UTILITY OF EXOSOMES IN THE DIAGNOSIS AND TREATMENT OF PANCREATIC ADENOCARCINOMA

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Disclosure: The authors have declared no conflicts of interest.
Received: 18.07.15 **Accepted:** 25.11.15
Citation: EMJ. 2016;1(1):77-85.

ABSTRACT

Pancreatic cancer is the most common lethal cancer, with annual incidence and mortality rates being approximately equal. This dismal prognosis can be attributed to late diagnosis making the cancers unresectable. These cancers respond poorly to chemotherapy and radiation, and surgical resection remains the most effective treatment available. Diagnostic tests that are sensitive, specific, and capable of early detection are urgently needed and would significantly impact upon pancreatic cancer treatment and outcomes. Exosomes, small membrane-bound vesicles which are fairly uniform in size (approximately 30–100 nm in diameter), contain messenger RNA, microRNA (miRNA), and proteins. They are ubiquitous and stable in most body fluids and exosomal miRNAs are also resistant to degradation by RNAses and DNAses. Expression profiles of serum exosomal miRNAs display sensitivity and specificity in the detection of pancreatic adenocarcinoma. Markers of pancreatic cancer-initiating cells are also expressed on serum exosomes. Exosomes exhibit key functions in addition to their distinct structural properties: they are involved in immune system modulation via the transfer of antigenic proteins, and through protease activity they modulate the extracellular environment prior to metastasis. Exosomes are being studied as potent gene delivery tools and dendritic cell exosomes are already used as cancer vaccines. This review focusses on the current state of exosomal research, particularly in relation to their applicability as diagnostic and therapeutic tools for patients with pancreatic adenocarcinoma.

Keywords: Adenocarcinoma, biomarkers, diagnosis, exosomes, pancreatic, vesicles.

INTRODUCTION

Pancreatic cancer is one of the most aggressive malignancies, resulting in poor prognosis for most patients.[1] It is considered the deadliest cancer and ranks fourth in cancer-related mortality.[2] The only effective treatment to date remains surgical resection. The poor prognosis for pancreatic adenocarcinoma (PaCa) is largely attributed to late detection and early metastasis. The 5-year survival rate for the earliest form of PaCa (Stage IA) is only 14%, and is a mere 1% for Stage IV.[3]

Pancreatic cancer research is now developing methods for early detection. Exosomes that are extracellular vesicles containing microRNAs (miRNAs), messenger RNA (mRNA), and proteins have recently been used as unique pancreatic cancer markers. miRNAs are non-coding RNAs that play a role in the regulation of post-transcriptional gene expression.[1] The miRNA expression profile of tumour-derived serum exosomes from PaCa patients differs significantly from those of healthy donors and patients with non-malignant disease.[2] Patients with cancer are thought to have exponentially higher numbers of circulating exosomes because they are secreted in large amounts during carcinogenesis.[4] In addition, exosomes released from tumour cells are readily detected in body fluids and have therefore emerged as potential non-invasive diagnostic tools capable of supporting earlier diagnosis due to their ubiquitous nature and structural stability.

STRUCTURE OF EXOSOMES

The classification of a particle as an exosome is based primarily on size, density, and membrane composition.[5] Exosomes are 30–100 nm vesicles of endocytic origin, and are secreted by a variety of cell types including cancer cells, mesenchymal cells, epithelial cells, haematopoietic cells, dendritic cells, mast cells, neurons, thrombocytes, and T cells.[5,6]

The first step in exosome biogenesis involves the inward budding of the plasma membrane to form early endosomes,[7] which then mature in the presence of several intracellular protein factors to become late endosomes, also known as multivesicular bodies (MVBs). These MVBs contain exosomes, which can be released from the cell or fused with intracellular lysosomes, leading to degradation of the contained exosomes.[7-9] More specifically, when releasing exosomes into the extracellular space, the MVBs fuse with the plasma membrane resulting in the release of exosomes by exocytosis.[7,10] Exosomes exhibit a lipid bilayer membrane carrying common exosomal marker proteins and cell type-specific markers,[11] which include tetraspanins such as CD9, CD63, CD81, and cytoplasmic proteins including actin, annexins, and Rab proteins.[11] The presence of Alix protein, tumour susceptibility gene 101, and tetraspanins reveals the MVB origin of exosomes,[5] which are packed with miRNA, mRNA, and proteins from the parent cell during formation. Kahlert et al.[6] demonstrated that pancreatic cancer cell-derived exosomes can contain fragments of double-stranded genomic DNA >10 kb in length. DNA samples from exosomes span all chromosomes, and mutations in the genes encoding K-ras and p53 can also be detected. In addition to the transfer of genes, Record et al.[8] describe the role of prostaglandin E2-rich, tumour-derived exosomes in tumour immune evasion and promotion of tumour growth by non-recognition.[8]

