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The Role Exosomes Played on Pancreatic Cancer

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Abstract

Pancreatic Cancer (PC) is one of the human digestive malignant tumor which has a poor prognosis. Exospores, containing proteins, nucleic acids and enzymes, are a class of extracellular vehicles defined as 40 nm to 150 nm diameter membrane nanovesicles of endocytic origin. As we all know that exosomes work as potential diagnostic markers for pancreatic cancer. Exospores in cancer initiation and progression are currently unclear. Our study focuses on the mechanisms and functions of exosomes that can accelerate pancreatic cancer cell proliferation, migration, and invasion. This review highlights our understanding of the interaction between exosomes and the malignant biology of pancreatic cancer. And we will also discuss the possible clinical application of exosomes.

Keywords: Exosomes; Pancreatic cancer; Tumor proliferation; Migration

Introduction

Pancreatic Cancer (PC) is one of the digestive malignancies which mainly arise from ductal epithelium and acinar cells. It has a very poor prognosis with a 5-year survival rate of less than 5%, and patients with advanced pancreatic cancer have a shorter survival time of about 3 to 6 months [1]. There were 432,242 new deaths in 2018 due to treatment delays caused by difficulties in early diagnosis of PC. Meanwhile, PC ranks 13th in prevalence worldwide, with 458,918 new cases last year and high rates in Europe and North America [2]. PC is mainly ductal cell carcinoma, a few of which are acinar cell carcinoma, acantho cutaneous carcinoma of the pancreas and cystadenocarcinoma. With the insensitivity of pancreatic cancer to chemotherapy, surgical resection remains the mainstay of treatment. Pancreatic cancer, however, is curable in only a minority of patients with locally respectable tumors, accounting for only 5% to 10%, with a survival rate of only 10% to 20% more than 5 years after surgery [3]. The reason why the poor prognosis of pancreatic cancer is that strong invasion ability, malignant biological mechanism has not been elucidated exosomes, containing proteins (cytoskeletal proteins, transmembrane proteins, and heat shock proteins, etc.), nucleic acids (DNA, mRNA, miRNA, long and short non-coding RNA) and enzymes (GAPDH, ATPase, pgk1, RAB, etc), are a class of Extracellular Vehicles (EVs) defined as 40 nm to 150 nm diameter membrane nanovesicles of endocytic origin [4-6]. The molecular contents of exosomes can reflect the nature and state of original cells, and it is these contents that alter the function of recipient cells [7]. Since the discovery and naming of exosomes by Johnstone et al. in 1987, the process of exosomes formation has been elaborated in detail, which is a more complex process: First, the membrane domain is endocytosed to form early endosomes, and then through the process of budding, forming intraluminal vesicles, which further become Multivesicular Bodies (MVBs) by encapsulating proteins, nucleic acids, and other substances. One part of these MVBs is degraded in lysosomes, and the other part fuses with the cell membrane and releases internal vesicles to form exosomes. The final steps in exosomes 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].

Recently, research on the role of exosomes in tumor growth and cancer metastasis has grown exponentially. From tumor growth to cell metastasis, the intricate exosomal communication networks between tumor and non-tumor cells directs every step of tumor biological change. Tumor cells develop exosome-based mechanisms that promote favorable microenvironments to support tumor growth by enhancing cell metastasis and avoiding apoptosis [10]. Besides, cancer exosomes also have the ability to induce neovascularization, which ensures the obtain of nutrients,

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oxygen, and removal of waste, and contribute to tumor cells sustained proliferation [7,11]. The invasion and dissemination of tumors is highly enhanced by cancer exosomes, which carry information that contributes to Extra Cellular Matrix (ECM) remodeling, cancer cell migration, and invasion [12,13]. Moreover, it has been demonstrated that exosomal communication contributes to tumor immune escape and metastatic niche preparation [14-18]. This review highlights our understanding of the interaction between exosomes and the malignant biology of pancreatic cancer. And we will also discuss the possible clinical application of exosomes.

Biological Characteristics of Exosomes

Exosomes, a subpopulation of small extracellular vesicles, arise from the membranes of Multi-Vesicular Bodies (MVB) and are released from the cell into the extracellular environment with the plasma membrane [18]. Including stroma cell, reticulocytes, epithelial cells, and tumor cells, almost all the live cells can release exosomes, which have been isolated from blood plasma, serum, urine, bile, saliva and breast milk [4,6,19-32]. Exosomes are small, membrane-enclosed vesicles (30 nm to 150 nm) that can deliver cargo (proteins, lipids, and nucleic acids) from the originating cells to the recipient cells [13]. Recently, a type of small vesicle released from Marrow Mesenchymal Stem Cell (MSC) - derived exosomes has been shown to transfer functional RNAs to recipient cells, indicating their promise as an alternative for cell-based therapy [14]. Especially, it was reported that exosomes could carry microRNAs (miRNAs), which are involved in cancer cell proliferation, differentiation, and apoptosis [15,16]. Moreover, as tumor suppressors or oncogenes, miRNAs regulate gene expression post-transcriptionally [17]. According to proteomic analyses, some proteins associated with cytosolic signaling proteins, cell surface receptors, antigen presentation, metabolic enzymes, Major Histocompatibility Complex (MHC), Heat Shock Protein (HPSs, as HSP70, HSP90, HSP60, HSC70), and tetraspanins (CD9, CD63, CD81, CD82) are selectively enriched in specific exosomes [33,34]. Some of the above proteins participate in the normal physiological activities of exosomes, while others 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]. And another class of proteins is associated with the specificity of original cell, such as melanoma exosomes express the tumor-associated protein Melanoma Antigen Recognized by T cell 1 (MART1); tumor exosomes of epithelial cell origin express Epithelial Cell Adhesion Molecule (EpCAM) [36,37].

