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Exosomes: Navigating a New Route in Pancreatic Cancer

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Abstract

Pancreatic cancer is a fatal disease, and even with an increased commitment to pancreatic cancer research, the 5-year survival rate remains at approximately 6%. However, in the last decade, there has been a rising interest in the role of small extracellular vesicles called exosomes in the cancer field. Accumulating evidence shows that exosomes participate in early processes of tumorigenesis, tumor progression, and metastasis by mediating communication between cells or between cells and their surrounding microenvironment. In pancreatic cancer, exosomes play key roles in building pre-metastatic niches in the liver, inducing immune evasion, changing metabolism, mediating crosstalk between tumor and stromal cells, and causing low chemosensitivity. Although research exploring the roles of exosomes in pancreatic cancer is still in its infancy, the studies presented in this review highlight their potential value in developing novel tools, such as lipid biomarkers, treatment targets, and efficient drug delivery devices, for cancer diagnosis and therapy.

Keywords: Exosomes; Pancreatic cancer; Pancreatic ductal; Adenocarcinoma; Biomarker

Abbreviations

PDAC: Pancreatic Ductal Adenocarcinoma; MIF: Migration Inhibitory Factor; KC: Kupffer Cell; DC: Dendritic Cell; TLR4: Toll-Like Receptor 4; AM: Adrenomedullin; PSC: Pancreatic Stellate Cell; CAF: Cancer-Associated Fibroblast; GPC1 crExos: Glypican 1 Circulating Exosomes

Introduction

Pancreatic cancer is a highly lethal disease; it is the fourth leading cause of cancer-related death, and in a decade, it is predicted to be the second leading cause [1]. It has a 5-year survival rate of approximately 6% and a median survival rate of approximately 6 months [2]. Pancreatic Ductal Adenocarcinoma (PDAC), which develops from the exocrine cells of the pancreas, accounts for more than 90% of all pancreatic tumors [3]. The poor prognosis of PDAC is due the difficulty of detecting the disease at an early stage, its high metastatic potential, and resistance to conventional therapies [4-6]. PDAC is difficult to detect largely because it is asymptomatic until it reaches an advanced stage. Currently, it is also difficult to obtain pancreatic cancer specimens; thus, monitoring postoperative recurrence and metastasis remains a challenge. Because of these obstacles, there is an urgent need to find early, reliable, non-invasive, or minimally invasive, pancreatic cancer biomarkers, and to develop novel therapeutic strategies.

Recently, small extracellular vesicles, called exosomes, have attracted scientific interest after being discovered as the crucial information transporters, or the "cellular postmen," between cells. Exosomes facilitate cell-cell and cell-microenvironment communication in both normal and malignant cells. Over the last decade, there has been an increase in exosome-related research that has implicated exosomes in a wide variety of cancer-related processes, including tumor cell proliferation, apoptosis inhibition, invasion and metastasis, immune tolerance, and chemoresistance [7,8]. Therefore, exosome research is a new direction in the identification of early detection methods and novel therapeutic strategies to fight pancreatic cancer.

Exosome Form and Function

Exosomes are lipid-bilayer-enclosed extracellular vesicles that can horizontally transfer diverse protein and nucleic acid cargo to recipient cells [9]. First discovered in maturing mammalian reticulocytes in the 1970s, exosomes can be secreted by a wide variety of mammalian cell types [10]. Exosomes have been shown to facilitate the exchange of genetic material between cells, allowing for the transfer of functional RNA molecules, such as mRNAs and microRNAs (miRNAs), which sparked interest in exosome research. Exosomes share several defining characteristics including: (1) Size (30–100 nm in diameter); (2) Density (1.13–1.19 g/ml); (3) Morphology ("cup" or "dish" shaped by transmission electron microscopy); and (4) Enriched protein markers [tetraspanins, tumor susceptibility gene 101 (TSG101), and heat shock protein 70 (HSP70)] [11]. In contrast with other types of extracellular vesicles, the hallmark of exosomes is their endosomal origin [9].

