Review Article

Extracellular vesicles in pancreatic cancer: a new era in precision medicine

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Abstract: Pancreatic cancer (PC) is a lethal disease that presents a considerable challenge to healthcare providers and patients, given its low survival rate. However, recent advancements in precision medicine and innovative technologies have transformed the management of this disease. Among these advancements, extracellular vesicles (EVs) have emerged as crucial players in cancer progression. In PC, EVs play a pivotal role by facilitating cell-cell communication, impeding immune response, promoting cancer cell proliferation and survival, and supporting angiogenesis and chemoresistance. Cancer-derived EVs have a distinct oncogenic composition supporting tumour development and progression. Hence, they are critical biomarker candidates for various cancers, including PC. Notably, EVs can be isolated from diverse biological fluids such as blood, urine, and saliva, making them an ideal minimally invasive diagnostic and monitoring tool for PC patients. Despite the promising findings in the field of EVs, clinical validation as biomarkers is lacking. Furthermore, EVs being biocompatible, can act as drug carriers, delivering therapeutic molecules directly to cancer cells while minimizing toxicity to healthy cells. Therefore, understanding the role of EVs as biomarkers and their potential as drug cargo vehicles may revolutionise early detection, prognostication, and treatment in cancer. This mini-review summarises the latest understanding of their role in intercellular communication, involvement as potential biomarkers and drug carriers.

Keywords: Extracellular vesicles (EVs); pancreatic cancer (PC); precision medicine

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Introduction

Pancreatic cancer (PC) remains one of the most aggressive malignancies mostly due to the lack of early detection and poor response to surgical and chemotherapeutic therapies. Surgical excision is the definitive treatment option when diagnosed early, whereas chemotherapy and radiotherapy are used in the adjuvant, neoadjuvant or palliative setting (1). Nonetheless, cancer recurrence is common after surgical intervention (2,3). The growth of a tumour is mediated by the interaction between cancer cells and the neighbouring cells in the microenvironment and distant organs (4). This could be mediated by direct cell-cell interactions, secretory proteins, and extracellular vesicles (EVs). Precision

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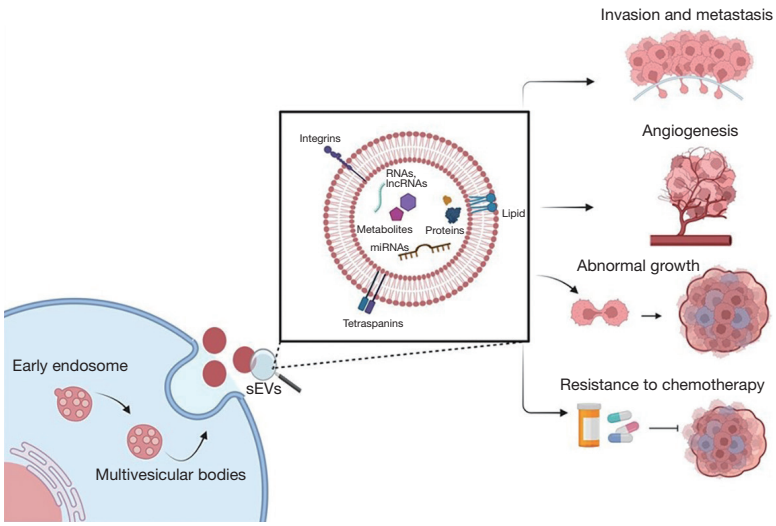

Figure 1 Functional role of pancreatic cancer-derived EVs. sEVs are produced by the fusion of multivesicular bodies with the plasma membrane containing bioactive cargo consisting of miRNAs, lncRNAs, and proteins and lipids. EVs derived from pancreatic cancer cells are crucial in tumour growth, angiogenesis, invasion, metastasis, chemoresistance induction, immunosuppression evasion, and inflammation. Figure made using BioRender.com. sEVs, small EVs; miRNAs, microRNAs; lncRNAs, long non-coding RNAs; EVs, extracellular vesicles.

