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# Advances in the application of extracellular vesicles in precise diagnosis of pancreatic cancer

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## Abstract

Pancreatic cancer is a highly malignant tumor with poor prognosis, emphasizing the need for accurate early diagnosis. EVs, as mediators of intercellular communication, carry DNA, RNA, and proteins that show differential but not tumor-specific expression patterns in pancreatic cancer. Studies have shown that combining RNA markers in EVs (such as miRNA, circRNA, and lncRNA) with serum CA 19-9 testing can significantly enhance diagnostic accuracy for pancreatic cancer. EV-associated proteins have exhibited favorable diagnostic performance in early-stage pancreatic cancer in preliminary studies, though their clinical applicability remains to be further validated. Furthermore, mutations in KRAS, TP53, and SMAD4 genes within EVs offer a promising avenue for non-invasive liquid biopsy. However, challenges such as standardization, low sensitivity, and specificity still hinder the clinical application of EVs. Future research should focus on strategies including multi-omics integration, AI-assisted analysis, multi-marker combined detection, and large-scale clinical validation to further improve the diagnostic capability for pancreatic cancer. Overcoming these obstacles may position EVs as a vital tool in the diagnosis of pancreatic cancer.

**Keywords** Extracellular vesicles, Pancreatic cancer, Diagnosis

## Introduction

Pancreatic cancer is one of the most aggressive malignancies worldwide, characterized by high incidence and exceptionally high mortality rates. Unfortunately, 80%–85% of patients are already at an advanced stage when diagnosed, resulting in a very poor prognosis, with a 5-year survival rate of only 12.8% [1–3]. In recent years, the incidence of pancreatic cancer has been steadily increasing. Current diagnostic approaches primarily rely on imaging modalities, serum tumor markers, and endoscopic ultrasound (EUS), often combined with fine-needle biopsy (EUS-FNB), to obtain cytological or histological confirmation. However, both methods demonstrate relatively low sensitivity and specificity for pancreatic cancer diagnosis [3, 4]. Imaging techniques often only allow for a definitive diagnosis once the cancer has significantly progressed, while serum tumor markers (such as CA 19-9) perform suboptimally, with limited specificity, particularly in differentiating pancreatic cancer from conditions like chronic pancreatitis [3, 4]. As a result, there is an urgent need for diagnostic methods with improved sensitivity and specificity.

The detection of biomarkers carried by extracellular vesicles (EVs) has emerged as a novel diagnostic approach. Since these biomarkers originate from parent cells, EVs have shown great potential in diagnosing

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various diseases. Notably, they have demonstrated exceptional diagnostic performance in pancreatic cancer, with diagnostic efficacy reportedly reaching up to 100% in some cases [5–8]. This review discusses the role of different biomarkers carried by EVs in pancreatic cancer diagnosis and explores potential future developments, aiming to provide insights for improving pancreatic cancer diagnostics.

## Overview of EVs

### Origin, classification, and formation mechanisms of EVs

Extracellular vesicles (EVs) are membranous vesicles secreted by cells and are present in various bodily fluids, such as blood, urine, and saliva. Based on their size and biogenesis pathways, EVs are classified into three major types: exosomes, microvesicles, and apoptotic bodies (Fig. 1) [9].

*Exosomes*: These vesicles range from 30 to 150 nm in diameter and originate from the intraluminal vesicles of multivesicular bodies. Exosomes are formed through either the ESCRT (endosomal sorting complexes required for transport) or non-ESCRT pathways and are released when the multivesicular bodies fuse with the cell membrane. They carry ESCRT-associated proteins and tetraspanins, a family of four-transmembrane-domain proteins involved in membrane organization, vesicle formation, and intercellular communication [9, 10].

*Microvesicles*: With diameters of 100 to 1000 nm, microvesicles are formed by direct budding from the plasma membrane. Their formation is typically associated with elevated cytosolic calcium levels and they are enriched with membrane proteins such as integrins, selectins, and phosphatidylserine [9–11].

*Apoptotic bodies*: These vesicles, measuring between 500 and 2000 nm, result from the process of apoptosis. They contain organelles and nuclear fragments and are primarily produced during the late stages of cell apoptosis [9, 12].