The precise mechanisms, underlying the sorting of various cargo into the Intraluminal Vesicles, (ILVs), or exosomes, are not fully elucidated. However, three different complex sorting pathways have

been identified: (1) The ceramide-dependent sorting complex pathway; (2) The endosomal sorting complexes required for the transport (ESCRT) pathway, including ALG2-interacting protein X (ALIX) and TSG101; and (3) The tetraspanin-mediated sorting pathway, including CD63, CD81, and tetraspanin 8 (TSPAN8) [12-15]. Moreover, although Ca^{+}, microenvironmental pH, heparanase, and hypoxia have been shown to affect exosome release [16-19], what precisely triggers cells to shed their exosomes remains unknown? Upon the release of exosomes into the extracellular environment, the expression of specific integrins or tetraspanins on the exosomal membrane appears to guide them to specific cells or organs [9,20]. Exosomes can also be transferred to other cells via fusion, micropinocytosis, and caveolin-mediated endocytosis. Enzymatic depletion of cell-surface Heparan Sulfate Proteoglycans (HSPGs), or pharmacological inhibition of endogenous proteoglycan biosynthesis, has been shown to attenuate exosome uptake, suggesting that HSPGs are required for exosome internalization [21]. Ongoing research continues to focus on the elucidation of the key players and mechanisms involved in exosome biogenesis, cargo loading, release, and uptake. This research is critical, because there is accumulating evidence for the role of exosomes in cell-cell and cell-microenvironment communication in tumor progression and metastasis.

Functions of Exosomes in Pancreatic Cancer

As shown in Figure 1, evidence suggests that exosomes, via cell-cell and cell-microenvironment communication, contribute to several key processes in pancreatic cancer progression, including: (1) Metastasis promotion [22], (2) Evasion of tumor cells from the immune response [23], (3) Metabolism alteration, (4) Tumor-to-stromal cell communication [24], (5) Chemosensitivity reduction [25].

