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Exosomes: Ideal Diagnostic Biomarkers and Therapeutic Nanodevices of Pancreatic Cancer

Hongpei Wu[1], Bojun Bao[1], Hui Cong[2], Jinxia Liu[1], Feng Jiang[1], Wenkai Ni[1], Lishuai Qu[1], Cuihua Lu[1], Runzhou Ni[1] and Mingbing Xiao[1,3]*

1 Department of Gastroenterology, Affiliated Hospital of Nantong University, Nantong, China
2 Department of Laboratory Medicine, Affiliated Hospital of Nantong University, Nantong, China
3 Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong, China

***Corresponding author:** Mingbing Xiao

xmb73@163.com

Department of Gastroenterology and Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong, China.

Tel: +86-0513-85052461
Fax: +86-0513-85052588

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Abstract

As the leading cause of morbidity and cancer related-death worldwide, pancreatic cancer poses a terrible threat to human health. Since therapeutic choices are limited at the advanced stage, screening and early diagnostic tools are indispensable for a better prognosis. Exosomes are nanovesicles which contain various biomolecules, such as nucleic acids, proteins and lipids. Besides, Exosomes are recognized as potential tools which can be used in tumor diagnosis and therapy, for they are involved in a multitude of pathological and biological processes, mainly for the roles they play in intercellular communication. Here, we will summarize the function of exosomes, mainly in pancreatic cancer (proliferation, metastasis, drug resistance and immune reaction in tumor microenvironment), and importantly, the potential functions of exosomes are also emphasized. Exosomes are not only biomarkers, but they can also work as a tool which is helpful for therapeutic armamentarium in pancreatic cancer. Besides, current challenges which may obstruct the clinical application of exosomes are also discussed in this paper. In-depth researches on the functions of exosomes in pancreatic cancer are still required.

Keywords: Pancreatic cancer; Exosomes; Biomarkers; Diagnosis; Treatment

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Introduction

Pancreatic Ductal Adeno Carcinoma (PDAC) is one of the most deadly malignancies and has a dismal prognosis with a five-year survival rate of <10% [1-3]. It's simply termed pancreatic cancer (PC). In 2017, the National Cancer Institute take stock of the situation of pancreatic cancer, it estimates that the incidence of pancreatic cancer is 3.2% in all kinds of cancers, and the death rate of pancreatic cancer accounts for 7.2% of all kinds of cancers [3]. In the past few years, the incidence of pancreatic cancer has been kept increasing, it is estimated that pancreatic cancer will become the second leading cause of death by 2030 [4]. Pancreatic cancer has the following characteristics: early diffusion, local diffusion and distant organ transfer. By the time pancreatic cancer is diagnosed, about 80% of the patients are presented with the surgical unresectable disease, and respond poorly to most chemotherapeutic agents [5]. Almost nobody can survive more than one year after diagnosis because of the high rate of development of the disease. Even for those who are diagnosed with localized disease and having radical curative therapy, the median survival still remains at a level of about 18 months [6,7]. Thus, early detection, respectable stage and effective therapy for pancreatic cancer are faced with great challenges.

Using traditional imaging methods to diagnose patients with early malignant tumors is getting much more ineffective [8]. Thus, imaging is often combined with other serum biomarkers. Recently, the preferred marker has been carbohydrate-associated antigen 19-9 (CA19-9), it plays an important role in the decision making and response monitoring. But it has known to exhibit low specificity and sensitivity for early PC examination [9,10]. Hence, new PC- specific biomarkers are urgently required to complement and improve the current pancreatic cancer diagnostic strategies.

The research of exosomes increased rapidly during the past

decade years, especially the research of the unique role of exosomes in different angles of cellular activity, particularly during cancer progression [10-13]. Exosomes contain genetic cargos, which could induce a number of changes in recipient cells at transcriptional, epigenetic or post-transcriptional levels [14-17]. Exosomes are endowed with important roles in cancer-related immune reactions, cancer metastasis as well as cancer-genesis [18-20]. The ability and function of exosomes in cancer development are shown as follows: exosomes can be simply isolated from bodily fluids like serum, making them a promising target for further interpretation of the molecular and cellular mechanisms which are related to the progression of pancreatic cancer [12,21-23].