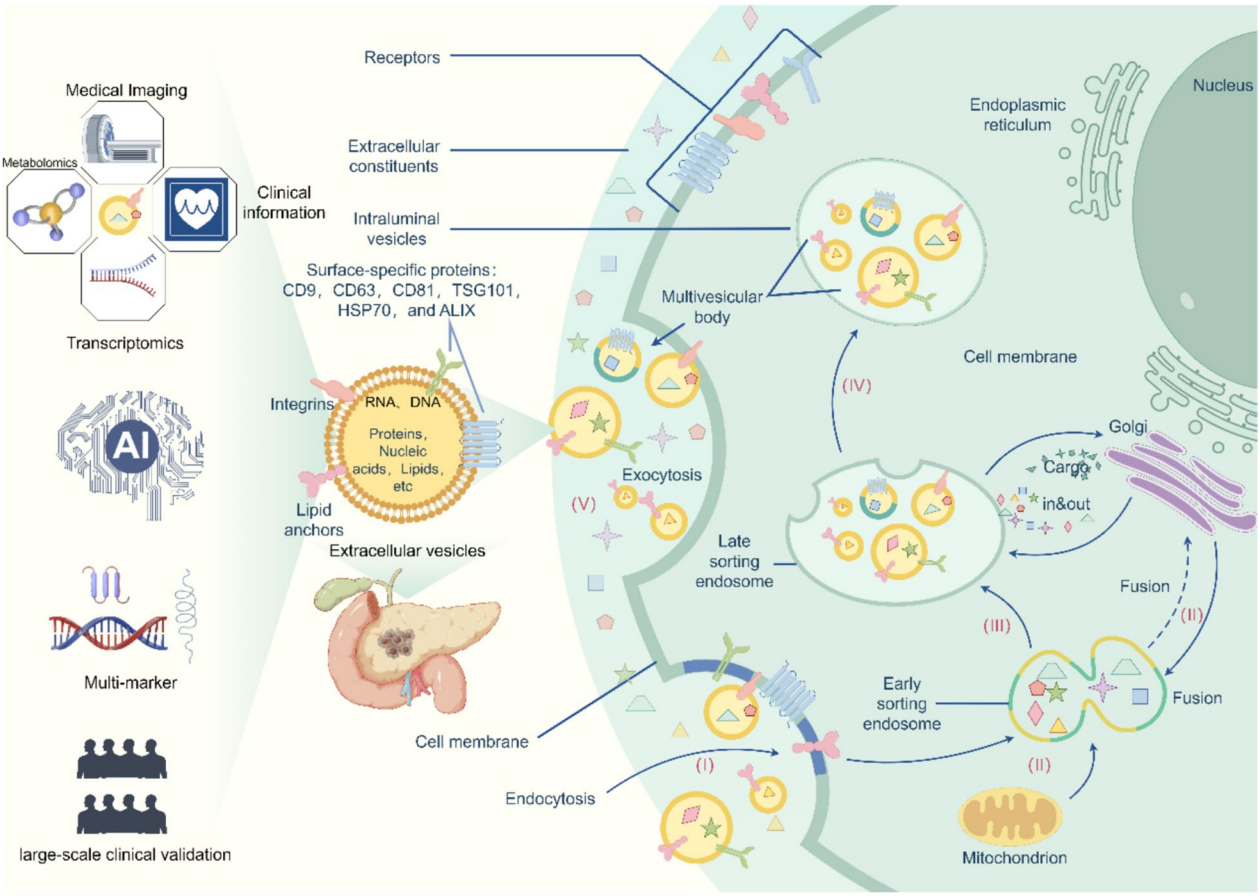


**Fig. 1** Biogenesis, Biomarker Cargo, and Emerging Applications of EVs (By Figdraw). Note: This figure focuses on exosomes and does not depict microvesicles or apoptotic bodies

### Isolation of EVs

The isolation of extracellular vesicles (EVs) is a crucial step in EV research, and different isolation methods have their own advantages and limitations (Table 1).

In recent years, a novel automated EV isolation system known as EXODUS has been developed. It utilizes a negative pressure oscillation system and a dual-coupled harmonic oscillation system applied to a nano-filtration chip to obtain high-purity EVs. This system can process rare samples, requiring only simple centrifugation before automatically extracting EVs. While it offers high purity, it is limited by the cost of consumables and the expensive equipment [17].

The future development of EV isolation technologies needs to focus on addressing the existing challenges, particularly achieving a balance between isolation purity, efficiency, and concentration. Gradually, automated extraction systems should be developed, and efforts should be made to establish standardized and regulated protocols for EV isolation.

