

Role of exosomes in pancreatic cancer (Review)

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Abstract. Pancreatic cancer (PC) is a malignant tumour of the human digestive system that has a poor prognosis. Exosomes contain proteins and nucleic acids, and constitute a class of extracellular vesicles defined as membrane-bound nanovesicles of endocytic origin, with a diameter of 40-150 nm. Exosomes are potential diagnostic markers of PC; however, their roles in cancer initiation and progression remain unclear. Previous studies have focused on the molecular mechanisms and functions of exosomes that allow them to accelerate PC cell proliferation, migration and invasion. The present review discusses the interactions between exosomes and the pathophysiology of PC. The potential clinical applications of exosomes are also discussed.

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1. Introduction

Pancreatic cancer (PC) is a digestive system malignancy that mainly arises from ductal epithelial and acinar cells (1). PC has a poor prognosis, with a 5-year survival rate <5%, and patients with advanced PC have a shorter survival time of 3-6 months (1). There were 432,242 new mortalities associated

with PC worldwide in 2018 due to treatment delays caused by difficulties in the early diagnosis of PC (1). In addition, PC ranks 13th in cancer prevalence worldwide, with 458,918 new cases in 2018, and the incidence rates in South Europe was 14% and North America was 13.5% (2). PC cases are mainly ductal cell carcinoma, with a few cases of acinar cell carcinoma, acanthocutaneous carcinoma of the pancreas and cystadenocarcinoma. Due to the insensitivity of PC to chemotherapy, surgical resection remains the mainstay of treatment (3). However, PC is curable in only a minority of patients with locally resectable tumours, accounting for only 5-10% of patients, and the survival rate more than 5 years after surgery is only 10-20%. The poor prognosis of PC is mainly due to its strong invasive ability (3), and the biological molecular mechanism underlying its malignancy has not yet been determined.

Exosomes, which contain proteins [cytoskeletal proteins, transmembrane proteins and heat shock proteins (HSPs)], nucleic acids (DNA, mRNA, miRNA, and long and short non-coding RNAs) and enzymes (GAPDH, ATPase, pgk1 and RAB), constitute a class of extracellular vesicles (EVs) defined as membrane-bound nanovesicles of endocytic origin, with a diameter of 40-150 nm (4-6). The molecular contents of exosomes can reflect the nature and state of their cells of origin, and these contents alter the function of recipient cells (7). Since Johnstone *et al* (8) discovered and named exosomes in 1987, the complex process of exosome formation has been elaborated in detail. First, the membrane domain is endocytosed to form early endosomes, and then, the process of budding forms intraluminal vesicles, which further become multivesicular bodies (MVBs) by encapsulating proteins, nucleic acids and peptide bands. Some MVBs are degraded in lysosomes, while other MVBs fuses with the cell membrane and release internal vesicles to form exosomes (8). The final steps in exosome biogenesis also involve Rab enzymes, which regulate the transport of MVBs and promote the fusion of MVBs with the plasma membrane, thereby releasing exosomes (8,9).

The number of studies investigating the role of exosomes in tumour growth and cancer metastasis has grown exponentially (8,9). From tumour growth to cell metastasis, the intricate exosomal communication networks between tumour and non-tumour cells direct all steps of biological changes in tumours (10). Tumour cells develop exosome-based mechanisms that promote favourable microenvironments to support tumour growth by enhancing cell metastasis and avoiding apoptosis (10). In addition, cancer exosomes have the ability

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to induce neovascularization, which ensures the acquisition of nutrients and oxygen and the removal of waste, and contributes to sustained tumour cell proliferation (7,11). The invasion and dissemination of tumours is highly enhanced by cancer exosomes, which carry information that contributes to extracellular matrix (ECM) remodelling, cancer cell migration and invasion (12,13). Furthermore, it has been demonstrated that exosomal communication contributes to tumour immune escape and metastatic niche preparation (14-18).

The present review discusses the interactions between exosomes and the malignant biology of PC. The potential clinical application of exosomes are also discussed.

2. Biological characteristics of exosomes

Exosomes constitute a subpopulation of small extracellular vesicles that arises from the membranes of MVBs, and are released from the cell into the extracellular environment with the plasma membrane (18). Almost all live cells, including stroma cells, reticulocytes, epithelial cells and tumour cells, can release exosomes, and such exosomes have been extracted from blood plasma, serum, urine, bile, saliva and breast milk (4,6,19-32). Exosomes are small, membrane-enclosed vesicles (40-150 nm) that can deliver cargo (proteins, lipids and nucleic acids) from the cells of origin to recipient cells (13). A type of small vesicle released from marrow mesenchymal stem cell (MSC)-derived exosomes has been demonstrated to transfer functional RNAs to recipient cells, illustrating their promise as an alternative for cell-based therapy (14). Notably, it has been reported that exosomes can carry microRNAs (miRNAs), which are involved in cancer cell proliferation, differentiation and apoptosis (15,16). In addition, as tumour suppressors or oncogenes, miRNAs regulate gene expression post-transcriptionally (14). Previous studies have demonstrated that exosomes from pancreatic cells play an important role in niche initiation prior to liver metastasis (15,16). A unknown pre-metastatic circuit has been described, through which pancreatic adenocarcinoma (PDAC)-derived exosomes induce the formation of pre-metastatic niches that promote the development of metastatic disease (17). Exosome-mediated metastasis of oncogenic miRNAs from pancreas cancer cells may change the biological characteristics of non-cancer cells; however, metastasis of tumour-suppressing miRNAs may inhibit the proliferation of pancreatic cells (18). According to proteomic analyses, some proteins associated with cytosolic signalling proteins, cell surface receptors, antigen presentation, metabolic enzymes, the major histocompatibility complex (MHC), HSPs (such as HSP70, HSP90, HSP60 and HSC70) and tetraspanins (CD9, CD63, CD81 and CD82) are selectively enriched in specific exosomes (33,34). Some of the aforementioned proteins participate in the normal physiological activities of exosomes, while other proteins mediate interactions between exosomes and recipient cells. For example, a family of tetraspanins or integrins on the exosomal membrane can selectively act on target cells or target organs (35). Another class of proteins is associated with the specificity of the cell of origin, such as melanoma exosomes, which express the tumour-associated protein melanoma antigen recognized by T cell 1, and tumour

exosomes of epithelial cell origin, which express epithelial cell adhesion molecule (EpCAM) (36,37).