In this current review, exosomes in the development and pancreatic cancer progression will be introduced. Furthermore, the potential clinical implications that exosomes may give in diagnoses and treatment phase will be discussed and explored.

Backgrounds of Exosomes

Most types of cells secrete exosomes which are lipid bilayer membrane-enclosed nano-sized (30~100nm) vesicles [24-26]. Formation of exosomes is through the following process: after cell endocytosis, early endosomes form and then become MultiVesicular Bodies (MVB) in which late exosomes are formed into intraluminal vesicles; and then plasma membrane fuses with MVB to release exosomes with the formation of budding [27]. Nowadays, scientists have already found exosomes in various biological fluids, such as breast milk, urine, bile, blood, lymph, saliva, under both morbid and healthy situations [28-30]. And exosomes are composed of primarily of proteins, lipids, RNA, and DNA and can work as cargo to transfer this information to recipient cells, which then play an essential role in transmitting information and carrying substances in carcinogenesis [26]. Exosomes which are secreted by different types of cells are enriched with identical proteins, including MHC class II proteins [31-33], heat shock proteins (Hsp70 and Hsp90), members of the endosomal sorting complexes required for transport (TSG101 and Alix), members of the tetraspanin family (CD9, CD63, CD81 and CD82). In terms of the ways of exosome isolation from culture medium, serum and plasma, there does exist several ways which are based on several features of their own, like electrokinetic potential, density, markers, content or size [34]. But due to the complex nature of the sample matrix and the physicochemical properties of exosomes, accurate isolation of exosomes from bodily fluids poses major challenges [35].

Exosomes were once thought to restore the dynamic and homeostatic cellular homeostasis conditions by getting rid of proteins which are not necessary and other molecules from cells, such as by eliminating transfer receptors during reticulocyte maturation and re-moving harmful DNA from the cytoplasm to avoid the senescence or apoptosis of normal human cells [36]. In the last few years, exosomes have been demonstrated that they are important in facilitating tumorigenesis by regulating tumor progression, angiogenesis, metastasis and immunity and. Circulating exosomes and especially their contents have become a potential source of message and also, they have been utilized as noninvasive biomarkers and liquid biopsies for early examination and treatment for cancer invalids [26].

The Role of Exosomes in Pancreatic Cancer

Proliferation

To a large extent, exosomes have already been depicted as promoters of cell proliferation in various cancers. Interstitial components of pancreatic cancer are more than those of other epithelial tumors [37]. Most pancreatic stellate cells (PSCs) distribute in the perivascular area of pancreas, which are a significant part of interstitial tissue [38]. In pancreatic cancer, activated PSCs release exosomes rich in miR-21, which can promote the transition process of epithelial to mesenchymal, the proliferation of tumor cells, and cell interstitial hyperplasia [39,40]. MiR-21, referred to as an “oncomiR”, is related to increased tumor proliferation and formation. Numerous studies have shown that over-expression of miR-21 is closely related to promote cell invasion and proliferation. Besides, the high level of miR-21 in exosomes is related to poor prognosis [41]. Meanwhile, PSCs also produce inflammatory mediators, like IL-15, IL-6, etc., and other nearby quiescent PSCs can be activated by them through an autocrine loop, which accelerates the progression of pancreatic cancer [42]. In addition, some studies have demonstrated that pancreatic cancer cells also promote cell interstitial hyperplasia by releasing exosomes rich in miR-155 [43].

Metastasis

According to the recent evidence, we can know that primary tumor is of great importance for the formation pre-metastatic niche [44]. Costa-Silva et al. demonstrated that exosomes would be secreted to the target organ(s) by primary cancer tissue(s), and then the target organ(s) would form a pro-metastasis ‘‘pre-metastatic niche’’ for later metastasis [45]. PDAC-derived exosomes helped promote the recruitment of bone marrow-derived neutrophils and macrophages through the formation of a fibrotic microenvironment. Kupffer cells can secrete growth factor β (TGF-β), and this microenvironment was established by converting the above TGF-β and through fibronectin up-regulation by hepatic stellate cells so as to response to the uptake of PDAC-derived exosomes, building a good environment for PDAC cells to metastasize and grow. Except the existence of various proteins in exosomes which are of great importance in the metastasis of PDAC, exosomal miRNA might also play a key role in the process. It was found that in an experiment of pancreatic cancer in rats that miRNA is existed in all exosomes which are from primary tumor, and the stromal cells are prepared for the distant pre-metastatic target organ for tumor cell hosting. The transferred miRNA mainly adjust expressions of genes which are involved in oxidative stress response, cell angiogenesis, adhesion and other essential phases in metastasis initiation [46].