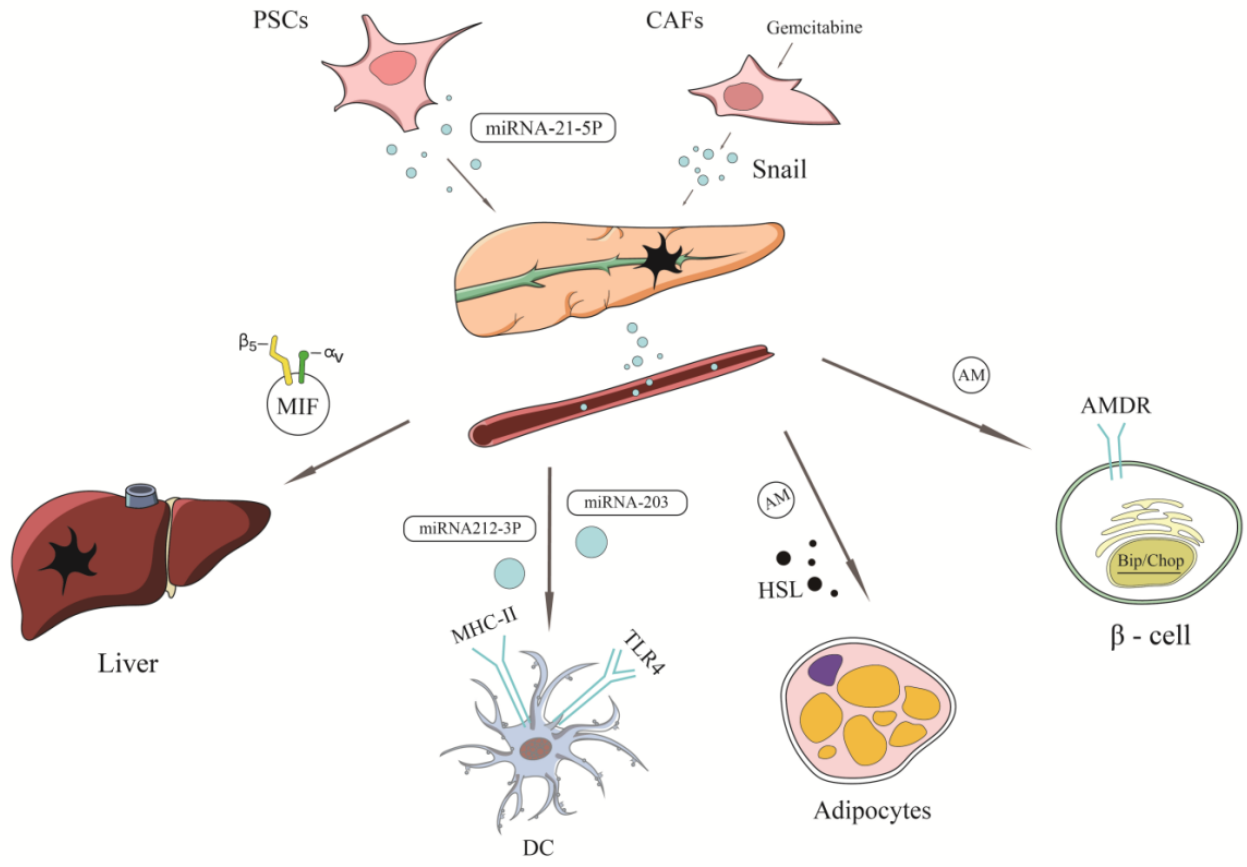

Figure 1: Sources and functions of exosomes in pancreatic cancer. MIF: Macrophage Migration Inhibitory Factor; DCs: Dendritic Cells; HSL: Hormone-Sensitive Lipase; PSCs: Pancreatic Stellate Cells; CAF: Cancer-Associated Fibroblast; AM: Adrenomedullin; ADMRs: Adrenomedullin receptors.

To induce pre-metastatic niches

Rapid metastatic progression is the most critical feature of pancreatic cancer; it involves co-evolution of the tumor and its microenvironment [26]. Patients most often succumb not to the primary tumor, but to the systemic fallout from its metastatic deposits. Prior to the influx of tumor cells, distinct ecosystems that favor the survival of "tumor seeds" are created and are referred to as pre-metastatic niches [27]. These pre-metastatic niches are conducive for tumor cell adhesion and invasion [28]. For instance, similar to the setting of a primary melanoma, the lung microenvironment becomes a hotbed of local molecular activity [28]. In 2015, Costa-Silva et al. reported that PDAC cell-derived exosomes induce the formation of liver pre-metastatic niches that foster the development of metastatic formation in the nude mice. This multistep process begins with the release of exosomes containing macrophage Migration Inhibitory Factor (MIF) from PDAC cells. Fluorescently-labelled exosomal MIF is preferentially taken up by Kupffer Cells (KCs) in the mouse liver, a phenomenon not observed in exosomes derived from healthy murine pancreas. MIF induces the release of transforming growth factor β (TGFβ) by KCs, which, in turn, promotes fibronectin production by the hepatic stellate cells. Fibronectin deposition subsequently promotes the recruitment of bone marrow-derived macrophages and neutrophils to the liver, ultimately leading to the formation of liver pre-metastatic niches that are more hospitable to circulating metastatic PDAC cells [29]. In 2015, the same research team also revealed the mystery of "metastatic organotropism," which is the propensity of metastasized tumor cells to target specific organs. They showed that exosomal Integrins (ITGs) direct organ-specific colonization by fusing with target cells in a tissue-specific manner, which promotes pro-migratory and pro-inflammatory S100 gene upregulation, thereby initiating pre-metastatic niche formation [20]. Different cancer cells carry different sets of integrins that direct the exosomes, and subsequent metastasis, to specific organs. For instance, the exosomal integrins α6β4 and α6β1 are associated with lung metastasis, whereas αvβ5 is associated with liver metastasis [20]. Taken together, these data show that tumor-derived exosomes help primary tumor cells leapfrog to specific distant sites, indicating that exosomes can be used to predict organ-specific metastasis and targeted to halt the metastatic spread.

To help pancreatic cancer cells escape immune responses

In several cancers, tumor cell exosomes interact with target cells via immune suppressive molecules, leading to the decreased proliferation of T lymphocytes and Natural Killer Cells (NKCs), or the differentiation of regulatory T lymphocytes [30]. However, in pancreatic cancer, exosomal miRNAs, which are small noncoding RNAs with diverse functions, play an important role in the immune escape of tumor cells. For instance, miR-203, which is upregulated in PDAC compared with normal pancreatic tissues [31], induces immune tolerance and facilitates cancer cell invasion and metastasis [8]. Dendritic Cells (DCs), which are the most typical Antigen Presenting Cells (APCs), express a wide range of toll-like receptors and cytokines, providing an essential link between the innate and adaptive immune responses [32]. Zhou et al. reported that pancreatic cancer-derived exosomal miR-203 suppresses the expression of toll-like receptor 4 (TLR4), and the downstream cytokines tumor necrosis factor alpha (TNF-α) and interleukin 12 (IL-12) in DCs, contributing to DC dysfunction [33]. Moreover, Okamoto et al. showed that TLR4 expression is important for the anticancer effect of DC-based immunotherapy [34]. Recently, Ding G et al. showed that pancreatic cancer-derived exosomal miR-212-3p inhibits the expression of regulatory factor X-associated protein (RFXAP), a key transcription factor for major histocompatibility complex II (MHC II) in DCs. RFXAP inhibition results in decreased MHC II expression and inactivation of $CD4^+$ T-lymphocytes, ultimately inducing immune tolerance [35]. These data describe a novel mechanism of pancreatic cancer immune tolerance, and suggest that eliminating cancer-derived exosomes can act as an immune agonist in pancreatic cancer immunotherapy. Interestingly, data from a recent study supported this hypothesis, showing that depletion of exosomal miRNAs by lysis and ultrafiltration increases immune activity via activation of DCs or Cytokine-Induced Killer Cells (CIKs) against pancreatic cancer cells [36]. Although these studies are promising, the precise functions of exosomes in the immune system remain poorly understood, and more comprehensive studies are needed.