medicine also called "personalised medicine" allows a tailored approach to an individual patient based on their risk profile and predicted response to therapy. Recent advancement in precision medicine has opened new avenues for therapeutic strategies, and one such emerging field in cancer research is the role of EVs. The EVs are described as lipid bilayer vesicles that are spontaneously released into the extracellular milieu by cells and lack the ability to replicate (5). They exhibit a broad spectrum of sizes, biogenesis, metabolic composition, and function, such as intercellular communication, immunological regulation, and disease progression. Based on the vesicle sizes and origin, EVs are often categorised into three categories: small EVs (sEVs) (30–150 nm), microvesicles (microparticles or ectosome, 50–1,000 nm), and apoptotic bodies (500–1,000 nm). Although EV release is a natural cellular process, a rapid increase in their release and a shift in their cargo's constituents, such as DNA, RNA, microRNA (miRNA), and proteins, can promote the development of cancer. sEVs are produced by tumour cells and adjacent stromal cells, such as cancer-associated fibroblasts (CAFs), tumour-associated macrophages (TAMs), and bone marrow mesenchymal stem cells, during tumorigenesis. Furthermore, EVs carry biological cargo that influences tubule formation and cell proliferation, thus facilitating cancer growth (6,7) (*Figure 1*). Understanding the role of EVs in PC will open the door to revolutionising precision medicine by providing novel insight into this disease's signatures, thus identifying potential therapeutic targets to improve patient outcomes, which we hope will be addressed by this mini review.

Role of PC-derived EVs in cancer progression

PC-derived EVs modulate angiogenesis

EVs are membrane-bound structures that are released by various cell types, including cancer cells (8). They contain various types of biomolecules, such as proteins, RNA, and lipids, which can modulate the function of neighbouring cells. In the context of PC, EVs have been shown to play a crucial role in regulating angiogenesis, the process by which new blood vessels form from existing ones. PC is known for its aggressive growth and poor prognosis, and the presence of new blood vessels is a hallmark of tumour progression. These new blood vessels help cancer cells to receive oxygen and nutrients to support their growth and spread to other parts of the body. Therefore, controlling angiogenesis has become an important target for the treatment of PC. Studies have shown that EVs derived from PC cells can modulate angiogenesis by affecting the expression of genes involved in this process. They

can communicate with neighbouring cells and activate pathways that promote or suppress the formation of new blood vessels, depending on the specific composition and quantity of EVs released by the cancer cells. In addition, EVs can also carry signalling molecules that can promote tumour growth, such as vascular endothelial growth factor (VEGF), which is a well-known angiogenic factor. PC-derived EVs stimulate human umbilical vein endothelial cells (HUVECs) cell growth, migration, and pro-angiogenic factor production (9). Studies suggest that the expression of CD151 and tetraspanin 8 (Tspan8) in PC contributes to tumour growth (10). Further, overexpression of D6.1A in PC increases angiogenesis *in vivo* by transferring D6.1A to endothelial cells (11). This stimulates the expression of angiogenic proteins such as CD31 and VEGF. Abnormalities in wound healing and tube formation have been observed in mice lacking the proteins, Tspan8 and CD151, with severe consequences reported in double-knockout mice (10,12). Interestingly, Tspan8/CD151-containing serum-derived sEVs can alleviate these events in genetically modified animals by promoting tube formation through signalling pathways involving receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs), eventually contributing to tumorigenesis *in vivo*. These findings suggest that blocking proteins such as Tspan8 and CD151 may have potential benefits in cancer therapy, as they play a role in tumour growth and development. The PC sEVs-derived miR-27a is found to significantly enhance angiogenesis in tumour-associated cells (13,14). This is achieved by increasing the production of proteins such as VEGFR, VEGF, matrix metalloproteinase 9 and matrix metalloproteinase 2. These proteins are involved in angiogenesis and cell migration. Additionally, annexin A1 (ANXA1) found in sEV cargo promotes endothelial tubular development and increases the motility of fibroblast and endothelial cells. Furthermore, the PKH-45H derived from sEVs has been found to induce angiogenesis *in vitro*. The angiogenic effect is mediated through the activation of specific molecular signalling pathways called extracellular signal-regulated kinase (ERK) and Akt, as well as a process called dynamin-dependent endocytosis. Additionally, tumour-derived sEVs improved the permeability of endothelial cells by transporting circular RNA molecules called circ-IARS into neighbouring cells (15). This triggers a chain of events inside the PC cells, resulting in tumour spread. Depleting myoferlin protein in PC cells has been shown to affect the production of sEVs and their transport to endothelial cells, resulting in reduced cell proliferation and propagation (16). Recent research revealed that cell signalling pathways involving DUSP2 and VEGF-C play a role in PC lymph angiogenesis and tumour invasion (17). Inhibiting DUSP2 increased the VEGF-C expression in PC-derived sEVs. In the PC animal model, VEGF-C-containing sEVs promote the proliferation of lymphatic endothelial cells and invasion of lymphatic vessels. In conclusion, the study of PC-derived EVs and their role in regulating angiogenesis has important implications for the understanding and treatment of this aggressive cancer. Further research in this area may lead to the development of new therapeutic strategies that can effectively control angiogenesis and prevent tumour progression.

