].

Natural product-based compounds can be an attractive strategy for the treatment of pancreatic cancer and could be integrated with NP approaches. For some of these, an inhibiting action against PCSCs has already been demonstrated (for example resveratrol, quercetin, and green tea catechins, and curcumin)[92], and for this reason they would deserve to be analysed as nanoparticle formulations. Among these natural compounds there are withaferin A (a major component of *Withania somnifora*) and carnosol (found in *Rosmarinus officinalis*, *Salvia carnosa*, and *Origanum vulgare*). They have suppressive effects on the proliferation, migration, and activation of c-Met in PCSCs[93]. A recent study investigated the role of α-mangostin (derived from the plant mangosteen) encapsulated NPs (Mang-NPs) in the inhibition of pancreatic carcinogenesis by targeting CSCs in human and transgenic mice. The data obtained indicated that Mang-NPs suppress PCSCs features (*i.e.*, EMT, cell proliferation, cell cycle, pluripotency, self-renewal, and apoptosis) and also target CSCs in mice[94]. A similar approach has been implemented for the investigation of the efficacy of anthothecol (an antimalarial compound) encapsulated by PLGA NPs (antho-NPs) against PCSCs.

Interestingly, it has been demonstrated that antho-NPs specifically inhibit PCSCs growth by modulating the SHH pathway[95].

Although significant progress has been made in the development of NPs, they are far from optimal. Indeed, there are already problems regarding the low drug loading capacity of some NPs. Liposomes are sometimes affected by drug diffusion through the liposome bilayer, and micellar drugs exhibit *in vivo* instability[90]. For these reasons, polymeric and nanogel drug conjugates, characterized by controlled drug release and higher drug loading capacity, provide a better strategy. Other challenges that must be addressed in the future for clinical use of NPs concern inefficient delivery, inherent toxicity, off-target effects, unfavourable biological distribution, and lack of clearance from the systemic circulation[96]. In conclusion, even if further research is needed for the development of efficient NPs, it is possible to speculate that the targeted delivery system for anti-cancer agents will be translated into clinical practice. It is tempting to imagine that in the near future modified NPs might serve as promising nanocarriers for site-specific drug delivery by targeting PCSCs and that protocol might be further improved for *in vivo* applications.

CONCLUSION

In conclusion, although further studies are needed, the new developments in targeting PCSCs are expected to have high impact in the treatment of PDAC in coming years. Nevertheless, some questions still need further investigation. While PCSCs represent an intriguing target for therapy, their complete characterisation is still needed. The identification of proteomic profiles and in particular of the deregulated pathways and proteins of PCSCs is fundamental to increasing our knowledge about pancreatic cancer and to identify new therapeutic approaches to eradicate PDAC stem cells that result in recurrence of the disease. Thus, enhanced biological knowledge of PCSCs, combined with the development of nanoparticle technology, promises to be key for the development of new effective treatments of pancreatic cancer.

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