Exosomes are stable under varying conditions, allowing them to survive in many body fluids.[6] In the tumour microenvironment, released tumour-derived exosomes can transfer proteins and RNAs with oncogenic activity to recipient cells. Exosomes are found in several body fluids of cancer patients, such as synovial fluid, cerebrospinal fluid, bronchial lavage fluid, breast milk, serum, saliva, urine, ascites, and malignant effusions.[7,10,12-15] The ubiquitous nature of exosomes make them promising for potential early diagnosis of PaCa.

FUNCTIONS OF EXOSOMES

Exosomes facilitate intercellular communication and are involved in several physiological and pathological processes, including coagulation, inflammation, tumour progression, immune response regulation, antigen presentation, and transfer of nucleic acids, proteins, and infectious cargo such as prions and retroviruses.[16,17]

Exosomes are thought to play a key role in facilitating cell–cell communication in the tumour microenvironment,[6] and are implicated in angiogenesis and promotion of cell proliferation and survival.[5] More specifically, exosomes play several roles in the tumour microenvironment: 1) suppression of immune function by inducing apoptosis of activated cytotoxic T cells or by promoting differentiation of regulatory T cells, thus enabling tumour progression; 2) stimulation of angiogenesis and migration leading to metastasis.[11] Previous studies have demonstrated that exosomes are actively released into the peripheral circulation by cancerous cells,[18] and biomarker studies have shed light on the possibility of using exosomal protein and RNA profiles in cancer diagnosis.[19,20]

Tumour-derived exosomes can alter the molecular profile of their microenvironment and help to establish a metastatic niche to aid tumour growth and metastasis.[5] It has also been reported that exosomes utilise vascular endothelial growth factor and cytokine cargo to enhance recruitment of endothelial and haematopoietic precursor cells to enhance neoangiogenesis in the tumour.[5] It has been suggested that exosomes influence planar cell polarity and the extracellular matrix to allow tumour cell mobilisation.[5]

Proteomic analysis has detected extracellular proteases, particularly metalloproteinases, in exosomes[21] and these membrane proteases may alter the surface of the recipient cell through ectodomain shedding. These cleaved and soluble proteins may then act in an autocrine and/or paracrine fashion. Other cell membrane proteins studied for their role in metastasis have revealed mechanisms that are more clear. CD151 and Tspan8 are exosome-based tetraspanins that promote metastasis in several tumour systems.[14] CD151 supports migration via integrin trafficking and activation of Ras, Rac1, and Cdc42 recruitment.[14] It also regulates cell motility via protease activity that enhances both adhesion and matrix degradation. Tspan8 contributes further to motility by associating

with α6β4 (an integrin laminin receptor).[14] Interestingly, tumours in CD151/Tspan8 knockout animals have been shown to lose metastatic potential, underlining the necessity of these tetraspanins in metastatic growth.

Exosomes are known to trigger apoptosis in anti-tumour immune cells through Fas ligand and tumour necrosis factor pathways.[5] Other immunomodulatory effects of tumour-derived exosomes include disruption of immune cell differentiation, such as maturation of $CD14^{+}$ and $HLA\text{-}DR^{low/neg}$ monocyte precursors into dendritic cells, and effects on monocytes leading to an altered inflammatory cytokine profile, which results in impaired stimulation of T cells.[5]

ISOLATION OF EXOSOMES FROM HUMAN SPECIMENS

Exosomes have been isolated from several physiological fluids, including blood plasma/serum, breast milk, saliva, and urine.[22] Isolating exosomes from serum and saliva is likely to serve as the best screening method for early diagnosis of pancreatic cancer because obtaining these specimens requires less invasive methods. In 2013, Lau et al.[13] showed that pancreatic cancer-derived exosomes isolated from saliva could be detected and used as salivary biomarkers in a mouse model.