### Identification and analysis of EVs

Current EV analysis primarily focuses on their physical characteristics and compositional components. The following table introduces common methods used for EV identification (Table 2).

## Application of EVs in the diagnosis of pancreatic cancer

### Biomarkers in EVs

The biomarkers found in pancreatic cancer-associated EVs primarily include RNA, proteins, and DNA. These biomarkers'specificity and stability make them powerful tools for the early diagnosis of pancreatic cancer. Below, we will discuss several key types of biomarkers.

#### *RNA*

RNA detection in EVs offers high specificity and sensitivity for diagnosing pancreatic cancer. The main RNA types include non-coding RNA (miRNA), circular RNA (circRNA), and long non-coding RNA (lncRNA). These RNAs carry biological information from the parent cells and have thus been extensively studied as potential biomarkers for the diagnosis of pancreatic cancer, although their use remains largely limited to experimental settings.

miRNA within EVs has shown high diagnostic performance for pancreatic cancer, with both sensitivity and specificity exceeding 80% in most studies [30–34]. Among the most reported miRNAs are miR-21 and miR-10b. Previous studies have demonstrated the effectiveness of using miR-21 as a diagnostic biomarker for pancreatic cancer. Except for Goto et al.'s study, which reported a specificity of 81%, other studies demonstrated specificity above 90%, with two reporting up to 100%. However, these findings were based primarily on mixed-stage or confirmed pancreatic cancer cohorts, and their performance in early-stage detection remains to be fully established [30–34]. miR-10b is highly expressed in pancreatic cancer patients, with significantly higher expression levels compared to healthy controls or pancreatitis patients. Elevated miR-10b levels in pancreatic cancer are associated with increased tumor invasiveness, promoting metastasis, and higher expression is linked to advanced-stage tumors [35–37]. Reese et al. studied a panel of 11

**Table 1** provides an overview of several common methods used for EV isolation [13–16]

| Isolation method | Steps | Advantages | Disadvantages |
|---|---|---|---|
| Ultracentrifugation | Gradually increase centrifugal force to remove impurities; isolate EVs with ultracentrifugation | Suitable for large sample volumes, low cost | Time-consuming, low purity, potential EV damage |
| Density Gradient Centrifugation | Centrifuge in a gradient medium, separating EVs based on density differences | High purity, effectively removes contaminants | Time-consuming, complex operation |
| Immunoaffinity Capture | Use antibodies to capture specific surface proteins on EVs, separate via magnetic beads or microporous plates | High specificity, suitable for small sample volumes | Requires development of antibodies for different EV types, high cost |
| Size-Exclusion Chromatography (SEC) | Separate particles of different sizes through a molecular sieve, EVs elute later through the column | Simple operation, preserves EV integrity | Low EV concentration, poor column reproducibility |
| Microfluidic Chip Technology | Samples pass through microchannels, separating EV subtypes based on physical properties | Fast, suitable for small samples | Expensive equipment, difficult to process large sample volumes |
| Polymer Precipitation | Add polymers like polyethylene glycol (PEG) to precipitate EVs, followed by centrifugation | Simple operation, low cost | Low purity, requires further purification |

**Table 2** Common Methods for EV Identification [6, 7, 18–29]

| Analysis type | Method | Advantages | Disadvantages |
|---|---|---|---|
| Physical characteristics | | | |
| | Nanoparticle Tracking Analysis (NTA) | Simultaneously measures particle size and concentration, simple operation | Requires high sample purity |
| | Dynamic Light Scattering (DLS) | Quickly measures particle size and size distribution | Difficult to differentiate EVs with small size differences |
| | Transmission Electron Microscopy (TEM) | High-resolution observation of EV morphology and structure | Complex sample preparation, expensive equipment |
| Molecular composition | | | |
| | Western Blotting | Strong specificity, validates EV presence and purity | Semi-quantitative, limited sensitivity |
| | Enzyme-Linked Immunosorbent Assay (ELISA) | High sensitivity, suitable for multi-sample quantitative analysis | Requires specific antibodies, cannot distinguish EV origins |
| | Mass Spectrometry (MS) | High-throughput analysis, identifies novel biomarkers | Complex sample preparation, requires expert data analysis |
| | Real-Time Quantitative PCR (qPCR) | High sensitivity, simple operation | Requires knowledge of target sequences, not suited for whole-genome analysis |
| Phenotype analysis | Nano-Flow Cytometry | Detects EVs as small as 40 nm, allows single-particle analysis | Expensive equipment, technically complex |
| Emerging technologies | | | |
| | Raman Spectroscopy | Label-free, provides molecular information | Weak signal, expensive equipment |
| | Electrochemical Sensors | Rapid and sensitive, suitable for on-site detection | Requires development of specific probes, needs improved interference resistance |
| | Protein Microarrays | High-throughput, allows multi-marker detection | Requires high-quality antibody matrix |
| | Digital PCR | High sensitivity, accurate quantification | Expensive equipment, complex operation |
| | Surface Plasmon Resonance (SPR) | Single-particle analysis, provides both size and membrane protein information | Requires specific antibodies, low throughput |