PC-derived sEVs promote chemoresistance

Despite advances in chemotherapy drugs and treatment strategies, many patients with PC eventually develop resistance to these drugs, leading to treatment failure and poor outcomes. Understanding the mechanisms behind chemotherapy resistance is crucial for implementing effective chemotherapy regimens and future drug development. Recent studies have shown that EVs, specifically sEVs, play a significant role in the development of chemotherapy resistance in PC (18). The sEVs derived from chemotherapy-resistant PC cells have been shown to promote resistance in other cancer cells by delivering signalling molecules and miRNAs. These signalling molecules and miRNAs can suppress the expression of genes involved in the cellular response to chemotherapy drugs. This suppression can reduce the effectiveness of chemotherapy drugs, enabling PC cells to survive and continue to grow. Additionally, sEVs can also stimulate the activation of signalling pathways that promote the survival and proliferation of cancer cells, making them more resistant to chemotherapy. They can carry cancer-promoting molecules, such as oncogenes, to other parts of the body, where they can seed new tumours and contribute to disease progression. This process can also contribute to the development of chemotherapy resistance, as the new tumours may have different drug sensitivities than the primary tumour. One approach being explored to overcome chemotherapy resistance in PC is the inhibition of sEVs release from PC cells (19). By blocking the production of sEVs, it may be possible to minimise the effect of chemoresistance and reduce the risk of relapse. Chemoresistance develops after prolonged exposure to chemotherapeutic drugs. The chemotherapy drug such as,

gemcitabine (GEM) alters tumour cell cargo, including sEVs. MiR-155 is an onco-miRNA that has been reported to be significantly expressed in the majority of PC cases and to correlate with poor PC patient survival (20). In *in vitro* experiments and nonobese diabetic/severe combined immunodeficiency (NOD/SCID) animals, GEM-induced overexpression of miR-155 in PC cells derived sEVs which can prevent GEM-induced apoptosis in other PC cells. Deoxycytidine kinase (DCK) is one of the targets that miR-155 aims to affect. During chemotherapy DCK has the ability to convert GEM into an active form that incorporates into DNA, hindering its replication and synthesis, causing tumour associated cell death. MiR-155 in PC derived sEVs downregulates DCK expression and enhances PC cell survival (21). Following exosomal miR-155 absorption, a pro-apoptotic p53 effector gene known as *TP53INP1* was observed to have a decreased expression level in PC cells. Furthermore, miR-155 overexpression serves as a tumour defence mechanism against chemotherapeutic drugs, indicating a poor prognosis. This phenomenon is further exemplified by the EphA2 protein, as chemo-resistant PC cells produce sEVs enriched with EphA2, which are then delivered to nearby PC cells to assist in their survival (22). GEM upregulates the expression of sEV-derived miR210, which can be delivered from chemoresistant to chemosensitive PC cells (23). In conclusion, sEVs play a critical role in the development of chemotherapy resistance in PC. Understanding the mechanisms by which these vesicles promote resistance is crucial for improving patient outcomes. Future research in this area may lead to the development of new therapeutic strategies that can effectively target sEVs and overcome chemotherapy resistance in PC.