Exosomes are secreted by various cell types and play important roles in cellular communication. However, the known underlying mechanisms by which microvesicles are released into the extracellular space are limited to three: Exocytose exosomes from the intracellular MVBs, SMVs from the PM and ABs from cells undergoing apoptosis. Exosomes have pleiotropic effects that influence the physiology of neighboring cells. Of these, the best studied (*in vitro*) are the roles of exosomes in various stages of the Immune response (interactions with immune cells). These range from exosomes being a vehicle for antigen presentation to antigen-independent roles that can inhibit (immunosuppressive properties) or promote immune responses (immune-activating properties). Additionally, exosomes play role in intercellular communication, being conveyors of proteins and lipids that affect downstream signaling events in recipient cells. They can also deliver genetic material that affects the physiology of recipient cells.

Some nucleic acids and lipids also show a highly selective enrichment [5,8,23-25]. Nucleic acids include microRNA (miRNA), messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and non-coding RNA (ncRNA). Among them, miRNAs, one kind of non-coding RNA with a length of 19~25 nt, 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 a variety of cells, and participate in important biological processes such as cell proliferation, differentiation, apoptosis, and metabolism [40]. As for the lipid molecules in exosomes, there is no doubt that they also show great research potential in pancreatic cancer. The majority of lipid molecules of exosomes are located on membranes, including sphingomyelin, cholesterol, ceramide and phosphatidylserine [6]. Studies have found that sphingomyelin and cholesterol can improve the stability of exosomal phospholipids bilayer. Besides, phosphatidylserine can promote the fusion of exosomes and target cell membranes, and can also participate in signal transduction as a signal molecule [41]. Meanwhile, it is found that exosomal lipids can induce apoptosis in human pancreatic cancer SOJ-6 cells by inhibiting the Notch1 pathway. Exosomal lipids can also induce drug resistance in human pancreatic cancer cells through the C-X-C motif Chemokine Receptor 4 (CXCR4)/Stromalcell-Derived Factor 1α (SDF1α) signaling pathway [42,43].

Exosomes are membrane vesicles that are released by cells upon fusion of multivesicular bodies 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 harbor unique subsets of proteins linked to cell type-associated functions. Exosome secretion participates in the eradication of obsolete proteins but several findings, essentially in the immune system, indicate that exosomes constitute a potential mode of intercellular communication. Release of exosomes by tumor cells and their implication in the propagation of unconventional pathogens such as prions suggests their participation in pathological situations.

Exosomes Work as Potential Diagnostic Markers for Pancreatic Cancer

Pancreatic cancer is a major threat to human health with very few effective therapies and prognoses [1-3]. As the fourth primary death cause of all different cancers, the survival rate of pancreatic cancer is incredibly low [3]. Despite improvements made to pancreatic cancer therapies, the mortality of the disease has remained more or less the same over the past several decades, largely due to the lack of adequate screening methods and biomarkers for early diagnosis.

Progress in the treatment of Pancreatic Aden Carcinoma (PDAC) remains elusive despite substantial time and resources invested in the attempt to improve the dismal prognosis. Pancreatic cancer is a malignant disease that develops rapidly and carries a poor prognosis. Currently, surgery is the only radical treatment. The American Cancer Society estimates that about 53,070 people will be diagnosed with and about 41,780 will die of pancreas cancer in 2016 with the most recent SEER database reporting a 7.7% five-year survival rate from 2006 to 2012. Early diagnosis of pancreatic cancer is difficult due to the lack of specific symptoms. At current stage of clinical treatment, we mainly rely on imaging examination for qualitative and positional diagnosis of PC. The serum marker CA 19-9 is also used, but the specificity of CA 19-9 for pancreatic cancer was not

high. Especially in patients with early pancreatic cancer, only 40% of patients with early pancreatic cancer have elevated serum CA 19-9 levels, while many patients are diagnosed with an advanced disease [44,45]. Therefore, the search for new early diagnostic markers, and can be used to differential diagnosis of pancreatic cancer and other benign lesions, has become the focus of pancreatic cancer diagnosis and treatment.

Glypican 1 (GPC1) is a lipid raft-heparin sulfate proteoglycan located on the cell surface that is involved in several important cellular signaling pathways [46]. It has been demonstrated that down-regulation of GPC1 expression in the pancreatic cancer cell line PANC-1 can slow down the growth rate of pancreatic cancer cells and reduce the ability of angiogenesis and metastasis in pancreatic cancer. That is both cancer cell- and host-derived GPC1 are crucial for full mitogenic, angiogenic, and metastatic potential of PC cells [47]. Using flow cytometry to detect and isolate GPC1 from serum exosomes of pancreatic cancer patients and mouse models of pancreatic cancer, Melo et al. demonstrated that GPC1 is enriched in exosomes from pancreatic cancer. Moreover, GPC1 in these exosomes has high specificity and sensitivity, which has the potential as a serological marker for initial diagnosis and prognosis of patients with early pancreatic cancer [28]. A recent study showed that high GPC1 crExos may be able to determine PDAC tumor size and disease burden. However, further studies using larger cohorts are needed to validate this conclusion [48].