Exosomes are secreted by different types of cells and play important roles in cellular communication (4,21). However, only the following three mechanisms by which microvesicles are released into the extracellular space are known: Exocytic exosome release from intracellular MVBs, single-membrane vesicle release from the plasma membrane and apoptotic body release from cells undergoing apoptosis (33). Exosomes have pleiotropic effects that influence the physiology of neighbouring cells. Of these effects, the best studied *in vitro* effects include the roles of exosomes in several stages of the immune response (interactions with immune cells). These roles range from exosomes acting as a vehicle for antigen presentation to antigen-independent roles that can inhibit (immunosuppressive properties) or promote (immune-activating properties) immune responses. In addition, exosomes play a role in intercellular communication by acting as conveyors of proteins and lipids that affect downstream signalling events in recipient cells (35). Exosomes can also deliver genetic material that affects the physiology of recipient cells (33).

Some nucleic acids and lipids also exhibit highly selective enrichment (5,8,23-25). Nucleic acids include miRNAs, mRNAs, transfer RNAs, ribosomal RNAs and non-coding RNAs (23-25). Among these, miRNAs, which are a type of non-coding RNA, 19-25 nucleotides in length, can post-transcriptionally inhibit the expression or translation of target genes (38,39). Furthermore, miRNAs can disrupt the stability of mRNA and inhibit its translation, regulate the expression of target genes in different types of cells, and participate in important biological processes, such as cell proliferation, differentiation, apoptosis and metabolism (40). In addition, the lipid molecules in exosomes exhibit great research potential in PC (41). Most lipid molecules in exosomes, including sphingomyelin, cholesterol and phosphatidylserine, are located on the membrane (6). Previous studies have demonstrated that sphingomyelin and cholesterol can improve the stability of exosomal phospholipid bilayers (42,43). In addition, phosphatidylserine can promote the fusion of exosomes with target cell membranes and participate in signal transduction as a signalling molecule (41). Exosomal lipids can induce apoptosis in human PC SOJ-6 cells by inhibiting the Notch1 pathway. In addition, exosomal lipids can also induce drug resistance in human PC cells through the C-X-C motif chemokine receptor 4 (CXCR4)/stromal cell-derived factor 1 α (SDF1 α) signalling pathway (42,43).

Exosomes are membrane vesicles that are released by cells upon the fusion of MVBs with the plasma membrane. Their molecular composition reflects their origin in endosomes as intraluminal vesicles. In addition to a common set of membrane and cytosolic molecules, exosomes harbour unique subsets of proteins linked to cell type-associated functions (40). Exosome secretion participates in the eradication of obsolete proteins; however, a number of studies, particularly those investigating the immune system, have demonstrated that exosomes constitute a potential mode of intercellular communication (42,43). The release of exosomes by tumour cells and their involvement in the propagation of unconventional pathogens, such as prions, are indicative of their participation in pathological situations (39).

3. Exosomes as potential diagnostic markers of PC

PC is a major threat to human health, with very few effective therapies and a poor prognosis (1-3). As the fourth leading primary cause of mortality among cancers, PC has an incredibly low survival rate (3). Despite improvements in PC therapies, the mortality of the disease has remained relatively the same over recent decades, largely due to the lack of adequate screening methods and biomarkers for early diagnosis (3).

Progress in the treatment of PDAC remains elusive despite the substantial time and resources invested in attempts to improve its dismal prognosis. PC is a malignant disease that develops rapidly and has a poor prognosis (2). Currently, surgery is the only radical treatment. The American Cancer Society estimates that ~56,770 people will be diagnosed with, and ~45,750 will die of PC in 2019. The most recent SEER database reported a 9% 5-year survival rate from 2008-2014 (3). The early diagnosis of PC is difficult due to the lack of specific symptoms (1). In the current stage of clinical treatment, imaging examinations are extensively used for qualitative and positional diagnosis of PC. The serum marker, CA19-9 is also used; however, it has a low specificity for PC (2). Only 40% of patients with early PC have elevated serum CA19-9 levels, and several patients are diagnosed with advanced disease (44,45). Thus, the search for novel early diagnostic markers that can be used to differentially diagnose PC and other benign lesions has become the focus of research concerning PC diagnosis and treatment.

Glypican 1 (GPC1) is crucial for metastatic potential of PC cells. GPC1 is a lipid raft-heparan sulfate proteoglycan located on the cell surface that is involved in several important cellular signalling pathways such as cellular division, differentiation and morphogenesis (46). It has been demonstrated that down-regulation of GPC1 expression in the PC cell line, PANC-1, can slow the proliferation rate of PC cells, and decrease angiogenesis and metastasis in PC (47). Thus, both cancer cell- and host-derived GPC1 are crucial for the full mitogenic, angiogenic and metastatic potential of PC cells (47). Using flow cytometry to detect and isolate GPC1 from serum exosomes from patients with PC and mouse models of PC, Melo *et al* (28) demonstrated that GPC1 is enriched in exosomes from PC. In addition, GPC1 in these exosomes has high specificity and sensitivity, and exhibits potential as a serological marker for the initial diagnosis and prognosis prediction of patients with early PC (28,48).