Drug resistances

In consideration of the role that exosomes play in information

transmission between different cells, they may play a quite essential role in drug resistance in the chemotherapy for pancreatic cancer. According to the previous researches, exosomes isolated from invalids with lung cancer undergoing cisplatin chemotherapy can deliver the generated drug resistance to other cells that have not received chemotherapy [44]. Similarly, exosomes which are isolated from the medium of breast cancer cells resistant to docetaxel or adriamycin can lead to the drug resistance of breast cancer cell line MCF-7 sensitive to chemotherapeutic drugs [47]. In addition, it has been revealed that the resistance to docetaxel can be regulated by the delivery of exosomes-mediated multidrug resistance protein-1 (MDR-1) [48]. Therefore, the drug resistance in pancreatic cancer is likely to be closely related to exosomes, which, however, still needs more relevant experimental evidence.

Immune reactions

Exosomes get involved in quite a few aspects of the immune response, including immune activation [49], immune tolerance [50], immune suppression [51], immune activation [52]. A multitude of researches have demonstrated that tumor cell-derived exosomes have an immunosuppressive effect. They make a great difference in both the progression and occurrence of tumors mainly by directly inhibiting immune cell function or regulating the secretion and expression of correlative cytokines, and thereby promoting the immune escape of tumors. In exosomes of pancreatic cancer, the expression of interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α) as well as toll-like receptor 4 (TLR4) can be inhibited by miR-203, which results in abnormal immune activation of dendritic cells (DC), and thereby leading to tumor immune escape [53]. In addition, a few cancers can secrete exosomes which can inhibit the role of multiple immune cells such as T cells, natural-killer cells (NK cells), macrophages and B cells, causing immune tolerance and immune evasion of cancer cells [13,16,53,54].

Exosomes as Biomarkers in Pancreatic Cancer

Pancreatic cancer has been a main cause of cancer-related death all over the world. Though modern diagnostic techniques, new drugs and proposed improved supportive treatment are available, the prognosis effect of pancreatic cancer still needs improvement. If non-invasive strategy could examine early symptoms of the disease and guide therapy methods, then the effects could be improved. It has been suggested that exosomes could act as potential tools for tumor treatment and diagnosis [55-58], and it is not only because of its role of biomarkers, but also because of its abundance and steady. Thus, exosomes can be recognized as potential biomarkers for early examination and personalized treatment [57].

Exosomal proteins possess unique characteristics over traditional serological markers. First, exosomal proteins show a higher sensitivity compared with proteins directly detected in blood. Second, exosomal proteins are highly specific over secretory proteins. Third, exosomal proteins show a high degree of stability, which are protected from external proteases and other enzymes by the lipid bilayer, and it's feasible to separate phosphorylation proteins from exosomal samples and freeze it for five years [36]. For instance, Melo et al. demonstrates glypican-1 (GPC1) can be considered as a new pancreatic cancer biomarker. Glypican-1 (GPC1) is a glycoprotein which is present in enhanced quantities especially on the cancer-derived exosomes' cell surfaces [58]. In the serum of patients, GPC1-circulating exosomes (crExos) were examined with pancreatic cancer with 100% specificity and sensitivity. Thus, the existence of $GPC1^+$ crExos were applied to tell normal control subjects from invalids with benign pancreatic lesions. In addition, these $GPC1^+$ crExos are closely related to tumor burden, besides, they could act as the role of prognostic biomarker in pre/post-surgical invalids. However, this tumor marker has been deemed controversial and critics, because two significant limitations of this study have been exposed. First, most of the PC cases examined were at advanced stages, such as stage IIb, III and IV. Second, only a few premalignant lesions were included in this study [59]. On the other hand, in this research, only 8 serous cystadenomas (benign lesions) while zero mucinous cists were evaluated [60].