Several isolation methods have been described, including ultracentrifugation, ExoQuick™ precipitation, microfluidic device (ExoChip), and magnetic bead (DynaBeads®) isolation. Ultracentrifugation is considered the gold standard and is used largely in the research setting, although it is time-consuming, labour-intensive, and not ideal for clinical laboratories because it requires a large amount of starting material; exosome yields are typically low.[17,22]

Size-based isolation and exosome precipitation are additional methods. The first is completed using ultrafiltration, which is less time-consuming than other methods and does not require special equipment. It does not result in pure exosomes, however, but rather an exosome-rich sample because both cell culture media and body fluids contain a large number of nanoparticles within the same size range as exosomes. Conversely, exosome precipitation takes advantage of the differential solubility of exosomes in alternative solvents; ExoQuick, a proprietary reagent produced by System Biosciences (Mountain View, CA, USA) is an example. Rekker et al.[22] compared ultracentrifugation with ExoQuick and found that both methods are suitable for serum exosomal miRNA profiling. The miRNA profile was slightly affected by the method used, however, with the detection of two miRNAs (miR-92a and miR-486-5p) significantly influenced by the isolation method chosen. They also found that ExoQuick is more effective in precipitating exosomes from highly viscous biofluids, such as serum, regardless of origin.[22-24] Alvarez et al.[25] described similar results after comparing ultracentrifugation with precipitation-based exosomal isolation protocols using urine samples. These authors studied exosomal protein, miRNA, and mRNA levels and found that the highest exosome yield and RNA quantities were obtained by the ExoQuick precipitation-based method, while ultracentrifugation methods proved most suitable for protein analysis.

The ExoChip microfluidic device is an on-chip isolation, quantification, and characterisation method for circulating exosomes; it is functionalised with antibodies against CD63, an antigen commonly overexpressed in exosomes. Kanwar et al.[4] used the ExoChip method to isolate exosomes from the serum of both healthy and pancreatic cancer patients, and found this method to be suitable for diagnosis and screening of human cancer. The advantages of the ExoChip method include the rapidity and ability to simultaneously process a large number of samples.[4]

A more elaborate version of antibody-based exosome isolation are Dynabeads, magnetic beads that can be used to specifically isolate exosomes because they are coated in a specific antibody that is then used to isolate the molecule of interest. One disadvantage is that many washing steps may be required, potentially leading to unacceptable cell loss. In addition, unless a 'negative sorting' method is used, in which all biomolecules are removed using the beads, antibodies will remain attached to the cell if the exosomes are left in the supernatant.[26]

Taylor and Gercel-Taylor[18] have used antibodies against epithelial cell adhesion molecule to isolate tumour-derived exosomes as biomarkers of ovarian cancer using a modified magnetic activated cell sorting procedure. They compared current methods for exosome purification and found that exosomes isolated by ExoQuick precipitation produce exosomal RNA and protein in greater quantity and purity than chromatography, ultracentrifugation,

and DynaBeads. However, the authors cited a lack of specificity for the originating cell of the exosomes as a limitation of this method. Further analysis using techniques such as quantitative reverse transcription polymerase chain reaction (RT-PCR) profiling of miRNA, however, should adequately characterise these exosomes.

Considering the state of the current literature, it would seem that ExoQuick and ExoChip stand out as the preferred methods for exosome isolation in the clinical setting due to their specificity and ease of use.

USE OF EXOSOMES IN DIAGNOSIS OF PANCREATIC CANCER

Detection of exosomal biomarkers derived from pancreatic tumour cells in human serum that are sensitive and specific for PaCa would have a significant impact on patient outcomes. The ability of exosomal markers to predict tumour stage and degree of differentiation would also be beneficial. Very recently, Melo and colleagues[27] identified a cell surface proteoglycan, glypican-1 (GPC1), which is specifically enriched on cancer cell-derived exosomes. These exosomes, termed 'GPC1^{+}' exosomes, were monitored and isolated from the serum of patients and mice with pancreatic cancer using flow cytometry, and were detected with absolute sensitivity and specificity.[27] These exosomes were able to distinguish patients with early and late-stage PaCa from healthy individuals and patients with benign pancreatic disease. An additional finding was that the levels of GPC1^{+} exosomes correlated with tumour burden and with survival of pre and post-surgery patients.[27] It is noteworthy that GPC1^{+} exosomes were also detected in patients with breast cancer. The authors concluded that GPC1 is a pan-specific biomarker for cancer exosomes, and that many cancer cells overexpress GPC1, with the most abundant increases observed in PaCa.[27]

