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Review

Functions and clinical implications of exosomes in pancreatic cancer

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ABSTRACT

Pancreatic cancer is one of the most aggressive human malignancies and is associated with a dismal prognosis, which can be contributed to its atypical symptoms, metastatic propensity, and significant chemoresistance. Emerging evidence shows that pancreatic cancer cell-derived exosomes (PEXs) play critical roles in tumorigenesis and tumor development, as they are involved in drug resistance, immune evasion and metabolic reprograming, and distant metastasis of pancreatic cancer. Their numerous differentially expressed and functional contents make PEXs promising screening tools and therapeutic targets, which require further exploration. In this review, we focus on the functions of PEX contents and their clinical implications in pancreatic cancer.

1. Introduction

Pancreatic cancers, of which pancreatic ductal adenocarcinoma (PDAC) accounts for over 80%, are globally among the most lethal cancers [1]. The 5-year survival rate for pancreatic cancer patients was 7.8% between 2006 and 2012, in contrast to the 66.9% 5-year survival rate for cancers at all sites [2]. The high mortality rate is primarily attributed to its insidious onset, high metastatic potential, limited treatment options, and marked resistance to conventional therapies [3]. Surgical resection remains the only curative treatment option; however, only 20% of patients with pancreatic cancer are eligible for curative resection because patients are often diagnosed at an advanced stage because of the lack of early and clearly defined symptoms.

Exosomes, secreted by most cells, are small (30–100 nm) extracellular vesicles (EVs) that play a key role in intercellular communication [4–6]. Recently, research interest in cancer cell-derived exosomes has increased as they carry specific sets of proteins, nucleic acids, and lipids that reflect their source cells and that modulate the behavior of recipient cells and confer specific cell or tissue tropism [4,7]. Hence, exosomes represent an appealing source of diagnostic biomarkers and treatment targets for human cancers.

Pancreatic cancer cell-derived exosomes (PEXs) are important regulators of tumorigenesis and development of pancreatic cancer. PEXs facilitate tumor growth and metastasis through exosome-mediated intracellular communication [8–10]. However, in some studies, PEXs have been found to decrease tumor cell proliferation by inducing tumor cell apoptosis [11,12]. Further, it has been suggested that PEXs play roles in immune activation [13] and tolerance [14,15], metabolic disorders [16], and chemoresistance [17] in pancreatic cancer. Some PEX molecules are aberrantly expressed, making PEXs potential biomarkers for screening of cancer patients and predicting their prognosis. PEXs also provide multiple potential therapeutic targets as they contain numerous functional molecules. For example, PEX-CD151 enhances the metastatic ability of ASML cells, a rat pancreatic adenocarcinoma line, and thus is a potential metastasis-associated therapeutic target [18]. By modulating their composition, PEXs can be used to deliver certain molecules to specific cells to perform a certain function.

2. PEX biomolecules and pancreatic cancer

As mentioned above, PEXs contain numerous functional biomolecules, and their implications mostly depend on the intercellular communication established through the delivery of their contents. These biological molecules, including various proteins, a rich cargo of RNAs, mutant genomic DNAs, and structural lipids, are selectively enclosed into PEXs, and are involved in tumorigenesis, metastasis, immune evasion, and drug resistance in pancreatic cancer. In this section, we describe the known functions of PEXs on the basis of their contents.

2.1. PEX proteins and pancreatic cancer

In addition to proteins shared with other vesicles, PEXs carry cell

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type-specific proteins [19], which provide biomarker candidates and therapeutic targets in pancreatic cancer. One study identified 79 exosomal proteins that were differentially expressed between highly metastatic Panc01-H7 cells and weakly metastatic Panc01 cells [9]. Bioinformatics analysis indicated that most of these proteins were associated with tumor growth, invasion, metastasis, and metabolism-related signaling pathways through exosome-mediated intracellular communication. Further, Panc01 cells showed decreased adhesion and increased migration and invasion following the uptake of Panc01-H7-derived exosomes. Epidermal growth factor receptor (EGFR), a component of one of the main molecular pathways correlated with pancreatic tumorigenesis, is another example [20]. Apart from circulating in the serum (as a 110-kDa soluble form), EGRF can be incorporated into PEXs (named "PEX-EGRF"), either in a full-length intact receptor form (170 kDa) or in a processed fragment form (65 kDa) [21], which may indicate specific implications of using PEX-EGRF as a biomarker and therapeutic target in clinical practice. Additionally, some protein in PEXs have been identified as functional players, as they are implicated in tumor metastasis [10], glycometabolic disorders [22], and immune activation [13]. The biological functions of PEX proteins need to be further explored in future (See Figs. 1–3).

2.1.1. PEX proteins and pancreatic cancer metastasis

Most patients are diagnosed with advanced-stage pancreatic cancer, which is characterized by metastasis to 2–3 distant organs, resulting in a low curative surgical resection rate and poor prognosis [23]. Several PEX proteins have been reported to be involved in this process. Migration inhibition factor (MIF), a multipotent cytokine, is a well-known mediator of liver inflammation and fibrosis [24,25] that can be packaged into exosomes. Exosomal MIF (here referred to as "PEX-MIF") was elevated in both a mouse model of pancreatic cancer and PDAC patients and primes the liver for metastasis, which is driven by transforming growth factor β signaling, fibronectin deposition, and the recruitment of bone marrow-derived macrophages to the liver [10]. Notably, high PEX-MIF expression was detected in a mouse model with pre-tumoral pancreatic lesions and in patients with stage I PDAC, prior to liver metastasis, implying a promising prognostic value in PDAC. Tetraspanins, a family of 4-transmembrane proteins, are constitutive exosomal components that are engaged in exosome binding and uptake [26]. As members of tetraspanins, exosomal CD151 ("PEX-CD151") and Tspan8 ("PEX-Tspan8") have been described as metastasis-promoting proteins in several cancers [26–28]. One study provided strong evidence that both PEX-CD151 and -Tspan8 enhance the metastatic ability of rat ASML cells by comparing wild-type ASML, -CD151-knockdown, and -Tspan8-knockdown clones. PEX-CD151 and -Tspan8 were found to facilitate the degradation of EVs, which support tumor and host cell motility through their associations with integrins and proteases, to reprogram hematopoietic cells to take on an inflammatory phenotype, initiate protease- and chemokine-receptor expression in stroma cells, and contribute to epithelial–mesenchymal transition in non-metastatic tumor cells [18]. Another metastasis-related PEX protein is plectin ("PEX-plectin"), a cytosolic protein in normal physiology that is overexpressed on the surface of PDAC cells via exosome trafficking [29]. PEX-plectin has direct functional roles in the transition from pre-invasive to invasive disease and in the occurrence and development of primary and metastatic tumors by driving the proliferation, migration, and invasion of PDAC cells. Moreover, PEX-plectin plays an essential role in efficient exosome production and in modulating exosome content [29]. These findings indicate that PEX proteins are involved in the development of metastatic tumors in pancreatic cancer patients, and,