miRNAs and found that miR-200b and miR-200c were significantly overexpressed in serum exosomes of pancreatic cancer patients compared to healthy controls ($P< 0.001$; $P= 0.024$), and these miRNAs also differentiated pancreatic cancer from chronic pancreatitis ($P= 0.005$; $P= 0.19$). Combining the expression levels of miR-200b and miR-200c in total serum and EpCAM-positive exosomes with serum CA 19-9 levels achieved a diagnostic accuracy of 97% ($P< 0.0001$) for pancreatic cancer in the referenced study. However, the clinical applicability of this approach, particularly in early-stage detection, requires further validation [38].

The circular structure of circRNAs provides them with unique stability and resistance to exonuclease degradation, making them relatively stable in blood, urine, and other biological fluids. These characteristics render circRNAs promising biomarkers for malignant tumors such as pancreatic cancer [39–41]. Multiple studies have demonstrated that the expression levels of circRNAs in EVs from the blood of pancreatic cancer patients are significantly higher than in healthy individuals, highlighting their potential value in early diagnosis. For instance, detecting the expression of circPDK1 can significantly improve the sensitivity and specificity of pancreatic cancer diagnosis, which is particularly important for this highly aggressive and difficult-to-detect malignancy [42]. Additionally, the expression of circRTN4 is closely associated with the progression of pancreatic cancer. Its elevated expression in patients not only aids in diagnosis but also provides valuable information for assessing disease progression and prognosis [43]. The detection of circRNAs in EVs can be combined with other markers to further enhance diagnostic accuracy.

Studies have shown that hsa_circ_0006220 and hsa_circ_0001666 are highly expressed in the plasma EVs of pancreatic cancer patients compared to healthy controls. The diagnostic efficacy of hsa_circ_0006220 alone is 0.7817, and hsa_circ_0001666 alone is 0.8062, but their combined diagnostic value reaches 0.884. Moreover, clinical-pathological characteristics indicate that the expression of hsa_circ_0006220 in plasma EVs of pancreatic cancer patients is associated with CA19-9 levels ($P= 0.0001$) and lymph node metastasis ($P= 0.0005$). Similarly, hsa_circ_0001666 expression correlates with tumor

size ($P$= 0.0157) and CA19-9 levels ($P$= 0.0001) [41]. With advancements in detection technologies, circRNA testing in liquid biopsies is continuously being optimized, and the combination with other markers like CA19-9 is enhancing the sensitivity and specificity of pancreatic cancer diagnostics.

Long non-coding RNAs (lncRNAs) are non-coding RNA molecules longer than 200 nucleotides [44]. Aberrant expression of lncRNAs in EVs has been identified in various malignancies, including pancreatic cancer [45–47]. He et al. discovered four abnormally expressed lncRNAs in plasma EVs from pancreatic cancer patients using whole-transcriptome sequencing. These four lncRNAs were expressed at higher levels in pancreatic cancer patients compared to healthy controls and showed a positive correlation with CA19-9 levels. The area under the receiver operating characteristic (ROC) curve (AUC) for these four lncRNAs in diagnosing pancreatic cancer was 0.8421, 0.6544, 0.7190, and 0.6321, respectively. When combined, the diagnostic efficacy increased to an AUC of 0.8476, with a sensitivity of 0.72 and specificity of 0.89 [48]. In another case–control study involving 501 samples, the diagnostic performance of eight lncRNAs in plasma EVs was analyzed. The AUC for diagnosing pancreatic cancer was above 0.93 in both the training and validation sets, and the test successfully identified resectable stage I/II cancers (AUC = 0.949). Additionally, it outperformed CA19-9 in distinguishing pancreatic cancer from chronic pancreatitis (AUC 0.931 vs 0.873, $P$= 0.028) [49].