Certainly, recent studies have also focused on elucidating miRNAs that can be used as specific markers in PC by comparing miRNA expression between pancreatic cancer patients and healthy controls. The next generation sequencing and qRT-PCR analysis of exosomal micro RNAs from PC are important tools to identify biomarkers for 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 pancreatic cancer exosomes, which can be used as markers for early diagnosis of pancreatic cancer [49-52]. For miRNA-based RT-PCR assay, a recent study designed Bulge-Loop miRNA qRT-PCR Primer Sets (one RT primer and a pair of quantitative PCR primers for each set) for four kinds of miRNAs, miR-21, miR-17-5p, miR-155, and miR-196a. MiRNA in the serum of 49 patients was used, including 22 PCs patients, 6 benign pancreatic tumors, 7 ampullary carcinomas, 6 chronic pancreatitis and 8 healthy volunteers. Meanwhile, clinic pathological data were collected, and PC patients were classified according to the presence and absence of metastasis, tumor differentiation and advanced stage. As a result, compared with the control group, serum exosomal miR-17-5p and miR-21 were highly expressed in PC patients, further confirming that detection of micro RNAs in serum exosomes is a meaningful serum marker for PC diagnosis [53]. Interestingly, the expression profiles of exosomal miR-21 and miR-17-5p were significantly enhanced in pancreatic cancer patients compared to multiple controls, and this difference could be used to distinguish pancreatic cancer from non-malignant chronic pancreatitis patients [54]. Another study focused on evaluating whether exosomal miRNAs could indicate localized pancreatic cancer. Exosomes were collected from conditioned media of pancreatic cancer cell lines and plasma samples from patients with localized pancreatic cancer (stage I-IIA, n=15) and healthy subjects (n=15). The cells and exosomal miRNAs from pancreatic cancer cell lines were profiled by next generation sequencing and plasma exosome miRNA expression was analyzed by qRT-PCR. This experiment confirmed that miR-196a and miR-1246 are highly enriched in pancreatic cancer exosomes. Consistently, plasma exosomes miR-196a and miR-1246 were significantly higher in pancreatic cancer patients than controls. Further combined with cancer subtypes for analysis, plasma exosome miR-196a is a better indicator of Pancreatic Ductal Aden Carcinoma (PDAC), whereas plasma exosome miR-1246 is significantly elevated in patients with intraductal papillary mucinous neoplasm. On the contrary, both miR-196a and miR-1246 levels have no difference between patients with pancreatic neuroendocrine tumors and healthy subjects [51].

Madhavan et al. simultaneously examined both serum exosomal proteins and miRNA markers, which were derived from the supernatant of PC cell line and the gene microarray of PC patients, respectively. Pancreatic cancer initiating cell (PaCIC) markers CD44v6, Tspan8, EpCAM, MET and CD104 were then measured by flow cytometry. Serum-exosomes and exosome-depleted serum was tested for miR-1246, miR-3976, miR-4306and miR-4644 recovery by qRT-PCR. As a result, the concomitant evaluation of PaCIC and miRNA serum-exosome marker significantly improved the sensitivity (1.00, CI: 0.95 to 1), with a specificity of PC was 0.80 (CI: 0.67 to 0.90), and 0.93 (CI: 0.81 to 0.98) after excluding non-malignant tumors compared with all other groups. That is, assessing the expression levels of initial tumor cell markers and miRNAs in serum exosomes from pancreatic cancer patients can significantly improve the sensitivity of serological detection of pancreatic cancer, and also differentiate pancreatic cancer patients from healthy subjects, patients with non-malignant chronic pancreatitis and patients with benign pancreatic lesions with a specificity of about 93% [27]. Based on the study of Madhavan et al., a novel experiment recently explored whether it could be a biomarker for pancreatobiliary tract cancer by detecting exosomal miRNAs in saliva. Saliva from 12 patients with pancreatobiliary tract cancer was collected, exosomal miRNAs in the saliva were isolated and extracted, using quantitative real-time PCR (RT qPCR), and the results were that the expression of miR-1246 and miR-4644 was significantly higher in the cancer group than controls. This study suggests that miR-1246 and miR-4644 in salivary exosomes may be candidate biomarkers for pancreaticobiliary tract cancer [55]. In addition, macrophage Migration Inhibitory Factor (MIF), previously mentioned in this paper, is highly expressed in pancreatic cancer-derived exosomes, and its inhibition prevents the formation of pre-metastatic niches and the progression of PC. Compared with patients whose pancreatic tumors did not progress, MIF is significantly increased in patients with stage-I PC who later developed liver metastasis, indicating that exosomal MIF plays an important role in liver metastasis and may also be an indicator to predict liver metastasis in the future [56]. The above studies showed that components in exosomes are of great significance for PC diagnosis. However, due to the small study sample size and lack of universality, it remains to be further explored whether exosomal miRNA detection can be used as an early diagnostic marker for PC (Table 1).

Exosomes Regulate Proliferation of Pancreatic Cancer

Exosomes Promote Metastasis and Invasion of Pancreatic Cancer. Exosomes are released into body fluids by tumor cells and participate in the formation of tumor microenvironment. Generally, tumor patients have more exosomes in their blood than normal people [57]. These exosomes are rich in proteins, lipids, and nucleic acids, which play a pivotal role in cell-to-cell interactive information transfer. Kahlret et al. demonstrate that KRAS and p53 DNA are mutated

Table 1: Specific types and functions of different miRNAs and their associations with pancreatic cancer.