PC-derived sEVs in tumour proliferation and progression

The liver is the most common site for PC metastases. The physical location of the liver adjacent to the pancreas and the vascular supply enables tumour seeding via the portal vein and makes it a common metastatic site. Several research teams have investigated the involvement of EVs in enhancing the metastatic cascade in PC (24,25). One of the research teams has identified that PC-derived EVs trigger pre-metastatic niche formation in the liver, which increases the tumour load (26). Kupffer cells are responsible for the delivery of PC-derived EVs that contain the macrophage migration inhibitory factor (MIF) (27). These EVs can alter the hepatic milieu by triggering an inflammatory reaction and generating fibrotic pathways. These modifications promote tumour progression in the liver by encouraging liver cells to take up immune cells and enhance transforming growth factor (TGF) and fibronectin synthesis. Furthermore, PC-derived EVs supplemented in integrin v5 tend to migrate to the liver, whereas EVs enriched in integrins 64 and 61 are identified in the lungs (28). This selectivity of PC-derived EVs targeting distant sites, notably the liver, indicates that EVs are significant mediators of tumour proliferation and progression. More research is needed to comprehend the molecular pathways underlying distant site metastasis regulated by PC-derived EVs.

EVs *vs.* cancer biomarkers

Scientific efforts are being made to detect early symptoms of the disease in high-risk individuals. This is particularly important in PC, where understanding the disease tumorigenesis, monitoring therapy, and predicting the progress is essential. High risk individual comprises of family history, genetic mutation associated with PC, suffering from diabetes and chronic pancreatitis. Given the asymptomatic onset of PC, the paramount focus lies on early detection using reliable biomarkers. Minimally invasive biomarkers such as cell-free DNA and circulating tumour cells have yield promising results (29,30). These biomarkers, however, pose a challenge for large-scale applications due to their low concentration in blood plasma, difficult isolation techniques, and long-term storage and handling challenges. Moreover, EV cargo-derived RNA is considered to be more reliable than cell-free DNA as they are resistant to degradation (31). In addition, EVs are comparatively highly concentrated in the blood and EV-derived cargo is unique to disease type (32,33).

In order to fully harness the diagnostic and prognostic potential of EVs in PC, it is imperative that practical challenges need to be addressed. The variability in isolation of EVs and contamination of albumin in plasma/serum samples can affect downstream application of EVs. Utilisation of different isolation techniques may result in varying EV concentration, which consequently impacts the purity and yield of retrieved EVs. Further, EVs composition can evolve according to the pathological and physiological conditions of PC. Future studies need to focus on EV expression based on tumour staging and detecting PC in early stages. Additionally, there is a need to increase the sample size based on high-risk population, chronic pancreatitis/benign pancreatic conditions and existing

genetic predisposition (Table S1 provides a comprehensive list of potential EVs, highlighting their advantages and disadvantages in comparison to other biomarkers).

Role of EVs as potential PC biomarkers

Due to a lack of early symptoms, screening tools, and biomarkers, 80% of PC patients are identified at a late stage. Currently, carbohydrate antigen 19-9 (CA19-9) is the only biomarker available for PC (34); However, its limited sensitivity makes it unsuitable for early PC detection. As a result, innovative early diagnostic tools are crucial to identify PC patients at an early stage for treatment, thus having an impact on overall survival. Given the importance of EVs released by cancer cells in PC progression, the functional cargo carried by EVs shows potential as biomarkers for PC (Table S2). Using EV as an early diagnostic tool in PC has its advantages. The EVs are ubiquitously present in nearly all body fluids (such as serum, saliva, urine) making them easily accessible. Further, the changes in abundance and cargo profile of PC-derived EVs would make them applicable to detect early stages of cancer before the onset of symptoms. This will allow room for screening at-risk populations such as those presenting with chronic pancreatitis, family history or other PC hallmarks (5). In addition to early cancer detection, EV cargo can be used to monitor disease progression and treatment response thus allowing for patient stratification based on progression, treatment sensitivity and response. There are also promising studies being conducted to develop EV-based detection tests for PC that can be used in clinical settings (35,36). These tests have the potential to be highly precise for PC detection, offering a non-invasive and reliable method for disease monitoring. Several research teams have isolated and collected. EVs from the sample of PC patients and healthy controls and assessed specific miRNA-derived EV content using reverse transcription polymerase chain reaction (RT-PCR) and miRNA-specialised sensors to determine if EVs can be used as diagnostic tool (37-42). MiR-550, miR-21 miR-17-5p and miR-10b expression was found to be elevated in PC patients, implying that they can serve as potential markers for PC detection (41,43-46). Madhavan *et al.* examined blood EVs for five PC-initiating cell markers and four combination miRNAs (47). Comparing PC-initiating cell signatures and miRNA EV markers may facilitate PC detection and differentiation from healthy adults. Proteomic investigation of EVs obtained from pancreatic juice of PC patients identified possible diagnostic biomarkers including mucins (MUCs), cystic fibrosis transmembrane conductance regulator (CFTR), and multidrug resistance 1 (MDR1) proteins. However, these biomarkers are not confined to PC-derived sEVs (48-50). Further, a research team developed PC cancer mice model by implanting Panc02. They could suppress the secretions of PC derive sEVs by transfecting with dominant negative Rab-11 (51). This resulted in the transcriptome detection of salivary sEVs (DN-Rab11). Within malignant mouse saliva, 209 differently increased transcriptomic marker genes were discovered, with six proven to overexpress in PC peripheral blood and saliva derived sEVs, *beta-carotene oxygenase 31*, *Apbb1ip*, *Daf2*, *FoxP1*, *GnG2*, and *Incenp* (51). Interestingly, *Apbb1ip* a gene involved in actin structuring, has been found to be elevated in mice deficient in PC sEVs. However, the increase in gene expression is lower in these mice compared to the PC sEVs mouse model, which suggests that this gene may not be present solely in sEVs. Combining multiple biomarkers may increase specificity and sensitivity in cancer detection, as one constituent of sEV cargo might not prove to be sufficient to detect PC in at-risk patients. Glypican-1 (GPC-1) is a type of heparan sulphate proteoglycan present on cell membranes and in the extracellular matrix, where it signals cell growth and proliferation (33,52).