miRNAs, selectively concentrated into tumour-derived exosomes and differentially expressed, are suggested as potential markers for detection of early pancreatic cancer.[28,29] Zöller reported in 2013 that exosomal miRNA is derived from living cells, while free miRNA may mostly derive from dead cells, and as a result could change significantly during therapy or in late-stage PaCa.[28] miR-155 was reported to be a marker of early PaCa, while miR-196a correlated with disease progression. Evaluating a combination of conventional biomarkers, such as CA 19-9 and plasma miRNAs, in PaCa revealed that miR-155, miR-181a, miR-181b, and miR-196a differ significantly in patients with PaCa compared with healthy individuals.[28] In addition, only miR-16 and miR-196a allowed for discrimination from chronic pancreatitis.[28] In a study analysing serum exosomal miRNAs and their correlation with clinico-pathological features of PaCa patients, miR-17-5p, miR-21, miR-155, and miR-196a were selected for examination.[29] There were low expressions of exosomal miR-155 and miR-196a in serum samples from PaCa patients when U6 (a non-coding small nuclear RNA commonly used as an internal control in miRNA quantification by RT-PCR) was used as a control.[29]

The low expression of miR-155 and miR-196a reported in the study described above is in contrast to the results reported by Zöller.[28] Serum exosomal miR-17-5p was higher in PaCa patients than in non-PaCa patients and healthy participants. High levels of miR-17-5p also correlated with advanced stage and metastasis, which was inversely correlated with resectability.[29] MiR-21 has been reported to be strongly overexpressed in PaCa and contributes significantly to cell proliferation, invasion, and chemo-resistance in PaCa patients.[29] This study concluded that there was a high expression of serum exosomal miR-17-5p and miR-21 in PaCa patients compared with healthy participants and non-PaCa patients.[29] Madhavan and colleagues[2] demonstrated that a combined panel of proteins, markers of pancreatic cancer-initiating cells (PaCICs), and miRNA derived from PaCa-derived exosomes could serve as biomarkers for the diagnosis of PaCa. These proteins were described as markers of PaCICs by Wang et al.[30] in 2013. Diagnostic accuracy would be improved by the presence of markers specific for PaCICs because miRNAs could be abnormally expressed by other non-PaCa malignancies. These PaCICs are a small pool of cells with the capacity to self-renew and account for drug resistance, metastasis, and late recurrence after years of dormancy.[31] The study of tumour exosomes found that CD44v6, $\alpha 6\beta 4$, Tspan8, and CXCR4 were highly enriched in PaCICs and expressed in exosomes.[30] The combined evaluation of PaCIC protein markers and miRNAs expressed by serum exosomes of patients with PaCa displayed a sensitivity of 100%, and a specificity of 80% when compared with non-PaCa patients. The specificity rose to 93% when patients with other malignancies were excluded from the control group.[2] Evaluation of these serum

exosomal biomarkers as diagnostic tests for pancreatic cancer detection still requires large-scale, prospective clinical validation studies, and the optimal panel that provides the highest diagnostic accuracy remains to be determined. There are reports of altered miRNA expression in the tissue and pancreatic fluid of patients with premalignant lesions such as intraductal papillary mucinous neoplasms.[32,33] These panels of miRNAs have been found to differentiate between benign and premalignant, as well as high-risk from low-risk, cysts.[32,33]

While it has been reported that there is a correlation between tissue and serum miRNA levels, these findings have not been replicated in serum exosomes. Table 1 presents a list of studies evaluating exosomal biomarkers for the diagnosis of PaCa.[34,35]

Table 1: Studies and exosomal biomarkers used for the diagnosis of pancreatic cancer.