Author/Year	miRNA/Protein	Physiological function	References
Joshi GK et al., 2015	miR-10b	Early diagnostic markers of PC	[49]
Que R et al., 2013	miR-17-5p	Early diagnostic markers of PC	[53]
Charrier A et al., 2014	miR-21	1. Promote transformation of pancreatic epithelial cells into stromal cells.	[53,60,102]
		2. Promote metastasis of hypoxic tumor cells	
		3. Early diagnostic markers of PC	
Chen D et al., 2017	miR-23b-3p	1. Promote pancreatic cancer cell proliferation and migration, and upregulate CXCL1/CXCL2	[85]
		2. Correlate with CA19-9 levels	
Wu DM et al., 2019	miR-126-3p	Down-regulate ADAM9 and inhibit proliferation, invasion and metastasis of PC	[64]
Richards et al., 2017	miR-146a	Increased secretion due to Snail upregulation, and lead to the proliferation and chemoresistance formation of PC cells	[62,63]
Pang WJ et al ., 2015	miR-155	1. Promote the proliferation of pancreatic interstitial cells	[53,61,104-106]
		2. Early diagnostic markers of PC	
		3. involve in the formation of chemoresistancem m m	
Matsushit H et al., 2016	miR-196a	Sensitive index for early diagnosis of PDAC	[71,93]
Zhou M et al., 2014	miR-203	Down-regulate the expression of TLR4 as well as downstream cytokines in DCs, and promote formation of immune suppression	[97]
Ding G et al., 2015	miR-212-3p	Inhibit the expression of MHC II and induce the formation of immunological tolerance in DCs	[96]
Wang X et al., 2018	miR-301a-3p	Activate PTEN/PI3Kγ signaling pathway, and promote metastasis of PC cells	[82]
Li Z et al., 2018	miR-338	Regulate the expression of MACC1, and promote metastasis and invasion of PC cells	[83]
Takikawa T et al., 2017	miR-451a	1. Promote pancreatic cancer cell proliferation and migration, and upregulate CXCL1/CXCL2	[52,84]
		2. Early diagnostic markers of PC	
Taller D et al., 2015	miR-550	Early diagnostic markers of PC	[50]
Taller D et al., 2015	miR-1246	Sensitive index for early diagnosis of PDAC	[50]
Madhavan B et al., 2015	miR-3976, miR-4306, miR-4644	Early diagnostic markers of PC	[27]

in both pancreatic cancer cell lines and serum-derived exosomes. Then, it is confirmed that this kind of mutation is very common in pancreatic cancer. KRAS mutations mean the development of early intraductal tumors, and p53 mutations represent the transition of tumors from low to high grade [58,59].

Exosomes released from pancreatic cancer cells also have a stimulatory effect on Pancreatic Stellate Cells (PSC) which remains quiescent in normal human body. PSC are a characteristic type of pancreatic stromal cells, similar to the population of stellate cells in the liver or other organs, which can be converted into activated myofibroblasts upon appropriate stimulation. Activated PSC can release exosomes containing miR-21. These exosomes can promote the transformation of pancreatic epithelial cells into stromal cells, enhance their proliferation ability, and promote the proliferation of stromal cells [60]. In addition, it has been demonstrated that pancreatic cancer cells promote mesenchymal proliferation by releasing exosomes rich in miR-155 [61].

In recent years, studies on Cancer-Associated Fibroblasts (CAFs) have gradually increased. Cancer-Associated Fibroblasts (CAFs), develop from bone marrow-derived Mesenchymal Stem Cells (MSCs), are cellular components of the desmoplastic stroma characteristic to the tumor and inextricably associated with proliferation of pancreatic cancer cells. CAFs exposed to gemcitabine significantly increased exosome release and consequently increased cell proliferation and survival of pancreatic cancer cells. Mechanistically, correlative studies have demonstrated increased expression of Snail (Snai1) as well as the Snail target mRNA-146a in these exosomes. Furthermore, inhibiting the release of CAF exosomes can decrease the proliferation and survival of pancreatic cancer cells [62,63]. Interestingly, it is the same MSCs that inhibit the progression of pancreatic cancer. It has been demonstrated that MSCs down regulate metalloproteinase-9 (ADAM9) by over expressing exosomes carrying miR-126-3p, thus inhibiting the proliferation, invasion and metastasis of pancreatic cancer cells. The study aims to elucidate how non-tumor-derived exosomes may affect the proliferation, invasion and apoptosis of pancreatic cancer cell lines, and certainly highlights the potential of miR-126-3p as a novel biomarker for the treatment of pancreatic cancer [64]. A more advanced study has suggested a zinc protein, ZIP4 that slows pancreatic cancer progression by reducing the secretion of HSP70 and HSP90. Obviously, it is a subset of exosomes that undertake the transport of these heat shock proteins [65].

The studies above substantiate that the effects of exosomes on pancreatic cancer cell proliferation depend on the cells from which the exosomes are derived. Not all molecules carried by exosomes have a role in promoting the proliferation of pancreatic cancer. On the contrary, some signal molecules delay the progression of pancreatic cancer. There is no doubt that more exosomal signaling pathways remain to be further investigated and explored.

Exosomes guide pre-metastasis of pancreatic cancer

The liver is the most common metastatic site for pancreatic cancer. The liver pre-metastatic niche is composed of Kupffer cells, Hepatic Stellate Cells (HSC), bone marrow-derived cells, Extracellular Matrix (ECM), and soluble factors (such as cytokines and chemokine). Primary tumor cells secrete large amounts of cytokines and growth factors that promote mobilization and replenishment of bone marrow-derived cells to future metastatic sites and promote the

formation of the tumor microenvironment [66]. Costa Silva et al. reported that exosomes play a crucial role in the liver pre-metastasis of PDAC by injecting PDAC-derived exosomes into mice, ultimately leading to an increased metastatic burden in their liver. These exosomes, when ingested by Kupffer cells, cause TGF-β secretion and up-regulation of fibronectin, an ECM component produced by activated HSC. Meanwhile, this fibrotic microenvironment enhanced the recruitment of bone marrow-derived macrophages [56]. It is these bone marrow-derived cells that modulate the tumor microenvironment through ECM remodeling, immune suppression, and inflammation [67]. In a further step, this experiment Hawed that macrophage Migration Inhibitory Factor (MIF) is highly expressed in PDAC-derived exosomes. By blocking this signal molecule, all the successive steps of pre-metastasis niches in the liver were nipped off to prevent exosome-induced PDAC transfer. Also, experimentally demonstrated that MIF is elevated in plasma exosomes isolated from a mouse model of pancreatic cancer carrying Pancreatic Intraepithelial Neoplasia (PanIN) or PDAC injury (PKCy mice). Moreover, MIF is significantly increased in patients with stage-I PC who later developed liver metastases compared with patients whose pancreatic tumors did not progress [56].