Exosomal microRNAs are important tools to diagnose PC. Recent studies have also focused on elucidating miRNAs that can be used as specific markers of PC by comparing miRNA expression between patients with pancreatic cancer and healthy controls (28,48). Next-generation sequencing and reverse transcription-quantitative (RT-q)PCR analyses of exosomal microRNAs from PC are important tools used to identify biomarkers for the diagnosis of PC. For example, miR-10b, miR-550, miR-196a, miR-1246 and miR-451a have all been experimentally confirmed to be enriched in PC exosomes and can be used as markers for the early diagnosis of PC (49-52). Regarding miRNA-based RT-PCR assays,

a recent study designed Bulge-Loop miRNA RT-qPCR primer sets (one RT primer and a pair of quantitative PCR primers for each set) for four types of miRNAs, namely, miR-21, miR-17-5p, miR-155 and miR-196a. MiRNAs in serum from 49 patients, including 22 patients with PC, six patients with benign pancreatic tumours, seven patients with ampullary carcinomas, six patients with chronic pancreatitis patients and eight healthy volunteers, were used. The clinicopathological data were collected and the patients with PC were classified according to the presence and absence of metastasis, tumour differentiation and advanced stage. Patients with PC had higher expression levels of serum exosomal miR-17-5p and miR-21 compared with the control group, suggesting that the miRNAs in serum exosomes represent serum markers for PC diagnosis (53). Notably, the expression profiles of exosomal miR-21 and miR-17-5p were significantly enhanced in patients with PC compared to multiple controls, whereby this difference may be used to distinguish patients with PC from patients with non-malignant chronic pancreatitis (54). Another study focused on investigating whether exosomal miRNAs can be used to localize PC. Exosomes were collected from conditioned media of PC cell lines and plasma samples from patients with localized PC (stage I-IIA, n=15) and healthy subjects (n=15). The cells and exosomal miRNAs from the pancreatic cancer cell lines were profiled via next-generation sequencing, and the plasma exosome miRNA expression was detected via RT-qPCR analysis. This experiment confirmed that miR-196a and miR-1246 are highly enriched in PC exosomes. Consistently, the plasma exosome miR-196a and miR-1246 levels were significantly higher in patients with PC compared with the controls. The control group included patients with other pancreatic diseases. Furthermore, when combined with cancer subtypes in the analysis, plasma exosome miR-196a was a better indicator of PDAC, whereas plasma exosome miR-1246 was significantly elevated in the patients with intraductal papillary mucinous neoplasms. Conversely, miR-196a and miR-1246 levels did not differ between patients with pancreatic neuroendocrine tumours and the healthy subjects (51).

Madhavan *et al* (27) simultaneously assessed both serum exosomal proteins and miRNA markers derived from the supernatant of a PC cell line and a gene microarray of patients with PC, respectively. The PC initiating cell (PaCIC) markers, CD44v6, Tspan8, EpCAM, MET and CD104 were detected via flow cytometric analysis. The serum exosomes and exosome-depleted serum were assessed for miR-1246, miR-3976, miR-4306 and miR-4644 via RT-qPCR analysis. As a result, the concomitant evaluation of PaCIC and miRNA serum-exosome markers exhibited significantly improved sensitivity [1.00; 0.95-1], with a specificity for PC of 0.80 (CI, 0.67-0.90) and 0.93 (CI, 0.81-0.98) after excluding the non-malignant tumours, compared with all the other groups. Thus, assessing the expression levels of initial tumour cell markers and miRNAs in serum exosomes from patients with PC can significantly improve the sensitivity of the serological detection of PC, and differentiate patients with PC from healthy subjects, patients with non-malignant chronic pancreatitis and patients with benign pancreatic lesions, with a specificity of ~93% (27).

Table I. Specific types and functions of different miRNAs and their associations with pancreatic cancer.

Author/Year	miRNA/Protein	Physiological function	(Refs.)
Joshi <i>et al</i> (49), 2015	miR-10b	Early diagnostic markers of PC	(49)
Que <i>et al</i> (53), 2013	miR-17-5p	Early diagnostic markers of PC	(53)
Charrier <i>et al</i> (60), 2014	miR-21	1. Promote transformation of pancreatic epithelial cells into stromal cells 2. Promote metastasis of hypoxic tumour cells 3. Early diagnostic markers of PC	(53,60,102)
Chen <i>et al</i> (85), 2017	miR-23b-3p	1. Promote pancreatic cancer cell proliferation and migration, and upregulate CXCL1/CXCL2 2. Associated with CA19-9 levels	(85)
Wu <i>et al</i> (64), 2019	miR-126-3p	Downregulate ADAM9 and inhibit proliferation, invasion and metastasis of PC cells	(64)
Richards <i>et al</i> (62), 2017	miR-146a	Increased secretion due to Snail upregulation, and induce proliferation and chemoresistance formation of PC cells	(62,63)
Pang <i>et al</i> (61), 2015	miR-155	1. Promote the proliferation of pancreatic interstitial cells 2. Early diagnostic markers of PC 3. Involved in the formation of chemoresistance	(53,61,104-106)
Matsushita <i>et al</i> (71), 2016	miR-196a	Sensitive index for early diagnosis of PDAC	(71,93)
Zhou <i>et al</i> (97), 2014	miR-203	Downregulate the expression of TLR4, as well as downstream cytokines in DCs, and promote formation of immune suppression	(97)
Ding <i>et al</i> (96), 2015	miR-212-3p	Inhibit the expression of MHC II and induce the formation of immunological tolerance in DCs	(96)
Wang <i>et al</i> (82), 2018	miR-301a-3p	Activate PTEN/PI3K γ signalling pathway and promote metastasis of PC cells	(82)
Li <i>et al</i> (83), 2018	miR-338	Regulate the expression of MACC1, and promote metastasis and invasion of PC cells	(83)
Takikawa <i>et al</i> (84), 2017	miR-451a	1. Promote pancreatic cancer cell proliferation and migration, and upregulate CXCL1/CXCL2 2. Early diagnostic markers of PC	(52,84)
Taller <i>et al</i> (50), 2015	miR-550	Early diagnostic markers of PC	(50)
Taller <i>et al</i> (50), 2015	miR-1246	Sensitive index for early diagnosis of PDAC	(50)
Madhavan <i>et al</i> (27), 2015	miR-3976, miR-4306, miR-4644	Early diagnostic markers of PC	(27)

Macrophage migration inhibitory factor is highly expressed in pancreatic cancer-derived exosomes. Based on a study by Madhavan *et al* (27), a novel experiment recently investigated whether exosomal miRNAs in saliva can be used as biomarkers of pancreatobiliary tract cancer. Saliva was collected from 12 patients with pancreatobiliary tract cancer; the exosomal miRNAs in the saliva were extracted via RT-qPCR, and the results demonstrated that the expression levels of miR-1246 and miR-4644 were significantly higher in the cancer group compared with the controls. These results suggest that miR-1246 and miR-4644 in salivary exosomes may be candidate biomarkers of pancreatobiliary tract cancer. Macrophage migration inhibitory factor (MIF) is highly expressed in pancreatic cancer-derived exosomes, and its inhibition prevents the formation of premetastatic niches and the progression of PC (55). Compared with patients whose pancreatic tumours did not progress, patients with stage I PC who subsequently developed liver metastasis exhibited a significantly increased expression of MIF, indicating that

exosomal MIF plays an important role in liver metastasis, and may also be an indicator for predicting liver metastasis in the future (56).