Study	Year	Specimen type and isolation method	Biomarkers	Results	Comments
Adamczyk et al.[34]	2011	Cell culture supernatant Ultrafiltration and ultracentrifugation	Soluble and exosomal forms of EGFR	EGFR is released as a full-length, intact receptor (170 kDa) and a 65 kDa processed form in exosomes released from pancreatic cancer cells.	EGFR is overexpressed in the majority of pancreatic cancers, and is regarded as a potential target for therapy.
Lau et al.[13]	2013	Saliva (mouse) Magnetic bead exosome extraction (Panc02 cell medium, serum, and saliva)	Apbb1ip, Aspn, BCO31781, Daf2, Foxp1, Gng2, Incenp	Suppression of exosome biogenesis in C56BL/6 mice resulted in lack of development of discriminatory biomarker in saliva.	Markers were also found to be significantly upregulated in the mouse pancreata.
Zöller M.[28]	2013	Serum Not applicable	miR-155, miR-196a, miR-181a, miR-181b, miR-196a, miR-16	No experiments conducted.	Free and exosomal microRNAs were examined.
Que et al.[29]	2013	Serum Filtration and ultracentrifugation	miR-155, miR-196a, miR-21, miR-17-5p	Low expression of exosomal miR-155 and miR-196a in serum samples of PaCa patients. miR-17-5p higher in PaCa patients compared with healthy participants and non-PaCa patients.	miR-17-5p reported to be the most specific for pancreatic cancer.
Kahlert et al.[6]	2014	Serum Filtration and ultracentrifugation	K-ras, p53	Exosomes from human serum samples, which span all chromosomes and contain DNA with mutated KRAS and TP53 genes, contain genomic DNA.	Exosomal double-stranded DNA was isolated from serum exosomes.
Madhavan et al.[2]	2015	Serum Ultracentrifugation	CD44v6, Tspan8, EpCAM, c-Met, CD104, miR-1246, miR-4644, miR-3976, miR-4306	miR-1246, miR-4644, miR-3976, miR-4306 were significantly upregulated in 83% of PaCa serum exosomes, but rarely in control groups. Most patients with PaCa (95%) reacted with a panel of anti-CD44v6, anti-Tspan8, anti-EpCAM, and anti-CD104.	Combined panel of PaCIC markers and microRNAs.

Table 1 continued.

Study	Year	Specimen type and isolation method	Biomarkers	Results	Comments
Melo et al.[27]	2015	Serum Filtration and ultracentrifugation	Glypican-1	Circulating GPC1+ exosomes were detected in the serum of pancreatic cancer patients with absolute sensitivity and specificity.	GPC1 is a cell surface proteoglycan specifically enriched on cancer cell-derived exosomes.
Klein-Scory et al.[35]	2015	Cell culture media Ultrafiltration and ultracentrifugation	NT5E/CD73	Membrane proteins, glycoproteins, small GTP-binding proteins, and a further heterogeneous group of proteins are enriched in vesicles. Proteins playing a role in carcinogenesis and modulators of the ECM are components of affinity-purified ECV.	Expressed in both exosomes and ectosomes.

ECM: extracellular matrix; ECV: extracellular vesicle; EGFR: epidermal growth factor receptor; GPC1: glypican-1; GTP: guanosine triphosphate; miR: microRNA; PaCa: pancreatic adenocarcinoma; PaCIC: pancreatic cancer-initiating cell.

Table 2: Studies showing therapeutic applications of exosomes in pancreatic cancer.

Study	Year	Mechanism	Outcome	Comments
Ohuchida et al.[36]	2011	High levels of expression of miR-142-5p and miR-204 were predictive of response to gemcitabine after resection for PaCa.	Increased survival in the gemcitabine treatment group.	Tissue levels of miRNAs were assessed. Yet to be correlated with exosomal miRNA.
Record et al.[37]	2011	Suppression of exosome secretion by tumour cells using the anti-hypertensive agent dimethyl amiloride.	Enhanced *in vivo* antitumor activity of cyclophosphamide.	None.
Aspe et al.[38]	2014	Delivery of exosomal survivin-T34A built from melanoma cell lines and plated on PaCa cell line.	Increased apoptotic cell death, and increased sensitivity to gemcitabine cytotoxicity.	Carried out *in vitro* using PaCa cell lines.
Mahmoodzadeh Hosseini H et al.[39]	2014	Delivery of exosomal staphylococcal enterotoxin B.	Induction of apoptosis in PaCa cells after 24 hours.	0.5 and 2.5 μg/100 μL of exosomal staphylococcal enterotoxin B significantly stimulated apoptosis after 24 hours.

PaCa: pancreatic adenocarcinoma.