To change the metabolism of pancreatic cancer patients

In pancreatic cancer patients, diabetes and weight loss, which are paraneoplastic phenomena, precede cachexia onset by several months. Up to 40% of pancreatic cancer patients have diabetes, which is frequently presented as early as 2–3 years before pancreatic cancer diagnosis [37]. New-onset diabetes, with a duration of <3 years, conveys a 4–7-fold increase in the risk of developing pancreatic cancer [38,39]. A study by Javeed et al. showed that pancreatic cancer-derived exosomes, containing cancer antigen 19-9 (CA19-9) and Adrenomedullin (AM), which interacts adrenomedullin receptors (ADMRs) on β-cells, readily enter with β-cells via caveolin-mediated endocytosis or micropinocytosis. This exosomal uptake leads to the upregulation of endoplasmic reticular stress genes, such as Bip and Chop, which increases reactive oxygen/nitrogen species (ROS/RNS) production, resulting in failure of the unfolded protein response, inhibition of insulin secretion, and increased β-cell death [40]. Another study revealed a novel mechanism of exosome-induced lipolysis in pancreatic cancer. Sagar et al. demonstrated that AM-containing, pancreatic cancer-derived exosomes induce lipolysis in murine and human subcutaneous adipocytes [41]. AM binds to ADMR on the surface of normal adipocytes and activates p38 and ERK1/2 Mitogen-Activated Protein Kinase (MAPK) pathways, which promotes lipolysis by phosphorylating hormone-sensitive lipase and results in early weight loss in PDAC patients. Moreover, ADMR blockage abrogates the lipolytic effect of the exosomes [41]. These data provide insight into the early-onset paraneoplastic effects of pancreatic cancer and are valuable in identifying target risk populations and developing new strategies for improving patient quality of life.

To mediate crosstalk between stromal cells and tumor cells

Pancreatic Stellate Cells (PSCs) and cancer-associated fibroblasts (CAFs) are key components in the dense fibrotic stroma that confers aggressiveness in pancreatic cancer progression [42,43]. PSCs with an adipogenic phenotype contain an abundance of vitamin A lipid droplets within their cytoplasm. In response to insult, PSCs switch to an activated state, whereby they lose their cytoplasmic vitamin A droplets and undergo transformation into myofibroblasts that express the cytoskeletal protein Smooth Muscle Actin (SMA), which is the true hallmark of an activated PSC [44]. Activated PSCs play key roles in the development of a fibrotic pancreatic stroma by increasing the synthesis of extracellular matrix proteins, such as collagen and enzymes responsible for matrix remodeling. Furthermore, several factors secreted from PSCs, such as platelet-derived growth factor (PDGF),