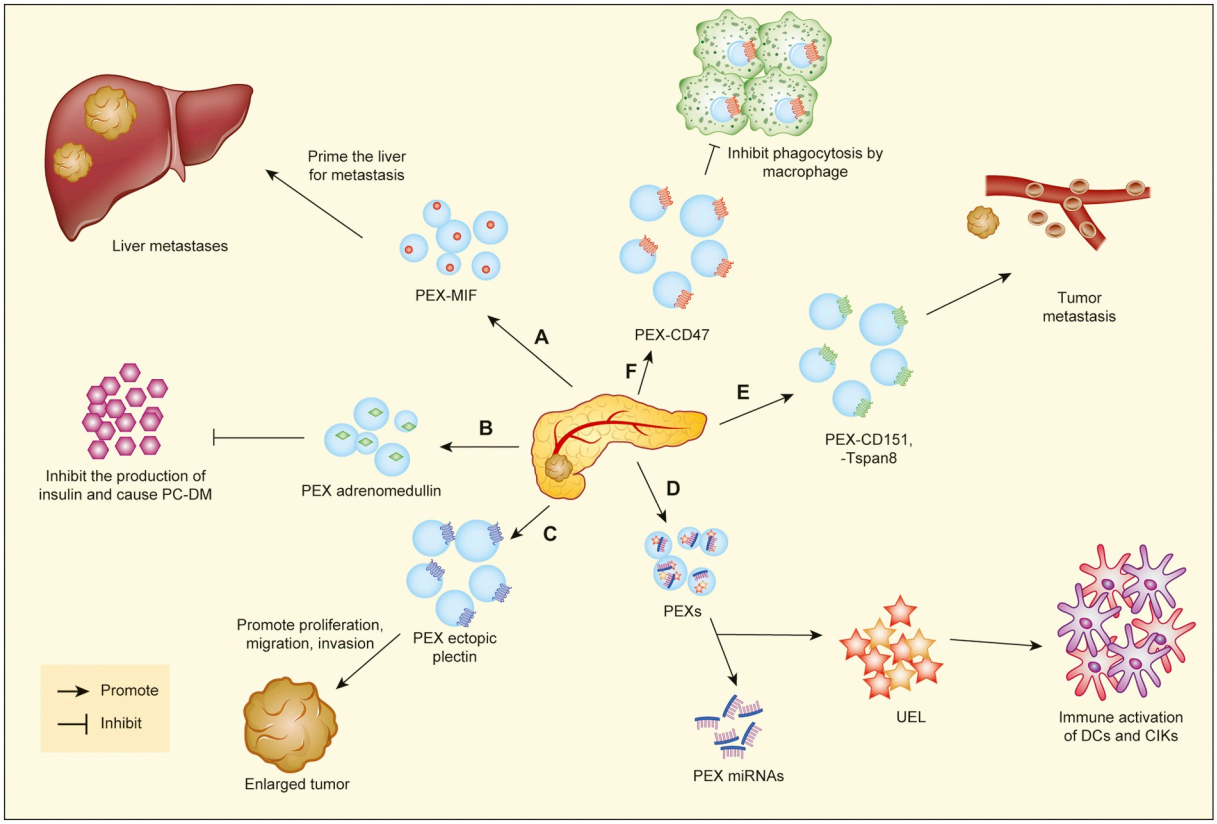

Fig. 1. Functions of PEX proteins.
(A) PEX-migration inhibition factor (MIF) primes the liver for metastasis. (B) PEX-adrenomedullin inhibits the production of insulin and causes pancreatic cancer-induced diabetes mellitus (PC-DM). (C) PEX ectopic plectin promotes the proliferation, migration, and invasion of pancreatic cancer cells. (D) Ultrafiltered exosome lysate (UEL), miRNA-depleted exosomal proteins, activates immune cells, including dendritic cells (DCs) and cytokine-induced killer cells (CIKs). (E) PEX-CD151 and -Tspan8 promote metastasis of pancreatic tumor cells. (F) PEX-CD47 protects PEXs from phagocytosis by immune cells, such as macrophages.