Role of EVs as a therapeutic tool

Rapid advancements in therapeutic tools have ushered in a new era of treatment strategies, where both biological and artificial vehicles are being harnessed for enhanced therapeutic outcomes (53-55). These remarkable tools share certain key physiological characteristics, such as their nanoscopic size and their capacity to efficiently deliver drugs to specific targets. Among these therapeutic tools, sEVs have emerged as particularly promising agents due to their unique capabilities (56). Notably, sEVs have demonstrated the remarkable ability to traverse the formidable blood-brain barrier, allowing for precise drug delivery to targeted sites. Unlike the artificial nanoparticles, the sEVs will not be eliminated by phagocytic system. sEVs constitute of range of proteins that help in bypassing immunological response. This intrinsic ability to elude immune detection makes sEVs an appealing choice for therapeutic interventions. Further, sEVs are comparatively more efficient as drug carriers than liposomes. As sEVs exhibit a distinctive characteristic of circulating in the bloodstream for prolonged time in comparison to liposomes. Moreover, sEVs offer distinct advantage as drug carrier by accommodating significantly

higher quantities of drugs compared to traditional liposomes.

EVs can be used to transfer therapeutic molecules, such as RNA and miRNA, directly to cancer cells, which can alter the physiology of the cancer cells and improve treatment outcomes. This approach presents significant challenges such as maintaining the stability of the vehicles. Using therapeutic vehicles can be challenging due to the need for specific targeting mechanism, maintain stability of vehicles in the body, and avoid toxicity. The investigation of the application of EVs in therapies is still in its early phase (Table S3). In 2019, Batista and Melo published an assessment of the approaches that have been investigated (57). One of the approaches focused on inhibiting the biogenesis or the release of EVs, thereby restricting the cancer-promoting signalling from the EVs.

EVs as active drug delivery tool

EVs are known to transport unique biomolecules that are effectively internalised by target cells. Furthermore, EVs possess the unique characteristic to exhibit negligible toxicity and minimal immunogenicity, such characteristics can make EVs a compelling option for becoming a targeted therapeutic agent (58,59). One of the research teams discovered that by modifying EVs to target a specific gene mutation, were able to reduce tumour growth and increase lifespan of mice (60). This study focused on using EVs to target the oncogenic mutation; KRASG12D which resulted in a greater reduction in tumour size compared to liposomes loaded with the same siRNA or shRNA. After 200 days of therapy, KRAS and KRASG12D expression was decreased in malignant tumours and pancreas, which was believed to be the cause of the observed results. These findings could potentially lead to the development of new targeted cancer therapies. The intervention effectively reduced the proliferation of the KRASG12D mutation in mice. According to a study conducted by Pascucci *et al.*, administration of paclitaxel to mesenchymal stromal cells resulted in secretion of EVs with cargo containing paclitaxel. These EVs inhibited PC cell growth *in vitro* (61). Subsequently, one of the scientific groups demonstrated the potential of employing EV loaded with paclitaxel as a treatment of multidrug-resistant cancer (62). Recent scientific efforts have engineered cells that can generate sEVs with a targeted surface protein content, including interleukin-12, targeted T- and natural killer-cells. The sEVs has the potential to be modified via the addition of a specific glycoprotein to enhance cellular function. Administering anticancer drugs can activate the stimulator of interferon gene (STING) pathway, which presents a promising strategy in the fight against cancer. However, further research is required to determine its effectiveness and potential clinical applications (63). EVs as therapeutic vehicles are promising area of research for the treatment of PC, and their continued development and optimisation holds great promise for improving the prognosis for PC patients.