galectin-1, stromal-derived factor 1, epidermal growth factor, insulin-like growth factor 1, and fibroblast growth factor, contribute to the increased invasiveness and proliferation of PDAC cells *in vitro* and *in vivo* [45,46]. PSCs have been shown to promote epithelial-to-mesenchymal transition (EMT) [47,48] and a stem cell-like phenotype in pancreatic cancer cells [49], which are key features in enhancing tumorigenicity, cell migration, and resistance to chemotherapy. CAFs are the main effector cells in the desmoplastic reaction, it display a myofibroblast-like phenotype, characterized by a spindle shape and the expression of α-SMA [50]. Based on the overlapping function of PSCs and CAFs, and their reported effects on pancreatic cancer cells, it is not surprising that crosstalk can occur among these cell types. For instance, Ali et al. demonstrated miR-21/miR-221 upregulation in PSCs and CAFs, which may contribute to increased pancreatic cancer cell aggression [43]. They further showed that antisense oligonucleotide (ASO)-mediated inhibition of miR-21 decreases migration and invasion in PSCs [43]. Recently, Masamune et al. showed that miR-21-5p is present in exosomes isolated from human PSCs, and when the PDAC cell lines PANC-1 and SUIT-2 are exposed to the PSC-derived exosomes, the pancreatic cancer cells show increased proliferation [51]. Moreover, PSC-derived exosomes stimulate the proliferation, migration, and mRNA expression of chemokine (C-X-C motif) ligands 1 and 2 in pancreatic cancer cells [51]. These data suggest that PSC-derived exosomes may be the primary driver of pancreatic cancer progression. Finally, Richards et al. showed that CAF-derived exosomes, exposed to gemcitabine, are critical regulators of epithelial cancer cell proliferation and survival [52]. Together, these studies facilitate our understanding of the roles of the sophisticated pancreatic cancer microenvironment, and provide a potential pool of novel targets to improve therapeutic sensitivity and prevent tumor progression.

To mediate resistance to chemotherapeutic drugs

Despite the significant advances in chemotherapy, drug resistance remains a major obstacle to successful treatment and contributes to poor prognosis in PDAC. Exosomes facilitate cell-cell communication and play a role in chemoresistance in many cancers, including lung cancer, breast cancer, glioblastoma, and myeloma [53-56]; therefore, it is logical to hypothesize that exosomes also play a role in the cytotoxic resistance in pancreatic cancer. Indeed, Richards et al. demonstrated that gemcitabine-treated CAFs secrete exosomes that increase expression of the chemoresistance-inducing factor Snail in pancreatic cancer epithelial cells, leading to their increased proliferation and chemoresistance. They further showed that treatment with GW4869, an inhibitor of exosome release, reduces survival in co-cultured epithelial cells [52]. This study demonstrated an important role of CAF-derived exosomes in chemotherapeutic drug resistance. This study indicates that more comprehensive research into the roles of exosomes in pancreatic cancer progression, particularly in the chemoresistance of pancreatic cancer cells, will offer promising methods for the development of precision medicine for pancreatic cancer diagnosis and therapy in the future.

Clinical Applications of Exosomes in Pancreatic Cancer

Exosomes are readily accessible in nearly all body fluids, including blood, urine, saliva, and ascites [57-60]; increasing evidence has shown that exosomes are secreted by cancer cells at higher rates than by healthy cells [61]. For clinical applications, exosomes have several advantageous features, including their stability over time and their inclusion of nucleic acids and proteins from the parental tumor. The identification of consistent differences between cancer cell-derived and normal cell-derived exosomes should improve the usefulness of exosomes as a non-invasive diagnostic tool for cancer [62]. These differences foster the hope that circulating exosomes can be widely used in clinical work. Exosomes are currently being used, or have the potential to be used, for the following clinical applications: (1) Lipid biomarkers, (2) Drug delivery devices, and (3) Novel treatments.

Exosomes as liquid biopsies

To act as promising biomarkers in early cancer detection: The sensitivity and specificity of non-biopsy tests, including conventional serum carbohydrate antigen 19-9 (CA19-9) and imaging examination, are not adequate to detect potentially resectable tumours <1 cm [63,64]. Moreover, although endoscopic ultrasonography-guided fine-needle aspiration (EUS-FNA) has a diagnostic accuracy of more than 85–90% for pancreatic cancer, it is highly invasive; thus, it is not feasible for large-scale screening [65]. However, exosomes offer a promising alternative for early pancreatic cancer detection via liquid biopsies. Using proteomics, Melo et al. showed that the membrane-bound protein glypican 1 (GPC1) is preferentially expressed on cancer-associated exosomes [62]. Subsequent work demonstrated an increased percentage of GPC1 circulating exosomes (GPC1 crExos) in mice and humans with breast or pancreatic cancer. GPC1 crExos measurements in PDAC patients, compared with those in healthy donors and benign pancreatic disease (BPD) patients, showed 100% sensitivity and 100% specificity. Interestingly, free circulating GPC1 measurements failed to achieve optimal discrimination among the patient groups. Using the PKT mouse model of PDAC, they further demonstrated that GPC1 crExos are present very early in tumorigenesis, detectable in some cases before any histologic abnormality was observed in the mouse pancreas. Similarly, the Costa-Silva study, using the PKCY mouse model of PDAC, demonstrated that exosomal MIF upregulation is an early event; thus, MIF can be detected in plasma-derived exosomes isolated from mice with pre-tumoral pancreatic lesions. Interestingly, high exosomal MIF levels are also present in plasma from patients with stage I PDAC, before liver metastasis [29]. Finally, proteomics analyses of affinity-purified exosomes from the pancreatic cancer cell lines PANC-1 and PaCa-44 identified proteins that are selectively enriched and cell-type specific; some of these proteins are candidates for use as detection markers [7]. For instance, TSPAN8, CD44v6, and α6β4 integrin are highly expressed in exosomes from pancreatic cancer-initiating cells [66].