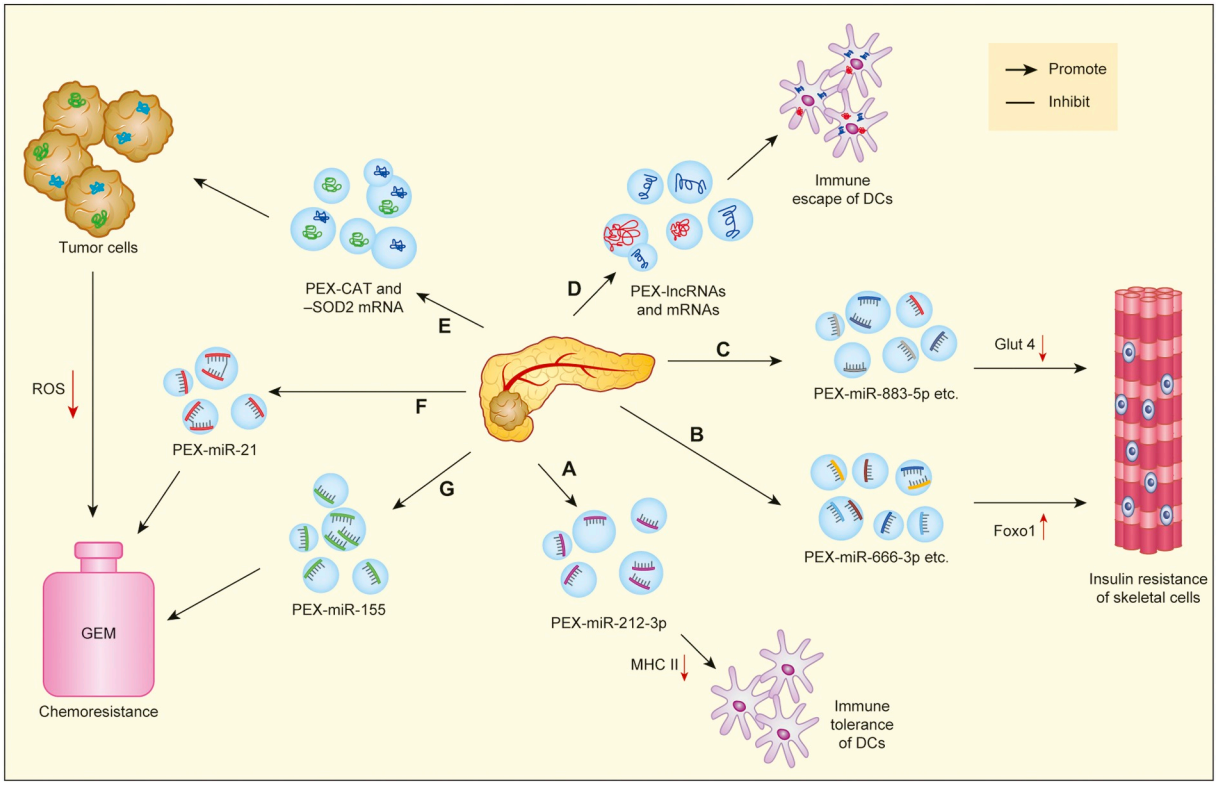

Fig. 2. Functions of PEX nucleic acids.
(A) PEX-miR-212-3p leads to immune tolerance of dendritic cells (DCs). (B—C) PEX-miRNAs, such as PEX-miR-666-3p and PEX-miR-883-5p, cause insulin resistance of skeletal muscle cells and even pancreatic cancer-induced diabetes mellitus (PC-DM). (D) PEX-lncRNAs and mRNAs are involved in immune escape of DCs. (E–G) PEX-mRNAs (CAT and SOD2 mRNA) and PEX-miRNAs (PEX-miR-21 and PEX-miR-155) are implicated in chemoresistance of pancreatic cancer.

upon confirmation, the functional proteins may serve as therapeutic targets in the near future.

2.1.2. PEX proteins and glycometabolic disorder in pancreatic cancer

The relationship between diabetes mellitus (DM) and pancreatic cancer is complex, but hyperglycemia and DM can occur as a consequence of pancreatic cancer [30]. Nearly 85% of pancreatic cancer patients have hyperglycemia, and the majority (45%–67%) have DM, which is frequently new-onset (75%) [31]. Although pancreatic cancer-induced DM (PC-DM) shares various features with type 2 DM, PC-DM often develops into ongoing, profound weight loss and lacks amyloid deposits in islets [32,33]. Given that DM precedes the diagnosis of PDAC by a few weeks to 2–3 years [30,34], PC-DM has been suggested as a potential platform for early diagnosis of pancreatic cancer.

Adrenomedullin (AM) is the most highly expressed protein in pancreatic cancer cell lines grown under low-glucose and low-oxygen condition, and AM present in PEXs (here referred to as “PEX-AM”) is involved in the onset of DM in pancreatic cancer [22]. Mechanistically, AM-containing PEXs enter β-cells through caveolin-mediated endocytosis or macropinocytosis. PEX-AM then interacts with adrenomedullin receptor on β-cells and increases β-cell dysfunction and death, possibly through AM-induced endoplasmic reticular stress and failure of the unfolded protein response. As a result, insulin secretion is inhibited, triggering the onset of PC-DM. Another study corroborated the important role of PEX-AM in the development of PC-DM [16]. The authors observed increased lipolysis in murine and human adipocytes upon exposure to PEXs, which could be attributed to PEX-AM, as adrenomedullin receptor blockade abrogated the effects of PEXs and activated the ERK1/2 and p38 MAPK pathways in subcutaneous adipocytes [16]. Hence, the authors suggested that the sudden weight loss observed in pancreatic cancer, which precedes the initiation of cancer-related symptoms, is due to adipose tissue lipolysis stimulated by PEX-AM.

2.1.3. PEX proteins and immune activation in pancreatic cancer

Tumor-derived exosomes are considered to be promising immune agonists because they carry numerous immune-regulating proteins [35]. Further, they contain many microRNAs (miRNAs), which can induce immune tolerance [14,15], thus impairing the immunocompetence of exosomes. Therefore, it has been reasoned that ultrafiltered exosome lysates (UEL), miRNA-depleted exosomal proteins prepared by lysis and ultrafiltration [13], can boost the immune activity of exosomes in activating immune cells against pancreatic cancer. Indeed, it has been shown that dendritic cells/cytokine-induced killer cells (DCs/CIKs) stimulated with UEL display a stronger killing activity than lipopolysaccharide- or PEX-stimulated DCs/CIKs, demonstrating that UEL can enhance the specific anti-tumor cytotoxicity of DCs/CIKs [13]. These discoveries confirmed that it is feasible to activate DCs and CIKs by stimulation with UEL, and exosomal proteins might be tumor vaccine candidates.

2.2. PEX nucleic acids and pancreatic cancer

In addition to the plethora of proteins selectively imported in PEXs and having their own functions in pancreatic cancer, PEXs contain plenty of nucleic acids, including miRNAs, messenger RNAs (mRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and some mutant genomic DNA fragments. Among these, miRNAs are the best studied and participate in chemoresistance, metabolic crosstalk, and immune tolerance in pancreatic cancer, whereas other nucleic acids have their own clinical significance (See Fig. 2).

2.2.1. PEX miRNAs

MiRNAs are highly conserved, small, non-coding RNAs that have crucial roles in numerous human diseases, including cancers [36]. MiRNAs can target conserved sites in the 3′ untranslated regions (3′