Taken together, these studies suggest that components of exosomes are of great significance for PC diagnosis. However, due to the small sample sizes and lack of generalizability, whether exosomal miRNA detection can be used as an early diagnostic marker of PC remains to be further investigated (Table I).

4. Exosomes regulate proliferation in PC

Generally, tumour patients have more exosomes in their blood compared with healthy individuals. These exosomes are rich with proteins, lipids and nucleic acids, which play a pivotal role in interactive cell-to-cell information transfer (57). Kahlert *et al* (58) demonstrated that KRAS and p53 DNA are mutated in both PC cell lines and serum-derived exosomes, which is a very common type of mutation in PC (59). KRAS

mutations indicate the development of early intraductal tumours, while p53 mutations indicate the transition of tumours from a low to high grade (58,59).

Exosomes released from PC cells also have a stimulatory effect on pancreatic stellate cells (PSCs), which remain quiescent in healthy individuals. PSCs constitute a characteristic type of pancreatic stromal cells, similar to the population of stellate cells in the liver or other organs and can be converted into activated myofibroblasts upon appropriate stimulation. Activated PSCs can release exosomes containing miR-21 (59). These exosomes can promote the transformation of pancreatic epithelial cells into stromal cells, enhance their proliferative ability and promote the proliferation of stromal cells (60). In addition, it has been demonstrated that PC cells promote mesenchymal proliferation by releasing exosomes rich with miR-155 (61).

Recently, studies investigating cancer-associated fibroblasts (CAFs) have gradually increased (62-65). CAFs, which develop from bone marrow derived MSCs, are cellular components of the desmoplastic stroma that are characteristic of tumours and inextricably associated with the proliferation of PC cells. CAFs treated with gemcitabine exhibit significantly increased exosome release, which consequently increases the proliferation and survival of PC cells (61). Mechanistically, correlative studies have demonstrated increased expression of Snail (Snai1) and the Snail target, mRNA-146a, in these exosomes. Furthermore, inhibiting the release of CAF exosomes can decrease the proliferation and survival of PC cells (62). Notably, the same MSCs inhibit the progression of PC. It has been demonstrated that MSCs downregulate metalloproteinase-9 by overexpressing exosomes carrying miR-126-3p, thus inhibiting the proliferation, invasion and metastasis of PC cells (63). This study aimed to elucidate how non-tumour-derived exosomes may affect the proliferation, invasion and apoptosis of PC cell lines, and highlighted the potential of miR-126-3p as a novel biomarker for the treatment of PC (64). A more advanced study suggested that a zinc protein, ZIP4, slows PC progression by decreasing the secretion of HSP70 and HSP90. A subset of exosomes mediate the transport of these HSPs (65).

These studies substantiate that the effects of exosomes on PC cell proliferation depend on the cells from which the exosomes are derived. Not all molecules carried by exosomes play a role in promoting proliferation in PC. Conversely, some signalling molecules delay the progression of PC. Undoubtedly, additional exosomal signalling pathways remain to be investigated.

Exosomes guide the premetastatic stage of PC. The liver is the most common metastatic site in PC. The liver premetastatic niche comprises Kupffer cells, hepatic stellate cells (HSCs), bone marrow-derived cells, ECM and soluble factors, such as cytokines and chemokines (66). Primary tumour cells secrete large amounts of cytokines and growth factors that promote the mobilization and replenishment of bone marrow-derived cells to future metastatic sites, and promote the formation of the tumour microenvironment. By injecting PDAC-derived exosomes into mice, Costa-Silva *et al* (56) demonstrated that exosomes play a crucial role in the liver pre-metastasis of PDAC, ultimately leading to an increased metastatic burden

in the liver. When ingested by Kupffer cells, these exosomes cause TGF- β secretion and the upregulation of fibronectin, which is an ECM component produced by activated HSCs (56). In addition, this fibrotic microenvironment enhances the recruitment of bone marrow-derived macrophages. These bone marrow-derived cells modulate the tumour microenvironment through ECM remodelling, immune suppression and inflammation. Furthermore, the experiment demonstrated that macrophage MIF is highly expressed in PDAC-derived exosomes. By blocking this signalling molecule, all successive steps of pre-metastasis niches in the liver were blocked to prevent exosome induced PDAC transfer. In addition, it was experimentally demonstrated that MIF is elevated in plasma exosomes isolated from a mouse model of PC with pancreatic intraepithelial neoplasia or PDAC injury (67). MIF expression in patients with stage I PC who subsequently developed liver metastases was significantly higher than that in patients whose pancreatic tumours did not progress (56).

Furthermore, Nielsen *et al* (68) demonstrated that metastasis-associated macrophages are exclusively derived from the bone marrow and can activate HSCs to transform into myofibroblasts, leading to the formation of a fibrotic microenvironment in the liver that supports the growth of metastatic PDAC. Simultaneously, another type of macrophages in the liver, embryo-derived tissue-resident macrophages (Kupffer cells), can promote the activation and fibrosis of HSCs, which is an important process in the formation of the premetastatic niche (69).

Tumour-derived exosomes also regulate the formation of premetastatic niches through the binding of integrins on their own membrane structure to specific target cells. For example, targeting integrins $\alpha 6\beta 4$ and $\alpha v\beta 5$ can decrease exosome uptake and decreased lung and liver metastasis, respectively. Notably, most PDAC-derived exosomes accumulate in the liver. Although exosomes were administered via retroorbital injection, they were unable to reach the lungs (70). The liver is the most common metastatic organ in PC, not only because of the anatomy associated with the entry of pancreatic blood into the liver through the portal vein but also because PDAC-derived exosomes are recruited into the liver (71). While melanoma cell-derived exosomes are taken up by liver macrophages and lung endothelial cells, the molecular mechanism by which pancreatic cancer-derived exosomes enter the liver remains unclear.