Furthermore, Nielsen et al. found 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 [68]. At the same time, another type of macrophage in the liver, embryo-derived tissue resident macrophage (Kupffer cells), and both of them can promote the activation and fibrosis of HSCs, which is an important process in the formation of pre-metastatic niches [69].

Tumor-derived exosomes also regulate the formation of premetastatic niches through the binding of integrins on their own membrane structure to specific target cells. Targets against integrins α6β4 and αvβ5, for example, can decrease exosome uptake, as well as reduce lung and liver metastasis, respectively [70]. Another interesting experimental result is that the vast majority of PDAC-derived exosomes accumulate in the liver. Despite the use of retro orbital injection of exosomes, they were unable to reach the lungs. It turns out that the liver is the most common metastatic organ for pancreatic cancer, not only because of the anatomy of pancreatic blood entering to the liver through the portal vein, but also some special mechanism [71]. Just as melanoma cell-derived exosomes are taken up by liver macrophages and lung endothelial cells, the mechanism by which pancreatic cancer-derived exosomes enter the liver remains to be investigated.

Mechanisms by which exosomes promote pancreatic cancer metastasis

For the high metastasis and invasiveness, the therapeutic outcome of pancreatic cancer is very poor in usual. Several studies have demonstrated the important role of exosomes in promoting metastasis and invasion of pancreatic cancer [66,72-74]. On the one hand, exosomes promote this process by modulating the tumor microenvironment, since the metastasis of tumor cells is closely related to the tumor microenvironment, and hypoxia and inflammatory cell infiltration (especially macrophages) are two important factors of these [75,76]. Hypoxia may contribute to tumor progression by modulating cell-to-cell communication through modifying exosome release [77]. Pancreatic cancer cells can produce miRNA-21-enriched exosomes to promote metastasis of hypoxic tumor cells. Moreover, stabilization and activation of Hypoxia-Inducible Factor (HIF) is a major mechanism by which pancreatic cancer cells respond to hypoxia, particularly HIF-1α and HIF-2α, which activate proto-oncogenes that promote tumor growth, angiogenesis, and cell metastasis [78,79]. Besides, macrophages are the most abundant infiltrating immune-related stromal cells around tumor cells. Depending on the microenvironment, macrophages can be polarized into classically activated type (M1) or alternatively activated type (M2). M1 macrophages, characterized by the expression of inducible Nitric Oxide Synthase (NOS), are pro-inflammatory, while M2 macrophages express higher levels of anti-inflammatory cytokines and a more active Arginase-1 (Arg1), which favors the growth of tumor cells [80,81]. The hypoxic microenvironment can activate the Phosphatase and Tensin Homolog (PTEN)/Phosphorinositol 3-Kinase (PI3K) gamma signaling path way by secreting miRNA-301a-3p-enrichedexosomes. M2 macrophage polarization is then stimulated in a manner that induces HIF1α or HIF2a, thereby promoting metastasis of tumor cells [82].

On the other hand, exosomes can also directly affect metastatic ability and invasiveness through certain signaling pathways. Recently, a circular RNA (circ-PDE8A) was extracted from liver metastatic PDAC cells by microarray analysis, and it was found that high expression of circ-PDE8A was associated with lymphatic invasion, TNM stage, and poor survival in PDAC patients. Further studies revealed that circ-PDE8A promotes invasive growth of PDAC cells by up regulating MET. Namely, circ-PDE8A regulates Metastasis-Associated in Colon Cancer-1 (MACC1) as a ceRNA of miR-338 and stimulates invasive growth through the MACC/MET/ERK or AKT pathways [83]. In addition, PSC can secrete exosomes carrying miR-451a that promotes pancreatic cancer cell proliferation and migration and up regulate the expression of chemokine ligands CXCL1 and CXCL2 [84]. Over expression of miR-23b-3p also promotes this process, and the expression levels of miR-23b-3p correlates with serum Carcinoembryonic Antigen 199(CA199) levels [85] (Figure 1).

Exosomes Promote Immune Tolerance in Pancreatic Cancer

As mentioned earlier, macrophages can be polarized into two key phenol types that is M1 and M2 macrophages. In specific functions, M1 macrophages express high levels of MHC I and MHC II antigens and secrete complement factors that promote complement-mediated phagocytosis [60,80]. At the same time, M1 macrophages also produce high levels of pro-inflammatory factors, such as IL-1, IL-6, IL-23 and TNF. In contrast, 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]. Some studies have shown that the invasion of pancreatic cancer is mostly M2 macrophages, which weaken the phagocytosis of tumor cells, and its number is also positively correlated with the degree of peripheral lymph node metastasis and early distant metastasis [86]. Furthermore, Di Caro et al. not only demonstrated that the majority of Tumor-Associated Macrophages (TAM) at the tumor-stroma interface in patients with PDAC were M2 type, but also that the prognostic relevance of postsurgical adjuvant chemotherapy for PDAC was associated with a decrease in the macrophage density of CD206 (+) and IL-10 (+) at the tumor-stroma interface [87]. In addition, exosomes extracted from the saliva of PDAC mice showed