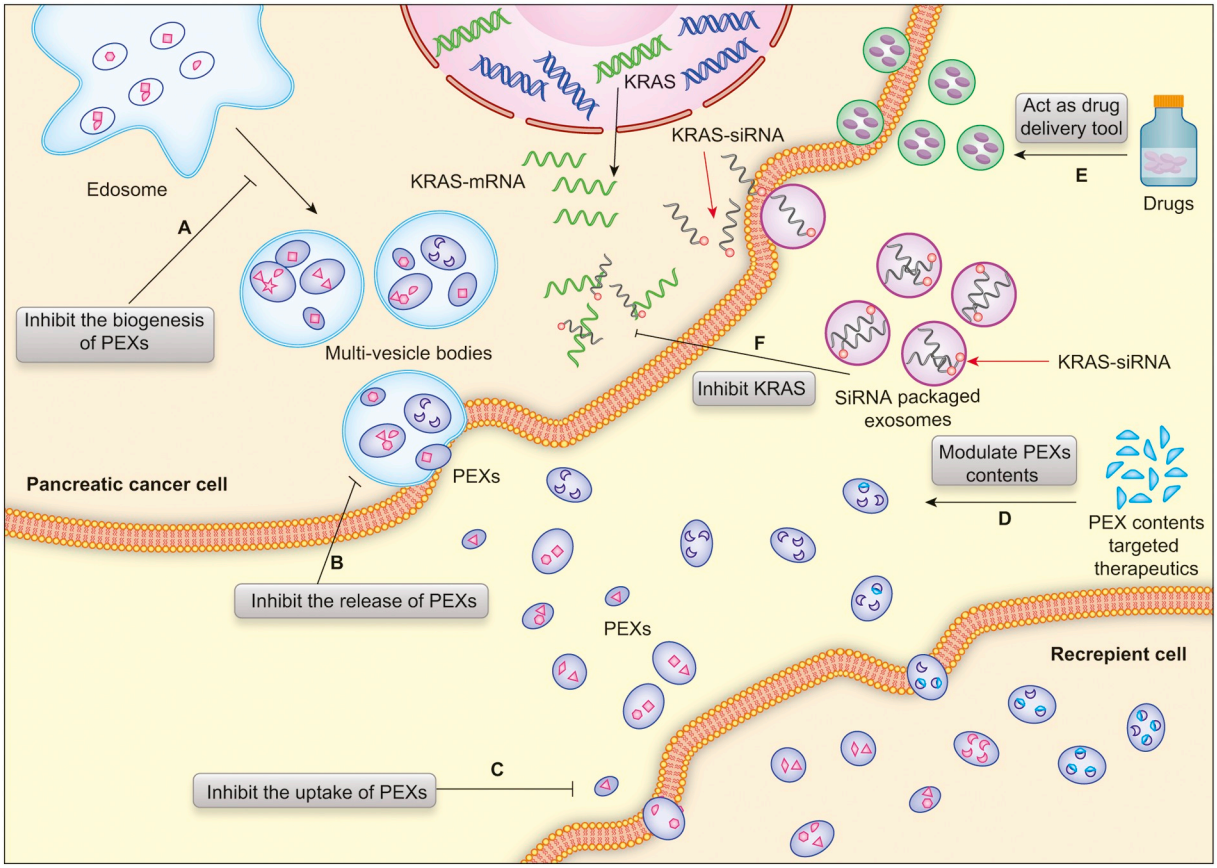

Fig. 3. Therapeutic implications of exosomes in pancreatic cancer, especially as carriers of siRNA to target KRAS mutation.
(A) Inhibition of PEX biogenesis. (B) Inhibition of PEX release. (C) Inhibition of PEX uptake. D) Modulation of PEXs contents. E) Drug delivery vehicle transferring, e.g., specific siRNA to inhibit KRAS mutation in pancreatic tumor cells. (F) Modulation of PEXs contents to abrogate their functions.

UTRs) of 5–100 mRNAs, affecting multiple cellular pathways either by repressing target mRNA translation or by mediating mRNA silencing. MiRNAs are potential diagnostic biomarkers given their marked stability and differential expression in patients. Most importantly, there is evidence that certain miRNAs can be selectively packaged into exosomes [3]. For example, the abundance of miR-10b in circulating PEXs has been quantified by using a novel, simple, label-free technique [37]. MiR-1290 and miR-1246 are also abundantly present in PEXs [38]. Exosomes protect miRNAs from degradation by RNA enzymes in body fluid and transport them to recipient cells, where they play key roles in the pathology of various diseases, including pancreatic cancer, by regulating gene expression and signal transduction. Below, we summarize known PEX-miRNA functions in pancreatic cancer.

2.2.1.1. PEX miRNAs and chemoresistance in pancreatic cancer. Chemoresistance, which is responsible for the poor prognosis of pancreatic cancer, poses a significant clinical problem. For example, gemcitabine (GEM), a key chemotherapy drug that is used either alone or in combination with other drugs depending on the health status of the patient, can extend the overall survival (OS) of pancreatic cancer patients only by approximately 6 months in most cases, as the majority of patients develop resistance to GEM after prolonged exposure [39]. Recently, several studies uncovered that this phenomenon is, at least in part, related to PEX miRNAs. For example, PEX-miR-21 is highly expressed in and has been identified as a chemoresistance marker of pancreatic cancer [17]. PEX-miR-155, another upregulated exosomal miRNA, has been found to be associated with GEM chemoresistance in pancreatic cancer patients. One study reported that PEX-miR-155 elicits resistance to GEM through a positive feedback process [40]. Specifically, long-term GEM treatment increases the secretion of miR-155, which, on the one hand, leads to GEM resistance via an anti-apoptotic pathway, and on the other hand, induces the release of exosomes. These exosomes in turn deliver chemoresistance-related substances, including miR-155, to other cancer cells, finally resulting in resistance to GEM chemotherapy in patients. Interestingly, exosomes have been identified as the major EVs in GEM-associated chemoresistance in pancreatic cancer [41]. Moreover, PEX-miR-155 can induce GEM chemoresistance by directly targeting the 3′ UTR of the deoxycytidine kinase transcript, thus downregulating the expression of this GEM-metabolizing enzyme [41].

2.2.1.2. PEX miRNAs and metabolic reprogramming in pancreatic cancer. As mentioned above, PC-DM, the main pathological component of which is insulin resistance of skeletal muscle, is a promising candidate for early diagnosis of pancreatic cancer [34], and PEX-AM is involved in the biogenesis of PC-DM. Intriguingly, a recent study indicated that PEX-miRNAs probably contribute to the onset of PC-DM as well, as suggested by their potential involvement in the metabolic crosstalk between cancer and peripheral tissues [42]. Mechanistically, murine PEXs can enter C2C12 myotubes and suppress insulin and PIK3/Akt signaling, thus maintaining insulin-induced Foxo1 nuclear exclusion and impairing glucose transporter 4 protein (Glut4) trafficking. As a consequence, glucose intake is inhibited and lipidosis of skeletal muscle cells is promoted, finally resulting in the development of insulin resistance and even PC-DM [42]. A miRNA microarray analysis showed that miR-666-3p, miR-540-3p, miR-125b-5p, and miR-450b-3p potentially facilitate Foxo1 expression, whereas miR-883b-5p, miR-666-3p, miR-450b-3p, and miR-151-3p may play essential roles in the downregulation of Glut4 expression [42]. In brief, this study demonstrated the roles of PEX-miRNAs in metabolic reprogramming of pancreatic cancer through stimulating insulin

resistance of skeletal muscle cells via the PI3K/Akt/Foxo1 signaling pathway. Furthermore, PC-DM patients have recently been shown to have strikingly lower expression of glucose-dependent insulinotropic peptide (GLP) and glucagon-like peptide-1 (GLP-1) [43]. These incretins, secreted mainly by enteroendocrine cells, are important for maintaining glucose homeostasis and are predicted to cause DM, including PC-DM [43–45]. However, PEXs produced by MIA PaCa-2 pancreatic cancer cells were reported to impair GLP and GLP-1 production in STC-1 mouse enteroendocrine cells [46]. The authors suggested that this result is mediated, at least in part, by four specific miRNAs, miR-6796-3p, miR-6763-5p, miR-4750-3p, and miR-197-3p, through the suppression of PCSK1/3 function in post-translational processing of Gip and proglucagon [46], which corroborates the involvement of PEX miRNAs in PC-DM.