Interrupting EV production and targeting molecular signalling

Chemoresistance is a key factor in the fight to treat PC effectively. GEM is currently one of the most utilised drugs. Unfortunately, the EVs produced following GEM therapy frequently cause an increased GEM resistance. Richards *et al.* reported in 2022 that GEM therapy significantly boosted the generation of EVs by CAFs (64). This proved EVs have a significant impact on PC progression and can be considered as a possible target for therapeutic intervention. Inhibiting EV secretion or uptake is widely considered a novel approach to PC therapy. Catalano *et al.* employed the drug GW4869 to inhibit the release of EVs harbouring miR-146a from CAFs (65). They observed that certain cell lines preserved their sensitivity to chemotherapy. Consequently, blocking EV uptake may elicit a suppressive impact on cancer cells. Research revealed that the protein REG3β released from pancreatic tissue interferes with EV uptake and internationalisation of the recipient cells, effectively restricting migration and metabolism in PC malignant cells (66). Further, PC cells treated with GEM enrich miR-155 expression and transfer miR-155 to other PC cells, inhibiting cell apoptosis. PC derived EVs possess the ability to mitigate the immune response by reducing the efficacy of antigen presentation. Depleting miRNAs from PC-derived EVs has been proven to increase cancer resistance in dendritic cells and other cells at distant sites (67). Interfering with EV production may reduce the effect of the tumour microenvironment and may enhance the efficacy of immunotherapy in PC. EVs have emerged as a promising tool for improving PC patients' overall survival. By engineering EVs to carry pharmacological drug and specific surface modification may enable them to selectively recognise and bind to PC cells. The pharmaceutical drug may include chemotherapeutic agents, novel targeted therapies or siRNAs are designed to inhibit signalling

pathways associated to PC progression. In conclusion, the PC derived EVs are small vesicles secreted by cells that can be engineered to deliver therapeutic agents directly to cancer cells. They can also traverse biological barriers and efficiently transport bioactive molecules that may interfere with the molecular mechanisms driving PC. Moreover, EVs can be analysed to provide diagnostic and prognostic value in PC. As research in EV-based therapies continues to evolve, it offers a beacon of hope for PC patients in need of more effective and tailored therapeutic options.

Future directions

PC is a challenging disease to diagnose and treat due to its advanced stage at presentation and systemic spread. The presence of EVs in bodily fluids such as urine, blood, pancreatic juice, and saliva makes them an ideal candidate as biomarkers. Current literature demonstrates the role of EVs biological cargo in PC progression, as they regulate tumour microenvironment, promote neo-angiogenesis and contribute to drug resistance (68). With the development of next-generation biotechnological tools such as single-cell and single-EV analysis, high-throughput, and shotgun proteomics, the capacity to assess possible candidate biomarkers may likely improve their utility further (69). The use of EVs in early diagnosis would provide an effective screening opportunity in high-risk individuals, leading to better outcomes for patients.

Conclusions

To summarize, EVs have great potential as biomarkers for PC. These bubble-like vesicles carry information related to advancement of the disease, therapy response and early detection. Nanoparticle-based EV analysis is a new technology that could enhance the sensitivity and specificity of EV-based diagnostic procedures and their usefulness as biomarkers. However, several obstacles must be solved before EVs may be used as a reliable biomarker for PC. These include increasing the specificity and sensitivity of current diagnostic tools, confirming the findings of more extensive clinical trials, and integrating EVs with existing diagnostic procedures such as imaging and biopsy to increase diagnosis accuracy. Despite the current challenges, ongoing research offers the potential to overcome these obstacles and make EVs a reliable biomarker in the fight against PC, ultimately improving patient survival rates.