5. Mechanisms by which exosomes promote PC metastasis

Exosomes promote metastasis and invasion in PC. Exosomes are released into bodily fluids by tumour cells and participate in the formation of the tumour microenvironment. Due to its high metastatic potential and invasiveness, PC has very poor therapeutic outcomes (66). Several studies have demonstrated the important role of exosomes in promoting metastasis and invasion in PC (66,72-74). On one hand, exosomes promote this process by modulating the tumour microenvironment since the metastasis of tumour cells is closely associated with the tumour microenvironment, and hypoxia and inflammatory cell infiltration (particularly macrophage infiltration) are two important factors (75,76). Hypoxia may contribute to tumour progression by modulating cell-to-cell communication by modifying

exosome release. PC cells can produce miRNA-21-enriched exosomes to promote the metastasis of hypoxic tumour cells (77). In addition, the stabilization and activation of hypoxia-inducible factors (HIFs), particularly HIF-1 α and HIF-2 α , which activate proto-oncogenes that promote tumour growth, angiogenesis and cell metastasis, are major mechanisms by which PC cells respond to hypoxia (78). Macrophages are the most abundant infiltrating immune-related stromal cells near tumour cells. Depending on the microenvironment, macrophages can be polarized into the classically activated type (M1) or alternatively activated type (M2) (79). M1 macrophages are characterized by the expression of inducible nitric oxide synthase and are pro-inflammatory, while M2 macrophages express higher levels of anti-inflammatory cytokines and a more active arginase-1, which favours the proliferation of tumour cells (80). A hypoxic microenvironment can activate the phosphatase and tensin homologue/phosphoinositol 3-kinase (PI3K) gamma signalling pathway via the secretion of miRNA-301a-3p-enriched exosomes (81). Subsequently, M2 macrophage polarization is stimulated in a manner that induces HIF1 α or HIF2 α , thereby promoting metastasis of tumour cells (82).

On the other hand, exosomes can also directly affect the metastatic ability and invasiveness through certain signalling pathways, such as circ-PDE8A (83). Recently, a circular RNA (circ-PDE8A) was extracted from liver metastatic PDAC cells via microarray analysis, and it was demonstrated that high circ-PDE8A expression is associated with lymphatic invasion, the TNM stage and poor survival in patients with PDAC (84). Further studies revealed that circ-PDE8A promotes the invasive proliferation of PDAC cells by upregulating MET. For example, circ-PDE8A regulates metastasis-associated colon cancer-1 (MACC1) as a ceRNA of miR-338, and stimulates invasive proliferation through the MACC/MET/ERK or AKT pathways (83). In addition, PSCs can secrete exosomes carrying miR-451a, which promotes PC cell proliferation and migration, and upregulates the expression of the chemokine ligands, CXCL1 and CXCL2 (84). Overexpression of miR-23b-3p also promotes this process, and miR-23b-3p expression is associated with the serum carcinoembryonic antigen 199 levels (Fig. 1) (85).

Exosomes promote immune tolerance in PC. Macrophages can be polarized into two key phenotypes, M1 and M2 macrophages. M1 macrophages express high levels of MHC I and MHC II antigens, and secrete complement factors that promote complement-mediated phagocytosis (60,80). M1 macrophages also produce high levels of pro-inflammatory factors, such as interleukin (IL)-1, IL-6, IL-23 and TNF (80). Conversely, M2 macrophages are characterized by lower pro-inflammatory cytokine production, leading to suppression of inflammatory responses, suppression of T cell proliferation and attenuation of adaptive immune responses (27). Previous studies have demonstrated that the invasion of PC is mostly supported by M2 macrophages, which exhibit decreased phagocytosis of tumour cells, and their number is also positively associated with the degree of peripheral lymph node metastasis and early distant metastasis (80,86). Furthermore, Di Caro *et al* (87) demonstrated that most tumour-associated macrophages at the tumour-stroma interface in patients with PDAC were of the M2 type, and that the prognostic relevance of postsurgical

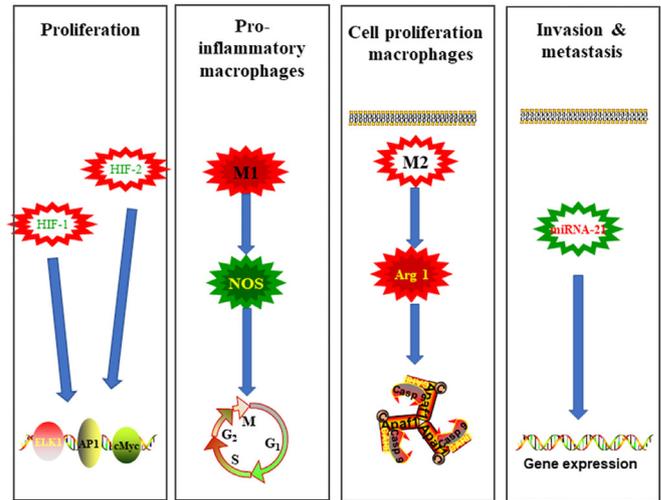


Figure 1. Mechanisms by which exosomes promote pancreatic cancer cell proliferation, invasion and metastasis.

adjuvant chemotherapy for PDAC was associated with a decrease in the density of CD206(+) and IL-10(+) macrophages at the tumour-stroma interface. In addition, exosomes extracted from the saliva of PDAC mice had inhibitory effects on immune surveillance and decreased the tumour-killing ability of NK cells (88). Exosomes inhibit the cytotoxicity of NK cells against tumour cells, which may be associated with the expression levels of TGF- β 1, MICA/MICB and myeloid blast markers (CD34, CD33 and CD117) (88). In particular, TGF- β , which downregulates the NK cell-activating receptor NKG2D, inhibits NK cell activity and cytotoxicity.

Fridlender *et al* (89) demonstrated that the switch of neutrophils to a protumour phenotype depends on TGF- β exposure, and that the recruitment of these cells to the tumour microenvironment is partially driven by macrophages. Neutrophils have also been demonstrated to be abundant at the invasive front of liver metastases in PC (90). Similar to the transformation mechanism of macrophages, neutrophils appear to adopt an alternative tumour-promoting phenotype to promote cell proliferation, angiogenesis, tumour invasion and suppression of the adaptive immune response (91). However, more cancer clinical data and additional evidence are required to determine the molecular mechanism by which exosomes regulate this type of cell.