2.2.1.3. PEX miRNAs and immune tolerance in pancreatic cancer. Tumor exosomal miRNAs reportedly are involved in tumor invasion and metastasis through immunological tolerance induction by inhibiting mRNA expression in recipient cells [47,48]. In pancreatic cancer, PEX-miR-203 inhibits the expression of toll-like receptor 4 in DCs, thereby inducing immune tolerance [15]. Based on these findings, one group showed that 12 miRNAs were increased more than 2-fold in PEX-stimulated compared to immature DCs, among which 9 miRNAs were also highly expressed in PEXs, indicating that miRNAs can be transferred from PEXs to DCs [14]. In addition, the expression of 208 mRNAs was inhibited by more than 4-fold in PEX-stimulated compared to immature DCs, and these mRNAs were considered as candidate targets of exosomal miRNAs. Bioinformatics analysis further suggested that regulatory factor X-associated protein, an important transcription factor for major histocompatibility complex II (MHC II) that influences immunity by activating CD4 + T-lymphocytes [47], is inhibited by PEX-miR-212-3p [14]. As a consequence, MHC II expression was reduced, which led to tolerance induction in pancreatic cancer DCs. These studies demonstrated that PEX miRNAs can affect the immune status of pancreatic cancer patients, which needs further investigation.

2.2.1.4. PEX miRNAs and metastasis of pancreatic cancer under hypoxia. The tumor microenvironment (TME), which is characterized by hypoxia and the infiltration of inflammatory cells, especially macrophages, plays crucial roles in tumor metastasis [49,50]. Considering that exosomes are important factors in the cross-talk between tumor cells and the microenvironment, one research team recently suggested that PEXs produced under hypoxia (“hypoxia PEXs”) activate the M2 polarization of macrophages, which leads to the progression of human pancreatic cancer [51]. Mechanistically, the authors demonstrated that miR-301a-3p, a miRNA upregulated in hypoxic pancreatic cancer cells as well as in hypoxia PEXs, induces macrophages to take on the M2 phenotype through the PTEN/PI3Kγ pathway, thus promoting cancer cell migration, invasion, and epithelial–mesenchymal transition in vitro, as well as lung metastasis in vivo. Moreover, hypoxia PEX miR-301a-3p has prognostic significance, as it is positively associated with invasion depth, lymph node metastasis, late TNM stage, and poor patient outcome [51].

2.2.2. PEX long RNAs

Although miRNAs in exosomes are comparatively well characterized and studied, the small quantity and lack of specific expression of miRNAs limit their extensive application. Functional long RNAs, such as mRNAs, lncRNAs, and circRNAs, also exist in cancer-derived exosomes [52]. LncRNA and mRNA expression is modulated in DCs upon treatment with PEXs [53]. This study revealed that mRNAs such as lgmn and lncRNAs such as ENST00000560647 may play critical roles in immune escape of PEX-treated DCs. In other words, PEXs can regulate the immune status of pancreatic cancer patients by affecting the lncRNA and mRNA expression profile of DCs. Other examples of exosomal-mediated lateral transfer of long RNAs are the catalase (CAT) and superoxide dismutase (SOD2) transcripts, both of which encode reactive oxygen species (ROS)-detoxifying enzymes. Induction of ROS generation is suggested to be one of the important mechanisms underlying the cytotoxic efficacy of chemotherapeutic drugs [54]. Cancer cells try to counter the abrupt increase in ROS by altering the expression of ROS-detoxifying enzymes. CAT and SOD2 mRNAs, after exosome-mediated transfer to cancer cells, are partly implicated in pancreatic cancer chemoresistance by suppressing basal and GEM-induced ROS production [41]. Importantly, this study also indicated that, among all EVs, exosomes are the only subtype that is involved in pancreatic cancer chemoresistance.

CircRNAs, including circ-IARS [55] and circ-PDE8A [56], have been detected in PEXs and have been suggested to have certain roles in pancreatic cancer. PEX-derived circ-IARS is upregulated in PDAC and can enter human microvascular vein endothelial cells and enhance endothelial monolayer permeability through the miR-122/RhoA/ZO-1 or the F-actin pathway, thus promoting PDAC invasion and metastasis [55]. Circ-PDE8A, which is also highly expressed in PEXs in PDAC, is correlated with PDAC progression, including duodenal invasion, vascular invasion, and TNM stage, via the miR-338/MACC1/MET/AKT or the ERK pathway, and is implied in the poor prognosis of PDAC patients [56]. In conclusion, long RNAs present in PEXs may be important factors in pancreatic cancer that are worth more research attention.

Recently, we published a database of exosomal long RNAs (termed “exoRBase”) containing RNA-sequencing data of human blood exosomes and experimentally validated published papers [52]. This database provides the annotation, expression level, and possible source tissues of long RNAs, which is helpful to identify specific molecular signatures of blood exosomes. As for pancreatic cancer, numerous PEX-associated long RNAs have been discovered, including 17,427 mRNAs, 11,376 lncRNAs, and 19,540 circRNAs, enabling further exploration of new exosomal biomarkers as well as their functional implications through customized browsing options in exoRBase.

2.2.3. PEX DNAs

Genomic DNA can be incorporated into exosomes, as exemplified by the double-stranded DNA fragments of 10 kb or longer that have been detected in PEXs. In addition to the facts that exosomal DNA originates from live cells and is protected from degradation in the circulation and only a low amount of serum is needed for detection, exosomal DNA may offer another advantage over cell-free DNA in that the larger fragments are more suitable for PCR-based detection of mutations [57,58]. In pancreatic cancer, KRAS and p53 mutations can be detected in PEX-derived DNA fragments. It is noteworthy that serum PEXs contain genomic DNA covering all chromosomes, implying the diagnostic and therapeutic values of PEXs for genomic DNA mutation detection [57]. Based on this study, a proof-of-concept study highlighted the value of PEX DNA for rapid, low-cost identification of cancer-driving mutations [59]. PEX DNA allows more sensitive detection of mutant *KRAS* than circulating DNA in local and metastatic PDAC [58]. Longitudinal monitoring of PEX DNA yielded exclusive information that allowed anticipating the outcome of neoadjuvant therapy in localized pancreatic cancer and disease progression in metastatic patients, implicating that PEX DNA may also be used as prognostic biomarker [58]. Notably, the detection of driver mutations in PEXs may not signify the presence of pancreatic cancer, as exosomal KRAS and p53 mutations can also be detected in intraductal papillary mucinous neoplasm and in healthy subjects [57–59].

2.3. PEX lipids

Lipids are essential components of the exosomal membrane, and specific lipids are enriched in exosomes compared to their source cells. Several studies have focused on the function of PEX lipids in pancreatic cancer. Based on the lipid composition of efficient exosomes, Beloribi-Djefaflia et al. designed synthetic exosome-like nanoparticles (SELNs)

in which the ratio of ordered to disordered lipids ranged from 3 to 6 (termed “SELN 3.0” and “SELN6.0”, respectively). They discovered that SELNs decrease the survival rate of human SOJ-6 pancreatic cancer cells by inducing cell death through inhibition of the Notch pathway [60]. Furthermore, the higher the ordered-to-disordered lipid ratio was, the lower the survival rate, which demonstrates the role of PEX lipids in evoking apoptosis of pancreatic tumor cells [61]. However, MIA PaCa-2 cells are resistant to SELN 6.0 because it decreases the expression of Notch intracytoplasmic domain in this cell line [60]. Mechanistically, SELN 6.0 induces the activation of nuclear factor (NF)-κB, which facilitates the expression and release of SDF-1α. SDF-1α then binds to its receptor CXCR4, activating the Akt signaling pathway to protect tumor cells from death. In conclusion, PEX lipids affect the development of pancreatic cancer, and may thus be a promising target for therapy.