#### *Proteins*

Proteins in EVs have gained significant attention as biomarkers for pancreatic cancer due to their specific expression and relative stability. A 2023 study conducted mass spectrometry analysis of EV proteins and identified several proteins differentially expressed between pancreatic cancer patients and healthy controls. This provides a strong basis for using EV proteins in the diagnosis of pancreatic cancer [50].

Glypican-1 (GPC1) is one of the most frequently reported protein biomarkers in EVs associated with pancreatic cancer. Melo et al. were the first to highlight the significance of GPC1-positive exosomes in the diagnosis of pancreatic cancer, demonstrating that GPC1-positive EVs could distinguish pancreatic cancer patients from healthy individuals with 100% accuracy. This finding presented a highly promising non-invasive method for pancreatic cancer detection [8].

However, subsequent studies have offered differing perspectives on the diagnostic value of GPC1 [51]. For instance, Li et al. reported that GPC1 showed lower sensitivity and specificity in pancreatic cancer, with values below 60%, possibly due to variations in study design, sample size, and detection methods [52]. Despite these discrepancies, the consistent identification of GPC1 across multiple studies suggests it remains a key biomarker for pancreatic cancer diagnosis. Notably, when combined with other protein biomarkers, the diagnostic performance of GPC1 can be significantly enhanced [53].

CD63, a member of the tetraspanin family, is highly expressed in EVs derived from pancreatic cancer cells. Studies have demonstrated that using CD63 in EVs to distinguish pancreatic cancer patients from healthy controls offers high diagnostic performance (AUC = 0.846). Moreover, CD63 shows significant diagnostic efficacy in both early and advanced stages of pancreatic cancer. When combined with CA19-9, the diagnostic performance improves further (AUC = 0.903) [54].

Additionally, CD63 levels in EVs can reflect tumor burden, as CD63 expression decreases rapidly following the removal of the primary tumor in pancreatic cancer patients [54]. This suggests that CD63 may not only aid in diagnosis but also in monitoring disease progression and response to treatment.

Epithelial Cell Adhesion Molecule (EpCAM) is an epithelial cell adhesion molecule that has been widely used in the diagnosis of pancreatic cancer. Its high expression in EVs from pancreatic cancer patients makes it a promising biomarker for early diagnosis. However, compared to other biomarkers, EpCAM has relatively lower specificity, often necessitating combination with other markers to enhance diagnostic accuracy. For instance, when used in conjunction with GPC1 or CD44v6, EpCAM significantly improves the ability to distinguish pancreatic cancer patients from healthy individuals [7, 55–57].

Ephrin Type-A Receptor 2 (EphA2) is a protein that plays a crucial role in the metastasis of pancreatic cancer. Studies have shown that EVs with high EphA2 expression are associated with tumor invasiveness and metastatic potential, making it useful not only for diagnosing pancreatic cancer but also for evaluating prognosis [55–58]. Li et al. used photosensitive beads to detect EphA2 protein in plasma EVs of pancreatic cancer patients and found that its expression was significantly higher than in healthy controls (P = 0.0051). This method demonstrated high diagnostic accuracy for pancreatic cancer (AUC = 0.865), and EphA2 expression was positively correlated with tumor progression. When EphA2 was combined with CA19-9 for diagnosis, the AUC improved to 0.958, indicating enhanced diagnostic performance [59].

In addition to the previously mentioned proteins, recent studies have identified other proteins within EVs that play an important role in pancreatic cancer diagnosis. Notably, Annexin A1 (ANXA1) and Claudin-4 (CLDN4) have shown potential diagnostic value. ANXA1

is highly expressed in EVs from pancreatic cancer cells and is closely associated with tumor invasiveness and metastasis, aiding in the identification of the malignancy level of pancreatic cancer [60]. CLDN4, a tight junction protein, is significantly upregulated in EVs from pancreatic cancer, and detecting CLDN4-positive EVs helps distinguish pancreatic cancer from other benign pancreatic conditions [61]. Additionally, Heat Shock Protein 70 (Hsp70), widely studied in pancreatic cancer, is highly expressed in EVs from pancreatic cancer cells. Its expression is strongly linked to the stress response and survival capacity of tumor cells [62–64]. The combined detection of these EV-associated proteins shows potential for enhancing diagnostic accuracy in pancreatic cancer, particularly in early-stage identification and assessment of tumor aggressiveness.