Exosomes also contain nucleic acids that can serve as early detection markers. Valadi et al. demonstrated that exosomes contain mRNAs and miRNAs, called "exosomal shuttle RNAs," and mediate transportation of nucleic acids between cells [67]. Exosomal miRNAs and their functions have been extensively studied in several human solid cancers [68-71]. Although there are relatively few studies on miRNAs in pancreatic cancer-derived exosomes, current data indicate that exosomal miRNAs may have great diagnostic value in pancreatic cancer. Dysregulated expression of RNAs, particularly miRNAs, has been observed in pancreatic cancer, compared with that in chronic pancreatitis and healthy controls [72-74]. Moreover, Que et al. found that the levels of serum exosomal miR-17-5p and miR-21 were higher in pancreatic cancer patients than in non-pancreatic cancer patients and healthy controls [75]. Importantly, Madhavan et al. reported that a combination of proteins, including CD44v6, TSPAN8, Epithelial Cell Adhesion Molecule (EpCAM), MET, CD104, and miRNAs, including miR-1246, miR-4644, miR-3976, and miR-4306, in circulating

exosomes can distinguish pancreatic cancer cases from non-cases with 100% sensitivity and 93% specificity; the patients included healthy controls, chronic pancreatitis patients, and individuals with benign pancreatic tumors, but excluded non-pancreas malignancies [76]. Another comprehensive study detected a wide range of biomarkers within the nucleic acid cargo of cancer-derived exosomes, including copy number profiles, point mutations, insertions, deletions, gene fusions, and mutational signatures [77]. In conclusion, liquid biopsies using circulating exosomes have great potential as a clinical tool for cancer early diagnosis.

To act as prognostic predictors: Melo et al. demonstrated that GPC1 crExos can be used to assess the burden of pancreatic cancer. Their study showed that GPC1 crExos levels are significantly higher in PDAC patients with distant metastatic disease than in patients with metastatic disease restricted to lymph nodes or no metastases, suggesting that GPC1 crExos can be used as an independent prognostic and predictive marker for disease-specific survival, and that it can act as a surrogate marker for monitoring disease progression [62]. Similarly, Costa-Silva et al. demonstrated that exosomal MIF levels are increased in pancreatic cancer patients compared with those in pancreatic cancer patients with no evidence of metastasis post-diagnosis, and those in healthy controls [29]. Other exosome-related proteins are also associated with the prognosis of pancreatic cancer patients, such as RAB27A, which belongs to the Rab family GTPases that regulate cellular vesicle trafficking, and TP53, which regulates the exosome secretion pathway. Pancreatic cancer patients with high levels of RAB27A and TP53 have poor overall survival [78]. Moreover, RAB27A expression was shown to be an independent prognostic marker for PDAC, indicating that the RAB27A-regulated exosome secretion pathway may represent a novel therapeutic target in pancreatic cancer. Consistent with these data, another study showed that RAB27B and p53 expression are negatively correlated with the overall survival of pancreatic cancer patients [79,80]. Together, as per Table 1, these surprising results predict a promising future for improved early diagnosis of patients with pancreatic cancer, and motivate continued exploration into using exosomes as a source of novel biomarkers.