In summary, PEXs do have a great influence on pancreatic cancer as they contain various functional molecules. They can affect tumor progression, invasion, and metastasis; modulate cancer immune; disturb glucose metabolism; and cause drug resistance. Notably, even though the majority of PEX-related studies suggest PEXs to be tumor promoters, several reports claimed that PEXs are tumor suppressors. Some studies revealed that PEXs suppress tumor cell proliferation by inducing tumor cell apoptosis via activation of the mitochondria-dependent apoptotic pathway and inhibition of the Notch-1 survival pathway [11,12]. This discrepancy might be attributed to the complexity of PEXs contents, differences between pancreatic cancer cell lines, and tissue-specific PEX functions, and warrants further investigation.

3. Non-pancreatic cancer cell-derived exosomes and pancreatic cancer

A main feature of the TME of pancreatic cancer is substantial desmoplasia in the tumor stroma. Desmoplasia is attributed to several cell types in the tumor stroma, including cancer-associated fibroblasts (CAFs) and pancreatic stellate cells (PSCs), which might play vital roles in the aggressiveness of pancreatic cancer [62]. Exosomes originating from stromal cells reportedly have active roles in regulating pancreatic cancer cell survival and proliferation. For example, miRNA trafficking and exchange between tumor stromal cells and pancreatic cancer cells via exosomes has been demonstrated [63]. The authors suggested that stroma-specific miRNAs, such as miR-145, can be packaged into stromal cell-derived exosomes and then delivered to adjacent pancreatic cancer cells, eventually suppressing pancreatic cancer by inducing apoptosis. In addition, exosomes stemming from cancer-initiating cells (CICs) are implicated in the development of pancreatic cancer [64].

3.1. CAF-derived exosomes

CAFs, the major component of stroma, are innately resistant to GEM and are associated with the regulation of chemoresistance in pancreatic cancer [65]. GEM-treated CAFs produce ample exosomes that contain chemoresistance-promoting factors, including mRNAs and corresponding miRNAs [65]. CAF-derived exosomes transfer these factors to recipient epithelial cells, supporting their growth and survival during GEM treatment. For example, the production of Snail (a chemoresistance-inducing factor) as well as its downstream target miR-146a is increased after GEM treatment, which promotes cancer cell proliferation and induces drug resistance. However, these effects can be repressed by treatment with GW4689, an exosome release inhibitor, demonstrating that exosomes from stromal CAFs contribute to chemoresistance in pancreatic cancer [65]. CAF-derived exosomes are also implicated in metabolic reprogramming of pancreatic cancer cells. Specifically, these exosomes can inhibit mitochondrial oxidative metabolism, promote glycolysis and glutamine-dependent reductive carboxylation, and facilitate tumor growth by smuggling metabolites into cancer cells [66].

3.2. PSC-derived exosomes and pancreatic cancer

PSCs have a great influence on the TME as they produce extracellular matrix components and interact with other types of cells, including pancreatic cancer cells [67]. Exosomes from PSCs stimulate the proliferation, migration, and chemokine gene expression of pancreatic cancer cells, which can be suppressed by GW4869 as well [67]. In addition, a variety of miRNAs, such as miR-21-5p, are incorporated into PSC-derived exosomes, and several miRNAs are selectively enriched in these exosomes as compared to their source cells. Interestingly, PEXs can in turn induce the activation and profibrogenic activities of PSCs [38]. Specifically, they facilitate the proliferation and migration of PSCs; upregulate the expression of cell activation-related and fibrosis-related genes in PSCs; and increase the expression of miR-1246 and miR-1290 in PSCs. In conclusion, exosomes might play a role in TME development by mediating the interactions between PSCs and pancreatic cancer cells.

3.3. CIC-derived exosomes and pancreatic cancer

CICs are closely correlated to metastasis, in which CIC-derived exosomes play a dominant role as they transfer CIC features into non-CICs [64]. The CD44 splice variant CD44v6 is a metastogen and a CIC marker of several cancers, including pancreatic cancer [64,68]. It can be imported into tumor-derived exosomes and has a great impact on tumors. CD44v6-enriched CIC-derived exosomes facilitate tumor progression by promoting the motility, invasion, anchorage-independent growth, and apoptosis resistance of pancreatic cancer cells in cooperation with integrin and proteases and upregulation of other CIC markers, especially, Tspan8 [64]. CD44v6 also is a key component in the formation of a premetastatic niche in pancreatic cancer [68]. This study showed that CD44v6 promotes the assembly of a tumor-derived soluble fraction with PEXs, thereby activating leukocytes, stroma, and endothelial cells in the metastatic organ.

4. Exosomes in other body fluids and pancreatic cancer

Exosomes can be produced and secreted by nearly all cells, which implies they exist in various body fluids. Although most exosome-related studies focused on plasmatic or serous exosomes, exosomes can also be collected from other fluids, including saliva [69], urine [70], breast milk [71], bronchoalveolar lavage fluid [72], and malignant effusions [73]. Considering that the collection of biofluids is non-invasive and easy, and exosomes contain a rich variety of materials, using these body fluids for early detection of systemic diseases is practical. For example, discriminatory salivary biomarkers with high specificity and sensitivity can be readily detected upon the onset of various diseases, including pancreatic cancer [74]. One research team proposed and validated the relationship between PEXs and discriminatory salivary biomarkers in the development of pancreatic cancer in mouse models [69]. Seven genes were significantly upregulated in the saliva of pancreatic cancer-bearing mice in comparison with non-cancer control animals, and they were also detected in exosomes derived from saliva, serum, and panc02 cells. In addition, transcriptome linearity does not exist between saliva supernatant and serum nor between serum and serum-derived exosomes, but it does exist between serum- and saliva-derived exosomes and between saliva and saliva-derived exosomes [69], partially explaining the difficulties in developing serum biomarkers for disease detection and supporting the utilization of saliva for cancer-specific biomarkers. More efforts are needed to clarify how PEXs relay discriminatory biomarkers from the pancreas to the distal oral cavity—whether they are merely direct transportation vehicles or also messengers that modulate the transcriptome of the salivary glands.

5. Clinical implications of exosomes in pancreatic cancer

Insights into PEXs and their functions pave new ways in the fight against pancreatic cancer, as PEXs greatly affect the diagnosis and treatment of this challenging disease through their content-based functions. In addition, exosomes are considered to be potent drug delivery vehicles.