#### *DNA*

In recent years, DNA mutations in EVs have also emerged as important biomarkers for pancreatic cancer. Mutations in genes such as KRAS, TP53, and SMAD4 can be detected in EVs, offering a non-invasive approach for the potential early identification of pancreatic cancer. These mutations not only assist in the early diagnosis but also provide valuable information for assessing disease progression and prognosis [65, 66].

KRAS and TP53 are the two most frequently mutated genes in pancreatic cancer patients [67–69]. A 2017 study found that among 48 serum EV samples from pancreatic cancer patients, the KRAS mutation rate was 39.6%, while the TP53 mutation rate was 4.2%. In contrast, only 2.6% of KRAS mutations and no TP53 mutations were detected in the serum EVs of 114 healthy controls. The concurrent detection of both KRAS and TP53 mutations in EV DNA may therefore improve the specificity of pancreatic cancer diagnosis, as this combination is typically absent in non-malignant individuals [69]. In a cohort study by Allenson et al., the detection rates of KRAS mutations in plasma exosomes were 7.4% in the control group, 66.7% in patients with localized disease, 80% in locally advanced cases, and 85% in metastatic pancreatic cancer patients. In comparison, the detection rates of circulating free KRAS mutations in plasma were significantly lower, at 14.8%, 45.5%, 30.8%, and 57.9%, respectively [70]. Recent studies suggest that TP53 mutations may be associated with patient survival. When assessed alongside KRAS and SMAD4 alterations, TP53 mutation status may provide additional prognostic insights [66, 69].

SMAD4 mutations are present in approximately 30% of pancreatic cancer patients, and these mutations or deletions are associated with tumor aggressiveness and metastatic potential. While not essential for monitoring advanced disease, detecting SMAD4 mutations may provide supplementary insights into tumor progression. Studies have indicated that when SMAD4 is assessed alongside other gene mutations, it may contribute to a more refined understanding of tumor behavior [65, 66]. Combining the detection of multiple gene mutations, such as KRAS, TP53, and SMAD4, in EV DNA significantly improves the sensitivity and specificity of pancreatic cancer diagnosis. This multi-gene panel approach shows promise not only in aiding the early detection of pancreatic cancer but also in predicting treatment response and patient prognosis [65, 66, 71, 72]. The combined analysis of these mutations allows for a more comprehensive evaluation of disease status and offers a promising strategy for personalized cancer management.

#### *Lipids*

EVs are typically rich in lipids such as cholesterol and sphingolipids, with a highly complex and variable composition, making detection challenging [73]. Currently, there is limited research on using the lipid components of EVs for pancreatic cancer diagnosis, and the diagnostic efficacy of lipid-based markers remains unclear. However, a 2019 study utilized liquid chromatography-mass spectrometry (LC–MS) to perform a comprehensive analysis of lipid content in serum EVs from pancreatic cancer patients. This study identified 37 lipid species that exhibited significant differences between pancreatic cancer patients and healthy controls [74].

## Challenges in using EV biomarkers for pancreatic cancer diagnosis

Despite the significant potential of EV biomarkers in pancreatic cancer diagnosis, several challenges remain:

*Sample collection and processing*: Different types of biological fluids (e.g., serum, plasma, urine) and their collection and processing methods can affect the content and stability of biomarkers in EVs. For instance, hemolysis in blood samples can interfere with the detection of EV biomarkers. Standardized and efficient protocols for sample handling are essential to ensure the accuracy of diagnostic results [45].

*Biomarker stability and detection sensitivity:* Although EV biomarkers are relatively stable, their concentrations may be low, particularly in the early stages of pancreatic cancer. Detecting these low-concentration biomarkers requires highly sensitive techniques. Conventional methods like qPCR or digital PCR may not be sensitive enough to detect low-abundance miRNAs or proteins [44].

*Specificity and diagnostic accuracy*: Some EV biomarkers, such as KRAS mutations or GPC1, are elevated in pancreatic cancer but may also be present in other tumors or inflammatory diseases. This lack of specificity

could lead to false-positive results when using a single biomarker [45]. Therefore, improving the specificity of these biomarkers or using a combination of markers is crucial for accurate diagnosis.