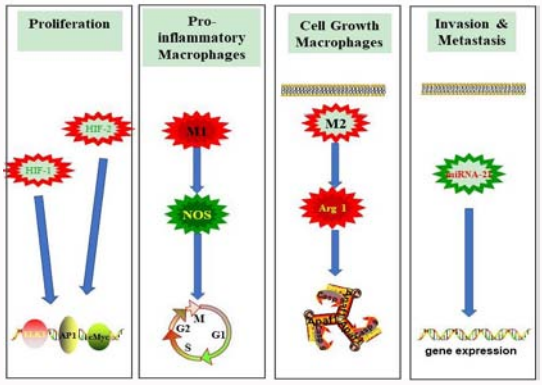

Figure 1: Mechanisms by which exosomes promote pancreatic cancer proliferation, invasion and metastasis.

inhibitory effects on immune surveillance and reduced tumor-killing ability of NK cells.30 Exosomes inhibit the cytotoxicity of NK cells against tumor cells, which may be related to the expression of TGF-β1, MICA/MICB and myeloid blast markers (CD34, CD33 and CD117). In particular, TGF-β, which down regulates the NK cell activating receptor NKG2D, inhibits NK cell activity and cytotoxicity [88].

Moreover, Friedlander et al. have also demonstrated that the switch of neutrophils to a protumor phenotype is also dependent on TGF-β exposure, and that the recruitment of these cells to the tumor microenvironment is partly driven by macrophages [89]. Neutrophils have also been shown 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 tumor-promoting phenotype to promote cell proliferation, angiogenesis, tumor invasion, and suppression of the adaptive immune response [91]. However, there are more cancer clinical data and evidence needed to prove the specific mechanism by which exosomes regulate this kind of cells.

Exosomes regulate adaptive innate immune responses in pancreatic cancer

Tumor-derived exosomes are widely present in the tumor microenvironment and plasma of tumor patients. These exosomes carry a variety of stimulatory and inhibitory molecules and deliver them to human immune cells, giving tumor cells the opportunity for immunosuppression and immune escape. Abundant evidence shows that T cells infiltration in the tumor microenvironment are closely related to patient outcomes. Tumors that can escape recognition by cytotoxic T cells generally have a poor prognosis. In patients with pancreatic cancer, it has been demonstrated that the ratio of high levels of tumor-infiltrating regulatory T cells (T-regs), defined as FoxP3 (+) CD4 (+) T cells, is significantly associated with shortened survival, whereas high levels of tumor-infiltrating CD4 (+) T and CD8 (+) T are significantly associated with prolonged survival [92]. Among them, T-regs are shown to support tumor growth and expansion by suppressing host immune responses and accelerating angiogenesis and tissue remodeling. The role it plays in the immune response from premalignant lesions to the established stage of PDAC suggests that the high prevalence of TR becomes a marker for evaluating poor prognosis [93]. There have been clear findings suggesting that tumor-derived exosomes have immunomodulatory properties, which are able to induce T-reg, promote T-reg expansion, and up regulate T-reg suppressive function and enhance T-reg resistance to apoptosis [94]. A critical role for TGF-β in FOXP3 expression in T-regs was further demonstrated by Wada et al., who isolated exosomes from malignant effusions of cancer patients to help maintain cultured T-regs [95]. As previously mentioned, exosomes can release TGF-β after treatment with Kupffer cells in pre-metastatic niches, although it is not possible to determine whether PDAC-derived exosomes themselves contain TGF-β, the above experiments demonstrate that exosomes can induce TGF-β production in immune cells, which may play an important role in maintaining tumor immune tolerance [54].

In addition, it is well-known that Dendritic Cells (DCs) also play an important role in activating immune responses. Studies have investigated how exosomal miRNAs derived from PC suppress mRNA expression in dendritic cells and induce immune tolerance. Compared with immature dendritic cells, 9 PC-related miRNAs were increased in exosome-stimulated dendritic cells and 208 mRNAs were inhibited. This result validates the experimental prediction that Regulatory Factor X-Associated Protein (RFXAP), an important transcription factor for MHC II, is inhibited by miR-212-3p transferred from PC-secreted exosomes, resulting in decreased MHC II expression. Moreover, miR-212-3p was negatively correlated with RFXAP in pancreatic cancer tissues. From 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 explore the effects of exosomes on toll-like receptors (TLR) in DCs. The effect of miR-203 on TLR4 and downstream cytokines was studied as an entry point. First established that miR-203 is expressed in PANC-1 cells and exosomes and that level are upregulated in exosomes-treated DCs. Then the results showed that TLR4 decreased in DCs treated by exosomes and miR-203, and conversely, TLR4 increased in exosomes-treated DCs by miR-203 inhibitors. The expression levels of Tumor Necrosis Factor-α (TNF-α) and Interleukin-12 (IL-12) also decreased under treatment of exosomes and miR-203, both of which increased in exosomes-treated DCs by miR-203 inhibitors. In conclusion, PC-derived exosomes down regulate TLR4 and downstream cytokines in DCs via miR-203 [97].

Exosome induced chemoresistance in pancreatic cancer

In the last few years, gemcitabine-based chemotherapy regimens have remained the mainstay of treatment for advanced or metastatic pancreatic cancer. However, with the activation of oncogenic miRNAs, anti-apoptotic enzymes and signaling pathways associated with cellular chemoresistance, PC cells have gradually developed resistance to chemotherapy. Moreover, the stromal tissue of PC is characterized by low blood perfusion and hypoxia, so that the dense stroma can affect the release of chemotherapeutic agents through physical barriers, high interstitial pressure, compression of blood vessels, and dense stromal cells [98]. Exosomes are important vehicles for intercellular communication of genes and signaling molecules. Prior to this, there had been a large number of evidences demonstrating the important role of exosomes in the chemoresistance of other types of cancer cells, such as lung, breast, prostate and gastric cancer [99-103]. Also, there are also many experiments showing that exosomes can improve the resistance of pancreatic cancer cells to chemotherapeutic drugs through a variety of mechanisms.