	Study (year)	Research subject	Exosome isolation	Exosome marker	Result	Ref.
Early detection	Melo et al. (2015)	321 (190 PC vs. 131 non-PC)	Ultracentrifugation	GPC1 protein	Sensitivity 100%; Specificity 100%	[62]
	Madhavan et al. (2015)	220 (131 PC vs. 89 non-PC)	Ultracentrifugation and immunoaffinity (anti-CD44v6, anti-TSPAN8, anti-EpCAM, and anti-CD104)	miR-1246, miR-4644, miR-3976, and miR-4306	Sensitivity 100%; Specificity 93%	[76]
	Que et al. -2013	49 (22 PC vs. 27 non-PC)	Ultracentrifugation	miR-21 miR-17-5p	Sensitivity 95.5%; Specificity 72.7% Sensitivity 81.5%; Specificity 92.6%	[75]
	Melo et al. (2015)	PKT mouse model	Ultracentrifugation	GPC1 protein	GPC1+ crExos increase at PanIN stage	[62]
	Costa-Silva et al. (2015)	PKCY mouse model	ELISA	Exosomal MIF	MIF increase at PanIN stage	[29]
Predictive prognostic	Wang et al. (2015)	265 pancreatic tissues (186 PC vs. 79 non-PC)	Immunohistochemistry	-	High RAB27A and TP53 indicate poor overall survival	[78]
	Zhao et al. (2015)	260 pancreatic tissues (186 PC vs.. 74 non-PC)	Immunohistochemistry	-	High RAB27B and p53 indicate poor overall survival	[79]
	Melo et al. (2015)	184 (32 distant metastases vs. 152 non-distant metastases)	Ultracentrifugation	GPC1 protein	Distant metastases show higher levels	[62]
	Costa-Silva et al. (2015)	37 (12 PDAC progressive after diagnosis vs. 25 non-metastases)	ELISA	Exosomal MIF	High exosomal MIF levels in PDAC progressive patients	[29]

Table 1: Clinical application of exosomes in liquid biopsy.

Exosomes as drug delivery vehicles

The lack of stability and targeted delivery, and the undesirable side effects, are the major limitations of chemotherapy agents used to treat cancer. The chemotherapy regimen FOLFIRINOX, or gemcitabine, are the current standards of care for pancreatic cancer patients; however, their efficacy is limited, often leading to an improvement in the quality of life rather than an effective cure of the disease [80]. Therefore, it is imperative to find new therapeutic strategies and valid pharmacological targets to improve the survival prospects of pancreatic cancer patients. The use of exosomes as drug delivery vehicles has gained considerable interest due to their excellent biodistribution and biocompatibility [81], which should enhance the

stability and specificity, and reduce the side effects and immunogenic response, of therapeutic agents. Indeed, Osterman et al. showed that exosome-bound curcumin is readily taken up by cultured PDAC cells and is effective in inducing cytotoxicity [82]. Moreover, Pascucci et al. demonstrated that mesenchymal stromal cell-derived exosomes can effectively deliver active drugs, such as paclitaxel, to inhibit tumor growth [83]. Interestingly, studies have shown that survivin resides in exosomes [84,85]. Survivin is a key member of the Inhibitor of Apoptosis (IAP) family of proteins and is a known stress-activated protein expressed in embryonic and fetal development and in most human cancers [86]. Galloway et al. combined low doses of exosomal survivin-T34A, which contains a threonine 34-to-alanine mutation in survivin, with gemcitabine (10 mM), and showed that this combination enhances the killing effects of gemcitabine [87]. Although these applications of exosomes have not yet been tested in the clinic, they clearly demonstrate the promising potential of using exosomes as drug delivery vehicles.

Exosomes as new treatment methods

Additional clinical treatment methods, involving exosomes, include the elimination of circulating exosomes and the use of exosomes in immunotherapy [88-90]. The elimination of circulating exosomes is an attractive treatment option to reduce or inhibit tumor cell proliferation, invasion, and metastasis. The potential for this treatment option was demonstrated by Costa-Silva et al., who showed that silencing of exosomal MIF, an immunostimulatory cytokine that promotes the formation of pre-metastatic niches in the liver, abolishes exosome education-induced liver metastasis of PDAC cells [29]. Increasing evidence has also shown the potential use of exosomes in cancer immunotherapy. For instance, DC-derived exosomes have been developed as immunotherapeutic anticancer agents [91]. Moreover, a study by Yang et al. found that injection of tumor-derived exosomes, containing IL-2, into mice with tumors largely inhibits tumor growth [92]; this effect is ascribed to an antigen-specific Th1-polarized immune response mediated by cytotoxic T-lymphocytes.