Exosomes regulate adaptive innate immune responses in PC. Tumour-derived exosomes are widely present in the tumour microenvironment and plasma from tumour patients. These exosomes carry and deliver various stimulatory and inhibitory molecules to human immune cells, giving tumour cells the opportunity to achieve immunosuppression and immune escape. Increasing evidence suggests that T cell infiltration in the tumour microenvironment is closely associated with patient outcomes. Tumours that can escape recognition by cytotoxic T cells generally have a poor prognosis (91). In patients with PC, it has been demonstrated that a high ratio of tumour-infiltrating regulatory T cells (T-regs), defined as FoxP3(+) CD4(+) T cells, is significantly associated with shortened survival, whereas high levels of tumour-infiltrating CD4(+) T and CD8(+) T cells are significantly associated with

prolonged survival (92). Among these cells, T-regs have been demonstrated to support tumour growth and expansion by suppressing host immune responses and accelerating angiogenesis and tissue remodelling (93). Their role in the immune response from premalignant lesions to the established stage of PDAC suggests that a high prevalence of T-regs can serve as a marker for evaluating a poor prognosis (93). Increasing evidence suggests that tumour-derived exosomes have immunomodulatory properties, which are able to induce T-reg polarization, promote T-reg expansion, upregulate T-reg suppressive function and enhance T-reg resistance to apoptosis (94). The critical role of TGF- β in FOXP3 expression in T-regs was further demonstrated by Wada *et al* (95), who isolated exosomes from malignant effusions from patients with cancer to help maintain cultured T-regs. Exosomes can release TGF- β following treatment with Kupffer cells in premetastatic niches, although it is not possible to determine whether PDAC-derived exosomes contain TGF- β (54). These experiments demonstrate that exosomes can induce TGF- β production in immune cells, which may play an important role in maintaining tumour immune tolerance.

In addition, it is well known that dendritic cells (DCs) play an important role in activating immune responses. Previous studies have investigated how exosomal miRNAs derived from PC suppress mRNA expression in DCs and induce immune tolerance (96). Compared with immature DCs, exosome-stimulated DCs exhibited upregulation of 9 PC-related miRNAs and the inhibition of 208 mRNAs. This result validates the experimental prediction that regulatory factor X-associated protein (RFXAP), which is an important transcription factor of MHC II, is inhibited by miR-212-3p transferred from PC-secreted exosomes, resulting in decreased MHC II expression. In addition, miR-212-3p was negatively associated with RFXAP in PC tissues (97). Based on this study, it appears that PC-derived exosomes inhibit RFXAP expression via miR-212-3p, thereby decreasing MHC II expression and inducing immune tolerance in dendritic cells (96). Another study aimed to investigate the effects of exosomes on Toll-like receptors (TLRs) in DCs. The effect of miR-203 on TLR4 and downstream cytokines was studied as an entry point. First, it was established that miR-203 is expressed in PANC-1 cells and exosomes, and that its levels are upregulated in exosome-treated DCs. The results demonstrated that TLR4 expression was decreased in DCs treated with exosomes and miR-203, while TLR4 was increased in exosome-treated DCs treated with miR-203 inhibitors. The expression levels of tumour necrosis factor- α and IL-12 also decreased following treatment with exosomes and miR-203, and increased in exosome-treated DCs treated with miR-203 inhibitors. In conclusion, PC-derived exosomes downregulate TLR4 and downstream cytokines in DCs via miR-203 (97).

Exosome-induced chemoresistance in PC. Recently, gemcitabine-based chemotherapy regimens have remained the mainstay of treatment for advanced or metastatic PC. However, with the activation of oncogenic miRNAs, anti-apoptotic enzymes and signalling pathways associated with cellular chemoresistance, PC cells have gradually developed resistance to chemotherapy (98). In addition, stromal tissues in PC are characterized by low blood perfusion and hypoxia; thus, the dense stroma can affect the release of chemotherapeutic agents

through physical barriers, high interstitial pressure, compression of blood vessels and dense stromal cells. Exosomes are important vehicles for intercellular communication between genes and signalling molecules (98). Previous studies have demonstrated the important role of exosomes in the chemoresistance of other types of cancer cells, such as lung, breast, prostate and gastric cancer cells (99-103). In addition, several experiments have indicated that exosomes can improve the resistance of PC cells to chemotherapeutic drugs through various molecular mechanisms (99).

Exosomes derived from PC can deliver multiple drug resistance-associated miRNAs and proteins to target cells to decrease chemotherapy efficacy. CAFs occupy most of the tumour volume in PDAC (98). Previous studies have demonstrated that CAFs are intrinsically resistant to gemcitabine and that CAFs exposed to gemcitabine significantly increase exosome release (62). These exosomes upregulate the expression of the chemoresistance-inducing factor Snail and the Snail target, mRNA-146a, in recipient epithelial cells and promote proliferation and chemoresistance in PC cells (98). Further treatment of gemcitabine-exposed CAFs with the exosome release inhibitor, GW4869, significantly decreases the survival rate of the exosomal recipient epithelial cells, indicating the important role of CAF exosomes in chemoresistance (62). Given that chemoresistance can spread to all PDAC tissues in a patient's body, it is assumed that a series of specific miRNAs are involved in this process, and that changes in their expression levels or related cellular communication factors can affect the development of chemoresistance (98). miRNA-155 is a typical miRNA that has multiple effects mediated by its downstream genes and is involved in several physiological and pathological processes, such as inflammation, immunity and tumour development. A recent study suggested the existence of an acquired chemoresistance mechanism in PC that is mediated by miRNA-155. Conditioned medium from PC cells pre-treated with gemcitabine provided significant chemoprotection against subsequent gemcitabine toxicity. A gene expression analysis demonstrated that superoxide dismutase 2 (SOD2) and catalase (CAT) are upregulated, while deoxycytidine kinase (DCK) is downregulated in PC cells following this pretreatment (62). Previous studies have suggested that exosomes may increase the level of the ROS detoxification gene expression products, SOD2 and CAT, by lateral transfer of their transcripts, resulting in chemoresistance in pre-treated PC cells (104,105). However, exosomes secreted by pre-treated PC cells can also interfere with the metabolic process of gemcitabine by inhibiting the activity of DCK via miRNA-155. Furthermore, chronic exposure to gemcitabine can increase miR-155 expression (105). The increase in miR-155 expression continues to promote the secretion of exosomes and the formation of chemoresistance capacity, thereby forming a positive cycle through which exosomal miR-155 regulates chemoresistance (106).