Exosomes derived from PC can deliver multiple drug resistance-associated miRNAs and proteins to target cells to reduce chemotherapy efficacy. Cancer-Associated Fibroblasts (CAFs) occupy the majority of the tumor volume in PDAC. Studies have demonstrated that CAFs

are intrinsically resistant to gemcitabine and that CAFs exposed to gemcitabine significantly increased exosome release. These exosomes upregulate the expressions of the chemoresistance-inducing factor, Snail, as well as the Snail target, mRNA-146a in recipient epithelial cells and promote proliferation and chemoresistance in PC cells. Further treatment of gemcitabine-exposed CAFs with exosome release inhibitor GW4869 significantly reduced the survival rate of exosomal recipient epithelial cells, indicating the important role of CAF exosomes in chemoresistance [62]. Since chemoresistance can spread to every PDAC tissue in a patient's body, it is assumed that there are a series of specific miRNAs and changes in their expression levels or related cellular communication can affect the development of chemoresistance. MiRNA-155 is a typical multifunctional miRNA, mediated by its downstream genes and involved in a variety of physiological and pathological processes, such as inflammation, immunity, and tumor development [104]. A recent study suggests the existence of an acquired chemoresistance mechanism in PC mediated by miRNA-155. Conditioned Medium (CM) from PC cells pre-treated with gemcitabine provided significant chemoprotection against subsequent gemcitabine toxicity. Gene expression analysis showed that Superoxide Dismutase 2 (SOD2) and Catalase (CAT) were up-regulated, while Deoxycytidine Kinase (DCK) were down-regulated in PC cells after this pretreatment. Studies have claimed that exosomes may increase the level of ROS detoxification gene expression product, SOD2 and CAT, by lateral transfer of their transcripts, resulting in chemoresistance in pretreated PC cells. On the other hand, 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 [105]. Furthermore, chronic exposure to gemcitabine is able to increase miR-155 expression. The increase in miR-155 continues to promote the secretion of exosomes and the formation of chemoresistance capacity, thereby forming a positive cycle for exosomal miR-155 to regulate chemoresistance [106]. Inhibitor of Apoptosis Protein (IAP) can promote the apoptosis of tumor cells, but its expression level is significantly reduced in various tumor cells [54]. In a study of PC tissues and cell lines, Asuncion Valenzuela et al found that the expression of IAP was significantly upregulated by nuclear factor-κB (NF-κB). Exosomes derived from PC contain associated mRNA for IAP, and upon chemotherapy, the levels of IAP protein or mRNA in the cytoplasm of PC cells remain unchanged or moderately upregulated. Therefore, exosomes may also enhance the resistance of PC cells to chemotherapeutic agents by inhibiting IAP expression [107]. Another study has found that exosomal lipids can induce chemoresistance in human pancreatic tumor cells (MiaPaCa-2) through the CXCR4 (chemokine receptor 4)-SDF-1α (stromal cell-derived factor-1α) signaling daxis [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 against gemcitabine, and exosomes that could be isolated from chemoresistant PANC-1 cells over expressed EphA2. But 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]. In summary, exosomes can promote chemoresistance in PC cells by regulating miRNAs, proteins, lipids, and signaling pathways. However, the regulatory mechanism of exosomes requires more relevant experiments to verify and explore (Figure 2).

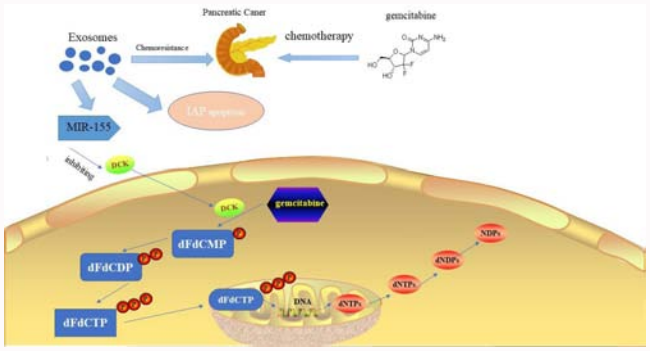

Figure 2: The mechanism of exosome induced chemoresistance in pancreatic cancer.

Exosomes and Novel Pancreatic Cancer Therapies

Exosomes have the potential to be targeted therapeutic carriers in processes such as cell signaling and material transport because of their low immunogenicity, non-toxicity, and highly stable biological characteristics in the blood. Compared with other carriers, exosomes not only hold a more stable lipid bilayer structure to protect their transporters, but also have a higher targeting delivery capacity, and its smaller diameter also ensures that it can selectively enter tumor tissues to achieve better therapeutic effects. More and more studies have focused on how exosomes, as a natural endogenous carrier, can be used to deliver certain interfering signals or transport cancer drugs [109].