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Footnote

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Supplementary

Table S1 Comparison of PC derived EVs *vs.* other cancer biomarkers

Potential biomarker	Sample type	Diagnostic value in PC patients	Advantages	Disadvantages	References
Carbohydrate antigen 19-9 (CA19-9)	Serum	Sensitivity: 79-81%; Specificity: 82-90%	Relatively easy collection; reliable marker for treatment response and monitoring	Poor screening marker; elevated expression in benign jaundice, pancreatitis, ovarian cancer or other malignancies	(1)
Circulating tumour cells (CTCs)	Serum/plasma	Sensitivity: 75%; Specificity: 96.4%; AUC: 0.867; 95% CI: 0.798-0.935	Correlated with poor prognosis	Low concentration in serum/plasma; Lack of evidence in large scale clinical setting; variable in isolation techniques	(2-4)
Cell free DNA (cfDNA)	Plasma	Combination of 5mC and 5hmC prediction model: Sensitivity: 93.8%; Specificity: 95.5%; AUC: 0.99	Correlated with poor prognosis	Utility is limited to identifying existing mutation in clinical setting; Lacks evidence in large scale clinical setting	(5)
Extracellular vesicles (EVs)	Plasma/serum/pancreatic juice	GPC1+ study: Sensitivity: 95-100%; Specificity: 95-100%	Correlated to early detection, prognostic marker and potential tumour staging marker	Variability in isolation techniques; Lacks evidence in high quality isolation in clinical setting	(6-17)

AUC, area under curve.

Table S2 Potential EV biomarkers in pancreatic cancer

Type of EV cargo	EV content	Experimental Approach	Sample Type	PC patient sample size	Sensitivity and specificity	Relevance to PC	Reference
Micro RNA (miRNA)	miR-1246, miR-3976, miR-4644, miR-4306	RT-PCR, qRT-PCR	Serum samples and PC cell lines	miR-1246, miR-4644 and miR-4306: Patients: 12. miR-3976: Patients: 131	miR-1246: Sensitivity: 66.7%, Specificity:100%, AUC: 0.814. miR-4644: Sensitivity: 75%, Specificity: 76.9%, AUC: 0.76	Elevated expression	(13,15,18)
	miR-18a	qRT-PCR	Patient plasma samples	Patients: 36	Not available	Elevated expression	(19,20)
	miR-17-5p	qRT-PCR	Patient serum samples	Patients: 22	Sensitivity: 72.7%, Specificity: 92.6%	Correlated to advanced stage of PC	(21)
	miR-122-5p	qRT-PCR	Patient plasma samples	Patients: 216	AUC: 0.81	Diagnostic marker	(7)
	miR-let7a	LC-MS/MS, qRT-PCR	Patient plasma samples	Patients: 29	Sensitivity: 100%, Specificity	Lower expression linked to PC progression	(22,23)
	miR-191, miR-451a and miR-21	qRT-PCR	Patient plasma samples	Patients: 32	miR-191: Sensitivity: 71.9%, Specificity: 84.2%. miR-451a: Sensitivity: 65.6%, Specificity: 85.7%. miR-21: Sensitivity: 80.7%, Specificity: 81.0%	Elevated expression	(24)
	miR-21	Western Blotting, TCLN biochip	Patient plasma and mouse serum samples	Patients: 36	Sensitivity: 95.5%, Specificity: 81.5%	Elevated expression	(25)
	miR-451a	qRT-PCR	Patient serum samples	Patients: 6	Sensitivity: 69.2%, Specificity: 70.8%	Elevated expression	(26)
	miR-196b/LCN2/TIMP1	RT-PCR	Patient serum and duodenal juice	Patients: 50	Sensitivity: 80%, Specificity: 80%, AUC: 0.93	Elevated expression	(27)
	miR-214	qRT-PCR	Patient plasma samples	Patients: 20	Not available	Lower expression linked to better survival rate. Diagnostic marker	(28)
	Ratio of miR-3940-5p/miR-8069	3D digital PCR	Patient urine samples	Patients: 43	Sensitivity: 58.1%, Specificity: 89.2%	Diagnostic marker	(29)
	miR-192-5p, miR-19a-3p, and miR-19b-3p	qRT-PCR	Patient serum samples	Patients: 159	Not available	Elevated expression; Diagnostic and prognostic value	(30)
	miR-10b, miR-21, miR-30c, miR-181a	LSPR-based assay	Patient plasma samples	Patients: 29	Sensitivity: 100%, Specificity: 100%	Diagnostic marker	(22)
Circular RNA (circ RNA)	has_circ_0000896 and has_circ_0000128	qRT-PCR	Patient plasma and cell culture samples	Patients: 8	Not available	Elevated expression	(31)
	circRNA-PDE8A	RNA binding protein immunoprecipitation assay, Biotinylated RNA pulldown assays	Patient plasma samples	Patients: 93	Not available	Elevated expression; correlated PC progression	(32)
	circ-IARS	qRT-PCR	Patient plasma, tissue and cell culture samples	Patients: 92	Not available	Elevated expression	(33)
	circRNA-0000069	Flow-cytometry, Western blotting, RT-qPCR	Patient tissue and cell culture samples	Patients: 179	Not available	Elevated expression	(34)