Inhibitor of apoptosis protein (IAP) can promote apoptosis in tumour cells; however, its expression significantly decreases in different types of tumour cells (54). In a study on PC tissues and cell lines, Asuncion Valenzuela *et al* (107) demonstrated that IAP expression is significantly upregulated by nuclear factor- κ B. Exosomes derived from PC contain the associated mRNA of IAP, and following chemotherapy, the protein

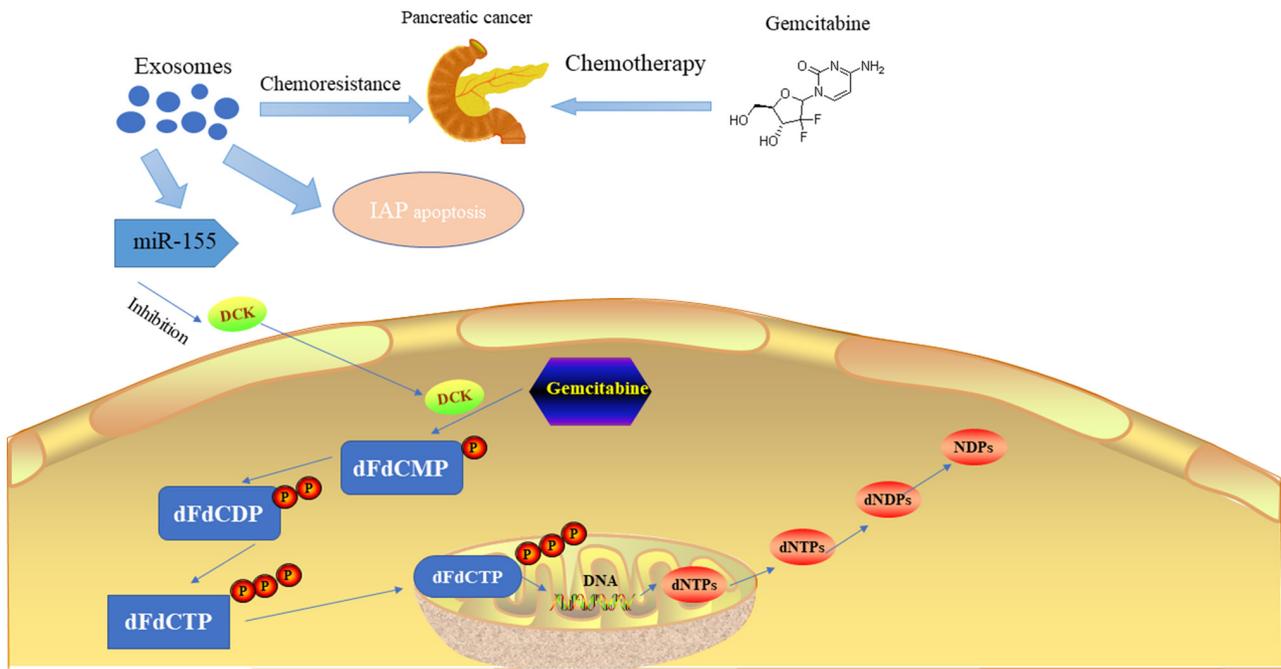


Figure 2. Mechanism of exosome induced chemoresistance in pancreatic cancer.

or mRNA IAP levels in the cytoplasm of PC cells remain unchanged or moderately upregulated. Thus, exosomes may also enhance the resistance of PC cells to chemotherapeutic agents by inhibiting IAP expression. Another study reported that exosomal lipids can induce chemoresistance in human pancreatic tumour cells (MiaPaCa-2) through CXCR4-stromal cell-derived factor-1 α signalling (43). A recent study identified a candidate chemoresistance transfer factor, Ephrin type-A receptor 2 (EphA2). Exosomes from chemoresistant PANC-1 cells increased the resistance of MIA PaCa-2 and BxPC-3 cells to gemcitabine, and exosomes can be isolated from chemoresistant PANC-1 cells overexpressing EphA2. However, treatment of MIA PaCa-2 and BxPC-3 cells with soluble EphA2 did not promote chemoresistance, indicating that membrane-borne EphA2 is important for the EphA2 chemoresistance effect (108). Collectively, exosomes can promote chemoresistance in PC cells by regulating miRNAs, proteins, lipids and signalling pathways. However, the regulatory molecular mechanism of exosomes requires verification and further investigation in additional experiments (Fig. 2).

6. Exosomes and novel PC therapies

Exosomes have the potential to be targeted therapeutic carriers affecting processes, such as cell signalling and material transport, because of their low immunogenicity, nontoxicity and highly stable biological characteristics in blood. Compared with other carriers, exosomes not only retain a more stable lipid bilayer structure to protect their cargoes but also have a higher targeted delivery capacity, and their smaller diameter further ensures that they can selectively enter tumour tissues to achieve better therapeutic effects (43). A previous study has increasingly focused on how exosomes as natural endogenous carriers can be used to deliver certain interfering signals or transport cancer drugs (109).

Mutations in the KRAS gene are widely present in several malignancies, and G12D and G12V mutations at codon 12 in the second exon are the most common in PDAC, accounting for 70-95% of cases (43). The combination of GTP enzymes and KRAS mutant genes is a key driver of PC. Normally, KRAS is inactivated immediately after activation (109). However, following KRAS gene mutation, the KRAS protein maintains a continuous activation state and no longer depends on stimulation by an upstream signal; thus, the KRAS protein is in a state of continuous binding with GTP, leading to abnormal activity in downstream signalling pathways, such as PI3K-AKT-mTOR, thereby promoting tumour cell proliferation, transformation, adhesion and survival (109). Buscail *et al* (110) targeted, silenced and delivered KRAS G12D via the exosome-mediated delivery of small interfering (si)RNA to PC cells. As a result, the proliferation and metastasis of the cancer cells significantly decreased. Recent studies have also investigated a novel approach to directly and specifically target oncogenic KRAS in tumours by using engineered exosomes known as iExosomes (110). siRNA targeting mutant KRAS was introduced into fibroblast-derived exosomes to generate iExosomes (104). As this exosome carries CD47, it decreases the clearance of iExosomes in circulation by monocytes and macrophages. Subsequently, the iExosome is delivered to cancer cells, thereby inhibiting KRAS GTPase activity and the activation of the downstream MEK-ERK or PI3K-AKT-mTOR signalling pathways, ultimately inhibiting tumour growth and metastasis (111).