5.1. PEXs and pancreatic cancer diagnosis

The insidious onset and lack of specific clinical manifestations of pancreatic cancers contribute to the fact that most patients are diagnosed in the advanced stage [3]. However, powerful diagnostic technologies and sensitive and specific molecular biomarkers are currently lacking. Conventional imageological examinations, such as CT scans and endoscopic retrograde cholangiopancreatography, are expensive and/or have potential risks [7]. CA19–9 is the only approved serum biomarker for pancreatic cancer; however, it lacks both sensitivity and specificity: approximately 14% of the general population do not express it, 25% of patients do not exhibit a rise in it, but patients with chronic pancreatitis (CP) and hyperbilirubinemia have increased CA19–9 levels [75].

Given the lack of ideal clinical biomarkers, significant interest has been shown in using PEXs as screening tools, as they offer several advantages over other detection tools. First, pancreatic cancer cells mainly produce PEXs rather than other EVs [31]; second, PEXs can be non-invasively collected from diverse body fluids and can be re-collected over time for monitoring [6]; third, PEXs are stable and their contents are protected from degradation by external nucleases and proteases [5]; fourth, in comparison with conventional biopsy, PEX molecules can be easily detected by sensitive technologies, including RT-PCR and next-generation sequencing [7]. However, the development of a standard isolation procedure to obtain sufficient exosomes of high purity is a primary challenge [7].

The aberrantly expressed biomolecules make PEXs promising for diagnostic purposes (See Table 1). For example, glypican-1 (GPC1), a membrane-anchored protein overexpressed in several tumor types [76], is re-expressed in pancreatic cancer patients through hypomethylation of its promoter [77]. In a study by Melo et al., $GPC1^+$ circulating PEXs were detected in all 190 PDAC patients, with 100% sensitivity and 100% specificity, and from early stages, indicating that it might be a potent early screening biomarker in pancreatic cancer [78]. In 2017, Yang et al. established a signature comprising 5 EV-based protein markers (EGRF, EPCAM, MUC1, GPC1, and WNT2) that provided higher sensitivity (86%) and specificity (81%) than the existing serum marker CA 19–9 or any single EV marker [79]. For example, GPC1 alone has a sensitivity of 82% and specificity of 52%, which is inconsistent with the findings of Melo et al. The discrepancy might be explained by the different GPC1 antibodies and vesicle isolation methods used in the two studies. In addition, zinc transporter protein (ZIP4), an exosomal protein derived from PC-1.0 cells (a highly malignant pancreatic cancer cell line), was identified as a novel diagnostic biomarker for pancreatic cancer as it efficaciously distinguished pancreatic cancer from benign pancreatic disease, biliary disease, and normal controls [80]. ZIP4 was also reported to promote PC-1 cancer cell (a less malignant cell type) progression both in vitro and in vivo, mainly through enhancing tumor growth.

Numerous PEX miRNAs have been shown to have potential diagnostic value as well. For instance, miR-196a and miR-1246 reportedly are highly enriched in PEXs and might be potential biomarkers for early detection of pancreatic cancer. Furthermore, PEX-miR-196a is a better indicator of PDAC, whereas PEX-miR-1246 is more suitable for intraductal papillary mucinous neoplasms (IPMN) [2]. High PEX-miR-10b, −miR-21, −miR-30c, and -miR-181a, and low PEX-miR-let7a levels differentiate PDAC from normal control and CP samples [3]. Most importantly, the authors confirmed that certain elevated PEX miRNAs decrease to normal values within 24 h following PDAC resection, implying that miRNA-rich PEXs are superior to PEX-GPC1 in terms of diagnostic ability. More PEX miRNAs might be utilized in pancreatic cancer screening. For instance, MiR-23b-3p, another miRNA upregulated in PEXs, promotes the proliferation, invasion, and migration of pancreatic cancer and is positively correlated to serum CA19–9 [81]. PEX-miR-191, −miR-21, and -miR-451a are also significantly overexpressed in pancreatic cancer and IPMN, and have 5%–20% higher diagnostic accuracy than their corresponding circulating miRNAs [17]. In addition, PEX-miR-451a is associated with mural nodules in IPMN. Moreover, miR-17-5p and miR-21 are upregulated in PEXs as well, and differentiate pancreatic cancer from CP and benign pancreatic tumors with high sensitivity and specificity [82].

5.2. PEXs and pancreatic cancer treatment and prognosis

Although research on PEXs is in its infancy, it indicates that PEXs could be a crucial therapeutic target for pancreatic cancer, as they possess multiple desirable qualities (See Fig. 3). PEXs contain numerous functional materials and can transfer them to tumor cells, which means that medical drugs blocking the production, release, or uptake of exosomes may have therapeutic potential. PEXs are also quite stable and thus can be stored long-term and are suitable for industrial production. Moreover, PEXs may serve as “mini-antigen presenting cells,” with potential application as novel immunotherapeutic agents [35]. Hence, PEXs are regarded as promising immune agonists and have progressed to clinical trials.

PEXs can be affected by certain molecules; thus, drugs targeting these molecules may prolong patient survival. For instance, GAIP-interacting protein C terminus (GIPC), a master regulator that blocks the autophagy of tumor cells, was found to regulate cellular trafficking pathways by affecting the biogenesis, secretion, and molecular composition of PEXs [83]. GIPC depletion stimulates the incorporation of ABCG2, a drug resistance protein, into PEXs. This turns ABCG2 inaccessible and non-functional, and thus, tumor cells remain sensitive to GEM. Obviously, GIPC is a potential therapeutic target for overcoming chemoresistance in pancreatic cancer. Other examples are cellular vesicle-trafficking proteins, such as RAB27A and TP53. Given that the exosome secretion pathway plays an important role in modulating the microenvironment and in invasive growth of tumor tissues, RAB27A is suggested to be associated with the aggressive behavior of pancreatic cancer and may be targeted in clinical therapy [84,85].

A few studies have reported the prognostic value of PEX components in pancreatic cancer (See Table 1). For example, PEX-GPC1 is correlated with tumor burden and survival in patients pre- and post-operation, suggesting its role as a prognostic biomarker in pancreatic cancer [78]. PEX-miR-17-5p predicts poor prognosis for pancreatic cancer patients because it is implicated in the inflammatory response or tumor immune escape [82]. MiR-21 is another PEX miRNA that has been identified as a candidate prognostic factor for overall survival in pancreatic cancer [17]. MiR-301a-3p selectively packaged into PEXs under hypoxia is positively correlated with depth of invasion, lymph node metastasis, and late TNM stage, and therefore, is a prognostic molecule in pancreatic cancer [51]. In addition, mice with pre-tumoral pancreatic lesions and in patients with stage I PDAC highly express PEX-MIF, suggesting that PEX-MIF is detectable prior to liver metastasis and thus, might have prognostic value. PEX DNA may also have prognostic implications. For example, longitudinal monitoring of PEX mutant KRAS allows prediction of the outcome of neoadjuvant therapy in patients with localized pancreatic cancer and of progression in patients with metastasis [58]. Clearly, the prognostic value of PEX contents deserves further study.