USE OF EXOSOMES IN PANCREATIC CANCER TREATMENT

Due to their unique structure and transport functions, exosomes are regarded as potential therapeutic agents/vectors in cancer treatment. The use of exosomes in cancer immunotherapy has been previously studied (Table 2).[36-39] In a 2011 review by Record and colleagues,[37] exosomes were reported as a means of amplifying dendritic

cell-mediated cytotoxic T-cell responses. Exosomal immunotherapy was referred to as a type of cellular therapy, but exosomes were reported to be more convenient to handle and more stable compared with whole cells.[37] It was reported that reducing tumour exosome production using dimethyl amiloride (an anti-hypertensive agent) enhanced the *in vivo* antitumour efficacy of the chemotherapeutic agent cyclophosphamide.[37] Some studies have shown that delivering antigens *in vivo* through small secreted vesicles such as exosomes is more immunogenic than delivery of soluble antigens alone.[40,41] Ohuchida et al.[36] identified 24 miRNAs whose expression was altered in gemcitabine-resistant cells, and also found that patients with high miR-142-5p and miR-204 expression had significantly longer survival times than those with low miR-142-5p and miR-204 expression in the gemcitabine-treated group. Although the miRNA levels were determined in paraffin-embedded tissue, it is known that miRNAs in circulating exosomes are representative of those expressed in the tumour.[37] This highlights the potential use of tumour-derived serum exosomal biomarkers as predictors of response to chemotherapy, and demonstrates that therapeutic applications of exosomes go beyond their use in cancer immunotherapy. A 2013 study by Aspe et al.[38] isolated exosomes from a melanoma cell line and enhanced the cytotoxic effect of gemcitabine on pancreatic cancer cells *in vitro* through exosome-mediated delivery of survivin-T34A mutant protein. This suggests that exosomes may be used as a vector for therapeutic agents that treat or enhance the effects of other treatments for PaCa. Some reports show the induction of apoptosis in pancreatic cancer cell lines via the delivery of staphylococcal enterotoxin B in purified tumour-derived exosomes.[39] Our research indicates that the use of exosomes as a therapeutic tool in PaCa is still investigational, and their use is yet to make a significant impact on clinical practice in general. Table 3 lists ongoing clinical studies involving the use of exosomes for the treatment of gastrointestinal cancers.

CURRENT TRENDS IN EXOSOMAL RESEARCH

Research exploring potential therapeutic applications of synthetic nanovesicles is currently underway. The dominant area of research concerns liposomes, the building blocks of synthetic nanovesicles, which permit modification of specific features such as lipid type, electrical charge, size, distribution, and location of antigens.[42] Scientists are able to engineer nanovesicles to exhibit one or multiple features of exosomes. One way in which nanovesicles are being used to identify and treat PaCa is through creation of synthetic nanovesicles capable of specifically targeting cancer cells. The expression of matrix metalloproteinases in pancreatic cells can signal tumour growth,[43] and scientists can use this biomarker to identify and attack tumour cells via the use of gemcitabine delivered by nanovesicles.[44] Nanovesicles have been used as carriers of glucose in patients with diabetes and insulin deficiencies,[45] and have also been used as carriers of ibuprofen.[44] Nanovesicles offer powerful mechanisms for drug delivery because of their ability to target recipient cells.[46] Although there remains a lot yet to be understood about the design and applications of synthetic nanovesicles, there is a clear potential for therapeutic use that would permanently alter the scope and practice of pancreatic cancer treatment.

Table 3: Ongoing clinical studies involving exosomes in gastrointestinal cancers.

National Cancer Institute registration number	Title of study	Study design	Sponsor
NCT02393703	Interrogation of Exosome-mediated Intercellular Signaling in Patients With Pancreatic Cancer	Prospective observational (cohort)	Memorial Sloan Kettering Cancer Center, New York City, New York, USA
NCT01294072	Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	Interventional (Phase I)	James Graham Brown Cancer Center, Louisville, Kentucky, USA
NCT01779583	Circulating Exosomes As Potential Prognostic And Predictive Biomarkers In Advanced Gastric Cancer Patients ("EXO-PPP Study")	Prospective (case–control)	Hospital Miguel Servet, Zaragoza, Spain

CONCLUSION

Exosomes possess unique molecular characteristics that hold promise in the development of biomarkers for early detection of pancreatic cancer. Further studies are needed in order to validate an optimal set of markers that could provide the best diagnostic performance. The ability of exosomes to transport proteins and nucleic acids makes them suitable for use in cancer therapy. The use of exosomes in the treatment of PaCa requires validation in human patients and subsequent large-scale, prospective clinical studies. Research involving synthetic nanovesicles and their use in PaCa treatment is ongoing.

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