Table S2 *(continued)*

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Table S2 *(continued)*

Type of EV cargo	EV content	Experimental Approach	Sample Type	PC patient sample size	Sensitivity and specificity	Relevance to PC	Reference
mRNA	CK18, CD63	Next Generation sequencing and qRT-PCR	Patient plasma samples	Patients: 89	AUC: 0.93	Detected in PDAC patients	(35)
	FGA, KRT19, HIST1H2BK, ITIH2, MARCH2, CLDN1, MAL2 and *TIMP1*	RNA sequencing analysis	Patient plasma samples	Patients: 284	AUC: 0.949	Detected in PDAC patients	(36)
Proteins	Glypican-1	UPLC-MS, Western Blot Analysis, qRT-PCR	Patient serum and tissue samples, Animal studies, cell lines	Patients: 190	Sensitivity: 100%, Specificity: 100%	Diagnostic/Screening tool	(6)
	MIF	Proteomics, RNA sequencing, tissue processing, immunofluorescence, SDS-PAGE, Western Blot, flow cytometry	Human peripheral blood samples, animal studies and Cell lines	Patients: 18	Not available	Initiates formation of pre- metastatic niche in the liver	(37)
DNA	NOTCH1, BRCA2	Next generation sequencing	Patient pleural fluid, blood, plasma	Patient: 3	Not available	Detected in patient exoDNA samples	(38)
	$KRAS^{G12D}$ and $TP53^{R273H}$	ddPCR	Patient serum samples	Patients: 171	Not available	Elevated expression	(39)
	KRAS	ddPCR	Patient plasma samples	Patients: 194	Not available	Detected in PC patients	(40)
	KRAS	ddPCR	Patient plasma samples	Patients: 88	Not available	Elevated expression	(41)

GPC1+, glypican-1; RT-PCR, reverse transcriptase polymerase chain reaction; qRT-PCR, quantitative real time polymerase chain reaction; LC-MS/MS, Liquid chromatography-mass spectrometry; TCLN biochip, tethered cationic lipoplex nanoparticle biochip; LSPR-based assay, localized surface plasmon resonance based assay; UPLC-MS, ultra performance liquid chromatography mass spectrometry; SDS-PAGE, sodium dodecyl-sulfate polyacrylamide gel electrophoresis; ddPCR, droplet digital polymerase chain reaction; MIF, migration inhibitory factor; AUC, area under curve.

Table S3 Preclinical and clinical trials of PC derived EVs

Disease Model	Trial	Pharmacological drug	Administration	References
Mouse	Preclinical	siPAK4	Intratumoral injection	(42)
Mouse	Preclinical	Gemcitabine	Intravenous injection	(43)
Mouse	Preclinical	siKRASG12D	Intraperitoneal injection	(44)
Mouse	Preclinical	siKRASG12D	Intraperitoneal injection	(45)
Mouse	Preclinical	siKRASG12D and miRNA-145-5p	Intratumoral injection	(46)
Human	Clinical (Phase 1)	siRNA KrasG12D	Intravenous injection	(47)

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