5.3. Exosomes as drug delivery vehicles

Compared to conventional synthetic nanoparticles, exosomes have

Table 1
Diagnostic and prognostic biomarkers in PEXs.

Diagnostic biomarkers in PEXs				
Classification		Sensitivity/specificity	Diagnostic value	Ref
Proteins	GPC1	100%/100%	Diagnose early pancreatic cancer	[78]
	Signature (EGRF, EPCAM, MUC1, GPC1, WNT2)	86%/81%	Diagnose early pancreatic cancer	[79]
	ZIP4	NM	Diagnose pancreatic cancer	[80]
MiRNAs	miR-196a	NM	Diagnose localized[a] PDAC	[2]
	miR-1246	NM	Diagnose localized IPMN	[2]
	Signature (miR-10b, −21, −30c, −181a; miR-let7a[b])	100%/100%	Diagnose PDAC (decrease to normal values within 24 h after resection)	[3]
	miR-23b-3p	NM	Positively correlated to CA19–9	[81]
	miR-191, −21, −451a	5%–20% more accurate than circulating markers	Diagnose pancreatic cancer and IPMN	[17]
	miR-17-5p	72.7%/92.6%	Diagnose pancreatic cancer	[82]
	miR-21	95.5% / 81.5%		
	Signature (miR-1246, −4644, −3976, −4306; CD44v6, Tspan8, EpCAM, MET, CD104)	100% / 80%	Diagnose pancreatic cancer	[93]
DNAs	Mutant KRAS	Positive in 39,6% PDAC and 28.6% IPMN	Detect cancer-driving mutations	[59]
	Mutant TP53	Positive in 39,6% PDAC and 28.6% IPMN		
Prognostic biomarkers in PEXs				
Classification		Prognostic values Ref		
Proteins	GPC1	Tumor burden; survival in patients pre- and post-operation		[78]
	MIF	Pretumoral lesion and stage I PDAC (prior to liver metastasis)		[10]
MiRNAs	miR-17-5p	Disease progression		[82]
DNAs	miR-21	OS and chemoresistance		
	miR-301a-3p[c]	Invasion depth, lymph node metastasis, late		
	Mutant KRAS	TNM stage		
		Neoadjuvant therapy in localized and progression in metastatic patients		[51] [45]

NM: not mentioned.
[a] stage I-II A.
[b] downregulated PEX miRNA.
[c] produced by PEXs under hypoxia.

similar size, a liposome-like membrane bilayer with customizable surface, and multifunctional capacity [86]. Nevertheless, exosomes possess unique properties, including innate stability, low degradability, strong protection of cargo, and ability to cross the blood-brain barrier. They also are less cytotoxic [87] than nanoparticles. Furthermore, exosomes avoid clearance by the human immune system as they express CD47 in their membranes. CD47 is a widely expressed integrin-associated transmembrane protein that protects cells from phagocytosis by monocytes and macrophages [88,89] through initiation of the “don't eat me” signal by binding with its ligand signal-regulatory protein alpha. This might explain why exosomes have a longer half-life in serum.

Several studies have focused on the usability of exosomes as delivery vehicles in pancreatic cancer. Chemoresistance is a major reason why GEM shows no significant improvement in patient survival [39]. Survivin-T34A, a dominant-negative mutant form of survivin, abrogates survivin-related chemo- or radiotherapy by activating caspase and inducing apoptosis [90]. Based on the superior features of exosomes to serve as delivery tools, Aspe et al. designed a YUSAC 2 (a melanoma cell line) tet-off system to generate survivin-T34A-containing exosomes, which effectively promoted cancer cell death, especially when combined with GEM [90]. In consideration of the high frequency of KRAS mutation, Kamerkar et al. engineered a new transporter (termed “iExosomes”) by using exosomes as carriers of small interfering RNA to precisely target oncogenic KRAS mRNA [91]. Subsequent functional studies showed that iExosomes markedly inhibit tumor cell proliferation and significantly increase overall survival in mouse models of pancreatic cancer. Of note, KRAS can enhance the macropinocytosis of iExosomes by pancreatic cancer cells, making iExosomes more effective in patients with KRAS mutation. Curcumin is a turmeric root derivative that has potent anti-cancer and anti-inflammatory effects in vitro and in vivo [92]. In pancreatic cancer cells exposed to curcumin, curcumin was packaged into PEXs, which transferred curcumin to cancer cells, thus inducing apoptosis. These results verified that exosomes have promising potential as effective delivery vehicles.

6. Conclusions and perspectives

PEXs are believed to be promising biomarkers and potential therapeutic targets in pancreatic cancer on the basis of their multiple functions and clinical implications. The discriminatory expression of numerous molecules endows PEXs with diagnostic and prognostic values. Through intercellular communication, PEXs are involved in proliferation and apoptosis, immune tolerance and metastasis, and metabolic reprograming of tumor cells, providing various potential therapeutic targets for the treatment of pancreatic cancer patients. Furthermore, exosomes might be a novel type of drug delivery tools, as they possess multiple unique properties in comparison with conventional nanoparticles.

However, several issues need to be fully addressed before clinical application of PEXs can be considered. First, it is difficult to compare findings among PEX-related studies because of the lack of standardized protocols. Therefore, the establishment of a set of non-controversial procedures, including start sample volume, exosome enrichment, cargo extraction and purification, and selection of a suitable internal control, are obviously required. Second, the effects of PEXs depend on their contents; some PEX molecules are tumor-promoting, whereas others are tumor-suppressing [13]. Balancing these effects is a key issue to solve. Third, most PEX-related studies were conducted in vitro, using cell lines cultured in conditioned media containing exosomes or exosome preparations from cells incubated with recipient cells. It is unknown whether the amount of a certain exosomal cargo delivered in these

conditions is within the physiological range. Fourth, when investigating the functions of PEXs in vivo, it is important to achieve specific induction of or interference with PEX release without affecting the release of other subtypes of EVs or other signaling molecules. Fifth, although a number of PEX cargos are biomarker candidates for pancreatic cancer, no proven biomarkers are available today. It is indispensable to verify candidates in large-scale, well-designed, multi-centered studies, to accelerate the translation of basic research into clinical practice. Sixth, limited attention has been paid to biofluids other than plasma and serum, and moreover, one study suggested that salivary exosomes [69] are a better diagnostic candidate than serum exosomes, indicating the need to focus more on unconventional biofluids in future studies. Finally, for exosomes to serve as drug delivery systems in pancreatic cancer, proper methods to improve drug encapsulation, organ tropism, and durability of exosomes must be developed. Clearly the research on PEXs is only in its infancy. Given the promising clinical implications of PEXs and the challenges in this research field, more efforts are required to gain a deeper understanding of PEXs to promote the treatment and prognosis of pancreatic cancer in the near future.

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