Mutations in the KRAS gene are widely present in a variety of malignancies, with mutations at codon 12 G12D and G12V, which occur on the second exon, being the most common in PDAC, accounting for 70% to 95%. The combination of GTP enzymes and KRAS mutant genes is a key driver of pancreatic cancer. Normally, KRAS is inactivated immediately after activation. However, after KRAS gene mutation, KRAS protein maintains continuous activation state, no longer dependent on the stimulation of superior signal, and is in the state of continuous binding with GTP, leading to abnormal activity of downstream signaling pathway, such as PI3K-AKT-mTOR, thereby promoting tumor cell proliferation, transformation, adhesion and survival. Buscail et al. targeted KRAS G12D, silenced it by exosome delivery of small interfering RNA (siRNA), and then delivered it to PC cells. As a result, proliferation and metastasis of cancer cells were significantly reduced [110]. Recent studies have also explored a novel approach to directly and specifically target oncogenic KRAS in tumors by using engineered exosomes (known as iExosomes). SiRNA targeting mutant KRAS was introduced into fibroblast-derived exosomes to make iExosomes. As this exosome itself carries CD47, it reduces the clearance of iExosomes in the circulation by monocytes and macrophages. The iExosome is then delivered into cancer cells, thereby inhibiting KRAS GTPase activity as well as activation of the downstream MEK-ERK or PI3K-AKT-mTOR signaling pathways, ultimately inhibiting tumor growth and metastasis [16].

Another idea of exosome-targeted therapy is to inhibit the uptake of exosomes by recipient cells. Heparan Sulfate Proteoglycans (HSPGs) serve as internalization receptors for tumor cell-derived EVs with exosome-like characteristics. Internalized exosomes colocalize with syndecan and glypican types of cell surface HSPGs,

and the uptake of exosomes is specifically inhibited by free HS chains, thus inferring that tumor cell-derived exosomes use HSPGs for internalization and functional activity [111]. In addition, syntenin genes can bind to the cytoplasmic tail of syndecans, which are internalized into sorting endosomes along with their intact Heparan Sulfate (HS) chains. On 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-bisphosphate (PIP2). Mouse fibroblasts isolated from syntenin knockout mice showed low amounts of HSPGs, which correlated with reduced uptake of exosomes, further confirming that the presence of HSPGs is essential for efficient internalization and function of exosomes. Meanwhile, syntenin gene is also a possible target for cancer therapy in the future [112].

Due to the non-toxicity and low immunogenicity of exosomes in serum, there are also recent experiments using them as a targeting carrier of chemotherapeutic agents to avoid causing chemotherapy toxicity in systemic tissues in order to achieve better therapeutic effects. Mesenchymal Stromal Cells (MSCs) have been proposed for delivering anticancer agents because of their ability to home in on tumor microenvironment. And MSCs can acquire strong anti-tumor activity after priming with Paclitaxel (PTX) through their capacity to uptake and then release the drug. Pascucci et al. used exosomes secreted by MSCs to load PTX to inhibit proliferation of PANC-1 cells, verifying the possibility of exosome packaging and delivery of active drug [113]. For further experiments, Kim et al. added PTX-loaded exosomes to drug-resistant cells and obtained good anticancer effects in mice model [114]. These experiments all suggest the feasibility of this novel system of exosomes for further development carrying other chemotherapeutic agents in the future.

Conclusions and Future Perspectives

The molecular composition of exosomes reflects the specialized function(s) of their original cells. Through their ability to bind target cells, they 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. Their particular lipid composition and the presence of protective proteins against complement such as CD55 and CD59 may contribute to their stability in the extracellular environment. The advantages of an exosomal - a cellular mode of communication should aid the development of diagnostic and therapeutic strategies: they are nonliving, they contain sorted sets of molecules involved in many different cellular processes, and they 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-tumoral immunotherapy are under development. The advantages however are in detriment to the potential consequences that exosomes pose to human health, where exosomes may be used by tumoral cells to invade normal tissue, and by pathogens such as prions and HIV to maximize their spreading in between cells.

This review highlights our understanding of the interaction between exosomes and the malignant biology of pancreatic cancer. And we also discuss the possible clinical application of exosomes. First of all, we introduce the biological characteristics of exosomes. And then we know that exosomes as diagnostic markers for pancreatic cancer. However, we all know that exosomes regulate proliferation metastasis and invasion of pancreatic cancer cells. There still many mechanistic questions remain, and much research work needs to be performed. Last but not the least, we introduce exosomes promote immune tolerance; induce chemoresistance and novel pancreatic cancer therapies in pancreatic cancer. Specifically, the establishment of new bio analytical technologies and novel experimental animal models could help researchers uncover the secrets of the exosomes. In this review, the mechanisms and functions of various exosomes are introduced in detail. We also attempt to identify the roles these proteins play in malignant cancer with the ultimate aim of determining the exact role of the exosomes in human cancer cells.

In the past few years, accumulating lines of evidence from studies conducted by different research groups in the field of pancreatic cancer have revealed that exosomes are linked to cancer and have the capacity to accelerate pancreatic cancer cell proliferation, migration, and invasion. It is believed that future research will provide more detailed information on the mechanism through which exosomes regulate the growth of tumor cells and will help us establish exosomes as new targets for the treatment of pancreatic cancer. It is believed that the application of the exosomes in the treatment of pancreatic cancer will be spectacular, and we believe that all human malignant tumors and related challenges will be conquered in the future.

Exosomes, as an important information communication and transport tool, mediate cell-to-cell information exchange by transmitting their carried molecules such as miRNAs and proteins to recipient cells, which in turn affect various physiological functions such as growth and metabolism of recipient cells. Recent studies on exosomes have fully demonstrated their important role in the regulation of PC proliferation, apoptosis, metastasis and other processes, providing a new perspective for understanding the mechanism of the malignant biological characteristics of PC, and are expected to provide a new direction for the treatment and early diagnosis of PC. Although the current research and experimental results are novel and exciting, there are still many doubts and questions about the exosome mechanism, which remain to be further explored.

Authors Contribution

Liu H and Huang S contributed to the design and writing of the review; all the authors read and approved the final manuscript.

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