Another potential, recently identified, therapeutic use of exosomes should facilitate treatment decisions for pancreatic cancer patients. Epidermal Growth Factor Receptor (EGFR) is overexpressed in a high proportion of PDAC tumors and can be detected in exosomes secreted by the epithelial component of tumours [93]. A key determinant of response to EGFR-targeted therapy is the presence of mutations in genes encoding downstream effector proteins such as KRAS [94]. Recently, it was demonstrated that exosomes from PDAC patients contain double-stranded genomic DNA fragments, including the KRAS gene, suggesting that it is possible to identify patients who would benefit from EGFR-targeted therapy without the need for invasive biopsies of the tumor [95]. However, the studies described in this section, investigating the application of exosomes in clinical treatment, are still in their infancy; thus, work continues in order to validate the use of exosomes for each of these treatment options in the clinic.

Future Directions for Exosomes in Cancer Diagnosis and Therapy

The clinical integration of these findings remains a challenge and requires further studies for validation. Future studies of exosomes will further elucidate their roles in pancreatic cancer pathogenesis and open new avenues for cancer diagnosis and therapeutics.

Identifying the "sweet spot"

Given the apparent early shedding of PDAC-associated exosomes, an important question is precisely how exosomes find the proper operative opportunity. The goal of markers is to identify patients with resectable tumors at the earliest point, when surgical resection offers the greatest benefit for survival. However, identifying the "sweet spot" for early detection of PDAC is difficult. Pancreatic Intraepithelial Neoplasia (PanIN) lesions are not rare in healthy individuals, and many adults have precursor lesions in their pancreas that do not appear to progress [96,97]. In addition, it may take 10-20 years for pancreatic cancer to progress from the initiation of the first malignant clone to metastatic disease, which provides a wide window for early detection [98]. Thus, if an early-stage PanIN is detected by a biomarker, without reference to the likelihood of its progression to PDAC, then the marker will have inadequate specificity for life-threatening invasive disease. Thus, further work is necessary to better delineate how circulating exosomes can narrow the PanIN-3/early PDAC window of opportunity for early detection.

Simplifying the purity process

Melo et al. used ultracentrifuges and flow cytometers, which are widespread and straightforward to use, to isolate exosomes [62]. Currently, in most laboratories worldwide, ultracentrifugation is one of the main methods for separating exosomes. Ultracentrifugation-dependent separation of exosomes is based on the differential densities of various particles in the serum. However, because other types of particles and small extracellular vesicles, such as lipoproteins and oncosomes, have similar gradient densities (1.11-1.19 g/ml) and sedimentation velocities to those of exosomes, they cannot be separated from the exosomes by conventional biophysical ultracentrifugation [99]. Furthermore, the ultracentrifugation process is lengthy, limiting its use in high-throughput clinical laboratories. Thus, future work should be aimed at identifying a more optimal purification process for exosomes.

Standardizing exosomal methodology

Standardization is another challenge for taking the potential exosomal therapeutics and diagnostics from bench to bedside. Exosome collection, isolation, and preparation, and platforms for the analysis of exosomal cargo, need to be standardized to facilitate the use of exosomes as clinical tools for pancreatic cancer [100]. However, the first blood-based cancer diagnostic to exploit free-floating exosomes became commercially available in the US on January 21st, which will serve to assess the validity of using exosomes in a broadly applicable platform.

Finding the optimal application pattern

The most promising biomarker identified in pancreatic cancer, GPC1 crExos, is also present in the serum of patients with breast cancer, leading to questions about the tissue specificity of this marker. Some research indicates that the combination of GPC1 crExos and MIF detection could be an attractive, non-invasive diagnostic tool to identify the very early stages of pancreatic cancer [101]. Future research should address whether a newly identified, ideal biomarker, or a combination of already existing proteins and nucleic acids, will provide the most effective diagnostic tool for pancreatic cancer.

Summary

Based on the rigorous work described in this review, circulating exosomes may be a promising target for early detection and treatment in pancreatic cancer. Although the understanding of exosome biology remains elusive, recent studies should accelerate our understanding and ensure a bright future for exosome research. We believe that these extracellular vesicles have great potential in the early detection, individualized medicine, and drug delivery vehicles, and may provide life-extending or life-saving treatments for pancreatic cancer. However, this critical endeavor to maximize the potential of exosomes in cancer therapy will require collaboration among investigators across varied backgrounds, as well as adequate funding support. Nevertheless, the potential to reduce mortality from one of the most lethal malignancies is enormous.

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Disclosure

The authors declare no conflicts of interest.

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