On May 01, 2017 Cancer Letters published an article entitle "A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer", which demonstrated that in terms of the diagnosis of pancreatic cancer, exosomal GPC1 is actually not a preferable one in view of high exosomal levels of miR-30c, miR-10b, miR-181a, miR-21. And low miR-let7a can easily tell PDAC from chronic and normal control pancreatitis (CP) samples [61]. Compared to GPC1, elevated exosomal miRNA levels declined gradually to normality within 24 hours after the resection of PDAC. The whole cases of PDAC showed dramatically elevated exosomal miR-30c and miR-10b levels, while 8 cases had slightly increased CA 19-9 levels or kept normal. Consequently, researchers conclude that miRNA signature consisting of elevated levels of miR-181a, miR-21, miR-30c, miR-10b, and low miR-let7a might have advantages over plasma CA 19-9 levels or exosomal GPC1 in forming a diagnosis of PDAC as well as distinguishing between CP and PDAC. In addition, the potential of combining miRNAs with proteins for early cancer examination has also been studied. The study results showed that in PDAC invalids, different miRNAs (including miR-4306, miR-1246, miR-3976 as well as miR-4644) are upregulated in the serum exosomes, besides, when combined with the measurement of proteins' panel, they could act as the role of PDAC biomarkers [11].

At the present, with whole genome, transcriptome sequencing and exome, research personnel have performed all-round profiling of exosomal RNA (exoRNA) and exosomal DNA (exoDNA), besides, some alterations in BRCA2 and NOTCH1 in invalid exoDNA samples have also been founded. Whether DNA variation can be biomarkers needs further investigation [62]. **Table 1** provides a concise sketch of reported biomarkers for pancreatic cancer.

Table 1 Exosomal markers in pancreatic cancer.

Biomarker type	Exosomal makers	Study(year)	Sample size	Sensitivity	Specificity	Ref
Diagnostic biomarker	CD44v6, Tspan8, EPCAM, MET, CD104	Wang et al. (2013)	220 (131PC *vs.* 89 non-PC)	100%	80%	[63,11]
	miR-21, miR-17-5p	Que et al. (2013)	49 (22 PC *vs.* 27 non-PC)	95.5%, 72.7%	81.5%, 92.6%	[64,65]
	miR-1246, miR-4644, miR-3976, miR-4306	Madhavan et al. (2015)	220 (131 PC *vs.* 89 non-PC)	100%	80%	[11]
	miR-10b	Jashi et al. (2015)	9 (3 PC *vs.* 6 non-PC)	100%	100%	[66]
	GPC1 protein	Melo et al. (2015)	322 (190 PC *vs.* 132 non-PC)	100%	100%	[58]
	Exosomal-DNA, Exosomal-RNA	San Lucas et al. (2016)	3 (2 PC *vs.* 1 non-PC	-	-	[62]
	ANXA6/LRP1/TSP1	Leca et al. (2016)	105 (78 PC *vs.* 27 non-PC)	-	-	[67]
Prognostic biomarker	Integrinxv	Hoshino et al. (2015)	40 (27 PC *vs.* 13 non-PC)	-	-	[79]
	Migration inhibitory factor	Costa-Silva et al. (2015)	55 (40 PC *vs.* 15 non-PC)	-	-	[45]

PC: Pancreatic cancer; non-PC: Including chronic pancreatitis, benign pancreatic tumors and healthy controls.

Exosomes for Therapy in Pancreatic Cancer

Recently, several exosome-based therapeutic approaches have been developed, especially using exosomes as drug delivery or nucleic acid carriers has attracted wide attention **(Figure 1A).** Exosomes are membrane-permeable, and the blood-brain barrier can be easily crossed by them [63-69]. Most of all, normal cell-derived exosomes are well tolerated and they have low immunogenicity [70]. Thus, in terms of the treatment of cancer and other diseases, exosomes are recognized as perfect candidate carriers. When it refers to the way of loading drug into exosomes, there are at least three ways which are shown as follows: 1) drug can be loaded into parental cells, and it then will be released in exosomes naive, 2) parental cells can be transfected/infected with DNA, and then, they will be released in exosomes, 3) exosomes isolated from parental cells can be loaded ex-vivo [71]. Exosomes can arrive at the site of action accurately and exert the pharmacological effect of drugs. *In vitro* experiments revealed that pancreatic cancer cells could actively absorb the exosomes implanted manually, which effectively induced cytotoxic reaction [68]. Paclitaxel was introduced into exosomes, which were then put into the culture media of prostate cancer cells, comparing with the free paclitaxel in culture media, this way increases the cytotoxicity [72]. Similar results were found in pancreatic cancer cell experiments [73]. But the efficacy of this method is unclear compared with the efficacy of albumin-bound paclitaxel or other chemotherapies, which needs further studies to confirm. Importantly, exosomes can also be designed to carry short hairpin RNA or short interfering RNA which is specific to oncogenic KrasG12D. In pancreatic cancer, oncogenic KrasG12D is quite common, it offers insight into the therapeutic potential of exosomes in particular target of oncogenic KRAS [74].