*Limited clinical validation:* Although EV biomarkers have shown promising diagnostic performance in some studies, most of these studies have involved small sample sizes and lack large-scale, multi-center clinical trials for validation. This limits the clinical application of EV biomarkers, and further research is needed to assess their suitability across diverse patient populations.

Overcoming these challenges is essential to fully harness the potential of EV biomarkers in the clinical diagnosis and management of pancreatic cancer.

## Future directions in EV-based diagnosis of pancreatic cancer

### Multi-omics integration analysis

By integrating multiple omics data from EVs, such as genomics, proteomics, metabolomics, and lipidomics, a more comprehensive understanding of the molecular characteristics of pancreatic cancer can be achieved. This multi-dimensional analysis not only provides deeper insights into the role of EVs in pancreatic cancer but also helps identify new biomarkers, thereby improving the sensitivity and specificity of early diagnosis. The combination of various omics layers allows for a more accurate molecular profiling of pancreatic cancer, leading to better diagnostic precision [75, 76].

### Artificial intelligence (AI)

With advancements in AI technology, machine learning and deep learning algorithms offer significant potential in pancreatic cancer diagnosis. By analyzing multi-omics data from EVs, AI can identify specific biomarker combinations unique to pancreatic cancer patients and construct more accurate diagnostic models. This approach can enhance diagnostic efficiency, enabling earlier detection and improving outcomes through the precise interpretation of complex data sets [77, 78]. AI-driven analysis can also adapt and refine diagnostic algorithms, making them more reliable and scalable for clinical application.

### Combined diagnostic strategies

Combining multiple EV biomarkers (such as RNA, proteins, and DNA) with traditional imaging techniques (e.g., CT and MRI) can enhance the accuracy and early detection of pancreatic cancer. This integrated approach can improve diagnostic models, offering more precise cancer staging and better support for treatment decisions [79]. The use of multi-biomarker detection alongside imaging provides a more comprehensive assessment, potentially leading to earlier interventions and improved patient outcomes.

### Clinical translation

To implement EVs in the clinical diagnosis of pancreatic cancer, larger-scale, multi-center clinical studies are needed to validate their performance. Establishing standardized EV detection protocols will help ensure consistency in results and improve the clinical feasibility of EV-based diagnostics. As research progresses, these standardized processes will be essential for incorporating EVs into routine diagnostic workflows, ultimately facilitating their translation into clinical practice [80].

## Challenges and future perspectives

Although extracellular vesicles (EVs) have shown considerable promise in the diagnosis of pancreatic cancer, several critical challenges continue to impede their clinical translation. The large-scale and high-purity isolation of EVs remains a critical bottleneck limiting both research and clinical applications, as current EV-based biomarker approaches are often associated with high costs and technical complexity. Additionally, the inherent heterogeneity of EVs—including differences in cellular origin, size, surface protein composition, and molecular cargo—complicates the identification and validation of consistent, disease-specific biomarkers [81, 82].

Among future research directions, advanced strategies such as single exosome profiling and exosome barcoding warrant particular attention. Single exosome profiling enables in-depth characterization of the phenotypic and molecular features of individual vesicles, which may facilitate the identification of more specific and clinically relevant diagnostic biomarkers. In contrast, exosome barcoding allows for the tracking and discrimination of distinct EV subpopulations, thereby enhancing analytical resolution and enabling multidimensional information capture—features particularly advantageous for early-stage diagnosis and longitudinal disease monitoring [83].

## Conclusion

EVs hold significant potential in the diagnosis of pancreatic cancer, as they carry a variety of biomarkers, including RNA, proteins, and DNA, providing detailed molecular information about the tumor. Through the integration of multi-omics analysis and the application of artificial intelligence, EV-based approaches offer promising potential for improving the early detection and personalized treatment of pancreatic cancer. Although challenges such as isolation, purification, and biomarker specificity remain, EVs present a promising future for non-invasive diagnostics. Future research will continue

to drive their clinical application, improving early detection and treatment strategies.

**Author's contribution**
HYY, XLC and YZ wrote the main manuscript text. HY Y prepared Fig. 1. YZ and XYD reviewed the article carefully and made suggestions for changes.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval**
This study is not applicable.

**Competing interests**
The authors declare no competing interests.

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