Another strategy for exosome-targeted therapy is inhibiting the uptake of exosomes by recipient cells. Heparan sulfate proteoglycans (HSPGs) serve as internalization receptors for tumour cell-derived EVs with exosome-like characteristics (111). Internalized exosomes colocalize with syndecan and glypican types of cell surface HSPGs, and the uptake of

exosomes is specifically inhibited by free heparan sulfate (HS) chains, thus suggesting that tumour cell-derived exosomes use HSPGs for internalization and functional activity (111). In addition, syntenin genes can bind the cytoplasmic tail of syndecans, which are internalized into sorting endosomes along with their intact HS chains (112). In target cells, syntenin genes are also involved in maintaining the pool of HSPG at the cell membrane by stimulating the recycling of the intact form of HSPG through direct interaction with phosphatidylinositol 4,5 biphosphate. Mouse fibroblasts isolated from syntenin knockout mice exhibited low amounts of HSPGs that were associated with the decreased uptake of exosomes, further confirming that the presence of HSPGs is essential for the efficient internalization and function of exosomes (112). In addition, the syntenin gene is a potential target for future cancer therapy (112).

Due to the nontoxicity and low immunogenicity of exosomes in serum, recent experiments have used exosomes as targeted carriers of chemotherapeutic agents to avoid causing systemic chemotherapy toxicity in off-target tissues and achieve better therapeutic effects (113). MSCs have been proposed for the delivery of anticancer agents because of their ability to home in on the tumour microenvironment. MSCs can acquire strong antitumour activity after priming with paclitaxel (PTX) through their capacity to take up and then release the drug (114). Pascucci *et al* (113) loaded exosomes secreted by MSCs with PTX to inhibit the proliferation of PANC-1 cells, verifying the possibility of using exosomes to package and deliver active drugs. Subsequently, Kim *et al* (114) added PTX-loaded exosomes to drug-resistant cells and obtained good anticancer effects in a mouse model. These experiments suggest the feasibility of this novel exosome system for further development to carry other chemotherapeutic agents in the future.

7. Conclusions and future perspectives

The molecular composition of exosomes reflects the specialized functions of their cells of origin. Through their ability to bind target cells, exosomes are likely to modulate selected cellular activities, such as vascular homeostasis and antigen presentation. The presence of exosomes in blood and tissues *in vivo* suggests their participation in physiological and/or pathological processes, such as PC. Their lipid composition and presence of proteins that protect against complement, such as CD55 and CD59, may contribute to their stability in the extracellular environment. The following advantages of an exosomal-acellular mode of communication should aid the development of diagnostic and therapeutic strategies: Exosomes are non-living, contain sorted sets of molecules involved in different cellular processes, have the capacity to transmit antigenic information and can be easily recovered from fluids. Based on these properties, diagnostic protocols, and clinical assays for anti-tumoural immunotherapy are under development. However, the advantages must be weighed against the potential consequences that exosomes pose to human health as exosomes may be used by tumour cells to invade normal tissue and pathogens, such as prions and HIV, to maximize their spread between cells.

The present review discusses the interactions between exosomes and the malignant processes of PC. The potential clinical applications of exosomes are also discussed. First, the biological characteristics of exosomes are introduced. Subsequently, exosomes as diagnostic markers of PC are discussed. In addition, exosomes regulate the proliferation, metastasis and invasion of PC cells. Several mechanistic questions remain, thus prospective studies are required to confirm current findings. Furthermore, exosomes that promote immune tolerance and induce chemoresistance are discussed in the present review, along with novel therapies for PC. The establishment of new bioanalytical technologies and novel experimental animal models may help researchers uncover the secrets of exosomes. In the present review, the molecular mechanisms and functions of several exosomes were introduced in detail. The roles of these proteins in malignancy were also discussed, with the ultimate aim of determining the exact role of exosomes in human cancer cells.

Recently, increasing evidence suggests that exosomes are associated with cancer and have the capacity to accelerate PC cell proliferation, migration and invasion (113,114). Prospective studies are required to provide detailed information regarding the molecular mechanism by which exosomes regulate the proliferation of tumour cells, and help establish exosomes as novel targets for the treatment of PC. It is speculated that the application of exosomes for the treatment of PC can be highly effective, suggesting that all human malignant tumours and associated challenges can be overcome in the future. Recent studies have demonstrated that some technical methods, such as engineering and scientific modification of exosomes may treat PC (113-115). Xu *et al* (116) developed a surface modification method for *in vitro* and *in vivo* exosome uptake research through Click Chemistry, which can be used as the basis for rational design of preclinical exosome therapy. A number of studies have reported the potential of using exosomes as nano-carriers to improve exogenous loaded drug therapy as a novel treatment strategy for PC (16,116-118).

As an important tool for communication and transport, exosomes mediate cell-to-cell information exchange by transmitting their carried molecules, such as miRNAs and proteins, to recipient cells, affecting several physiological functions, such as the growth and metabolism of recipient cells (16). Recent studies concerning exosomes have demonstrated their important role in the regulation of PC proliferation, apoptosis, metastasis and other processes, providing a new perspective for the understanding of the molecular mechanism underlying the malignant biological characteristics of PC, and are expected to provide a new direction for the treatment and early diagnosis of PC (119-121). Although current research and experimental results are novel and exciting, there are still many questions regarding the molecular mechanism of exosomes that remain to be further investigated.

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HL and SH contributed to the design and drafted the initial manuscript. HL and SQ revised the manuscript for important intellectual content. HL, XF, YG and YZ wrote and prepared the original draft of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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