In addition, some researchers tried to enhance the therapeutic effect of pancreatic cancer by blocking the release of exosomes (**Figure 1B**). Richards et al. found the following fact: the release of exosomes is greatly increased when PDAC-associated fibroblasts (CAFs) expose to gemcitabine. The expression of Snail is also

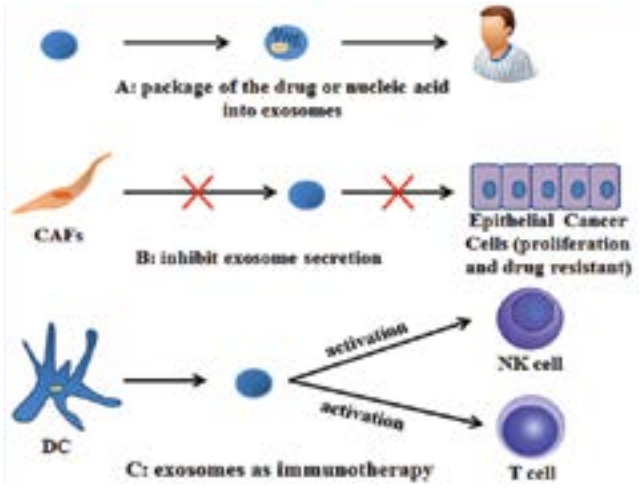

Figure 1 The clinical applications of exosomes as new drug targets in pancreatic cancer, including: **(A)** Exosomes as drug delivery or nucleic acid carriers; **(B)** Inhibition of exosome secretion; **(C)** Exosomes as immunotherapy.

enhanced by these exosomes, which has something to do with PDAC metastasis after polarity protein AF6' complete nuclear localization [75], promoting survival, proliferation and drug resistance [76]. The treatment of gemcitabine-exposed CAFs with GW4869 can inhibit the release of exosome, the survival rate of co-cultured cancer cells can be decreased when using this method. It indicates that the exosome inhibitors together with chemotherapy can overcome PDAC chemoresistance as well as invasive behavior [75].

Moreover, some scholars proposed the use of exosomes as a medium of immunotherapy to induce anti-tumor immune response **(Figure 1C).** For instance, it has already been proved that exosomes isolated from dendritic cells exposed to tumour peptide *in vitro* were impactful in provoking a tumour directed immune response in a 3 subcutaneous tumour model [77]. Although current progress is limited, it's still a quite emerging field and will be studied in other aspects for the treatment of pancreatic cancer [78,79].

Conclusions and Future Challenges

Over the past several years, exosomes in pancreatic cancer has been a hot spot of research. They are important in the proliferation of pancreatic cancer cells, and the drug resistance of pancreatic cancer, immune regulation, diagnosis, and treatment, etc. Today, several challenges remain to be solved, although exosomes have great potential for future pancreatic cancer researches. Firstly, due to the complex nature of the sample matrix and the physicochemical properties of exosomes, accurate isolation and detection of exosomes pose great challenges. Currently, among the various methods of extracting exosomes from cell culture media and bodily fluids, differential and buoyant density centrifugation is the most extensive used one. Despite dramatically advances in detection strategies, quantification and routine detection of exosomes are still tricky problems because the rapid, sensitive, reproducible and low-cost methodologies are lacking [35]. Second, only a few cases were included in the relevant clinical studies. We may manipulate exosomes if we can have a further understanding of the function that exosomes have in PDAC. More concretely, we may use them to achieve diagnostic purposes (like blood-based biomarkers) and therapeutic gain.

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Conflicts of Interest

The authors have no conflict of interest to declare.

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