Chapter

From Fluids to Forecasts: The Promise of Small Extracellular Vesicle miRNAs in Revolutionising Cancer Diagnostics

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Abstract

Small extracellular vesicle (sEV) RNAs, particularly microRNAs (miRNAs), have emerged as pivotal biomarkers for cancer diagnosis and prognosis. Encapsulated within sEVs, these miRNAs reflect specific cellular characteristics and disease states, offering a window into cancers' molecular underpinnings. Notably, miRNAs, such as miR-7977, miR-98-3p, miR-620, and miR-17-5p in lung cancer and miR-373, miR-1246, miR-223-3p, and miR-21 in breast cancer, have been identified in sEVs extracted from various bodily fluids, including blood, urine, and saliva. Their remarkable stability and ease of isolation make them prime targets for non-invasive cancer detection strategies. The fold change of these miRNAs is intricately linked with cancer progression, metastasis, and therapeutic responses, underscoring their potential as diagnostic and prognostic markers. Traditional detection methods like quantitative reverse transcription-polymerase chain reaction (qRT-PCR) have been foundational; however, recent biosensing technologies, such as nanopore sequencing and microfluidic chips, offer enhanced sensitivity and specificity for detecting miRNAs in clinical samples. These innovative approaches refine the detection process and pave the way for real-time monitoring of disease progression and treatment efficacy. Overall, the collective evidence positions sEV miRNAs as robust indicators for cancer, signalling a shift towards personalised cancer care that emphasises early detection and tailored treatment strategies.

Keywords: extracellular vesicles, miRNA, mRNA, personalised medicine, RNA biomarkers

1. Introduction

Cancer remains a significant health challenge, necessitating early and adequate diagnostic measures. The advent of non-invasive cancer detection techniques heralds a new era of clinical diagnostics, enabling frequent monitoring of treatment effectiveness and facilitating adjustments to treatment plans for personalised care [1, 2]. Such advancements significantly enhance patient outcomes, allowing for early screening and diagnosis before the onset of symptoms and improving survival rates

by treating cancers at stages when they are more responsive to therapy [3]. Despite progress in biomarker research aimed at alleviating the cancer burden, current diagnostic methods still grapple with issues of specificity, sensitivity, prolonged processing times, and invasiveness. The exploration of liquid biopsies marks a promising direction, yet the quest for cell-specific signatures with high reliability presents a formidable challenge [4]. Consequently, researchers increasingly focus on innovative approaches, especially developing biomarkers derived from minimally or non-invasive bodily fluids such as blood, urine, and saliva [4–6].

sEVs, such as exosomes, have emerged as critical players in this context. Originating from cellular endosomal compartments, these nanoscale membrane-bound vesicles (30–200 nm) reflect distinctive molecular signatures based on their cell of origin, carrying specific cargoes of RNAs, proteins, and lipids into the extracellular space [7, 8]. Contrary to earlier perceptions of sEVs as mere carriers of metabolic waste, ongoing research underscores their significant role in both physiological processes and pathological conditions [9]. The ability to non-invasively detect these vesicles in bodily fluids offers a window into cellular and disease processes, making sEVs a valuable tool in diagnosing, prognosis, and monitoring various cancers [8, 10].

The focus on cellular RNAs, particularly miRNAs, circRNAs, and lncRNAs, has intensified, given their roles in cancer pathophysiology [11–13]. The intersection of sEV cargo with miRNAs has garnered significant attention for its potential in cancer biomarker research. The transfer of sEV miRNAs can profoundly influence tumour progression, highlighting their importance in the disease molecular landscape [14, 15]. However, the field faces specific challenges, including technical difficulties in sEV miRNA detection and the need for standardised methodologies to ensure reliability and reproducibility across studies. Addressing these challenges is crucial for advancing the clinical application of sEV miRNAs as biomarkers. Recent studies exemplify the potential of sEV miRNAs in cancer diagnosis. For instance, research has demonstrated the efficacy of miR-21 encapsulated in EVs as a prognostic marker for non-small cell lung cancer, offering insights into tumour aggressiveness and patient survival outcomes [16]. Another study highlighted the role of sEVs miR-155 in breast cancer, correlating its levels with disease progression and response to treatment [17].

This chapter discusses the complex process of sEVs formation, selective packaging of miRNAs and their significance in cancer diagnosis. It provides a comprehensive overview of the biogenesis of sEVs from the plasma membrane to their release into the extracellular space, as well as the critical role played by various proteins in this process. The discussion also covers standard sEV isolation methods, such as ultracentrifugation, precipitation, and microfluidics, as well as recent advances in miRNA detection techniques including digital droplet PCR (ddPCR) and nanoflares. Further, this chapter also examines the advantages and drawbacks of these techniques and explores the analysis of sEV miRNA expression profiles in different types of cancer. This highlights the potential of sEV miRNAs as non-invasive biomarkers for early detection, diagnosis, and prognosis of cancer. Consequently, it provides valuable insights into tumour development, progression, and response to therapy.

2. sEVs biogenesis and miRNA packaging

sEVs are formed through a complex biogenesis process that unfolds in distinct stages. This process begins with the formation of endocytic vesicles from the plasma membrane, which then undergoes inward budding to form multivesicular bodies (MVBs) that house

intraluminal vesicles (ILVs). Following their formation, MVBs can merge with lysosomes for degradation or fuse with the plasma membrane, releasing sEVs into the extracellular space (**Figure 1**). During these stages, various cytoplasmic biomolecules, including nucleic acids and proteins, are selectively incorporated into the lumen of EVs [18, 19].

Central to the biogenesis of sEVs is the action of the endosomal sorting complexes required for transport (ESCRTs), which are organised into four main complexes: ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III. The process starts with ESCRT-0, which is responsible for gathering cargo molecules. ESCRT-I and ESCRT-II then contribute to the budding of the membrane, encapsulating the selected cargoes within. Following this, ESCRT-III is crucial in cutting the membrane to release ILVs packed with cargo into the MVB [18, 20, 21]. Accessory proteins, notably ALIX and TSG101, play essential roles in the efficient packaging of cargo and the overall biogenesis of EVs. ALIX aids in encapsulating cargo and forming vesicles by attracting ESCRT-III to the site, which is necessary for ILV formation. On the other hand, TSG101 is vital for forming MVBs triggered by EGF, a step critical for generating sEVs. These mechanisms ensure the selective packaging of molecular contents into sEVs, highlighting the sophisticated nature of exosome biogenesis and release [20, 22].

The process of sorting microRNAs (miRNAs) into sEVs involves a sophisticated interplay of RNA-binding and membrane proteins (**Figure 1**), as detailed in the 2020 review by Michael et al. [23]. Key RNA-binding proteins such as Heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1), Argonaute 2, Y-Box Binding Protein 1, Major Vault Protein (MVP), and La Protein play crucial roles in this process. For instance, hnRNPA2B1 binds to miRNAs, aiding their incorporation into sEVs. Argonaute 2 transports miRNAs into sEVs through the KRAS-MEK-ERK signalling pathway. Similarly, Y-Box Binding Protein 1 facilitates miRNA loading into sEVs, while MVP and La Protein are responsible for the direct transfer of miR-193a into sEVs.

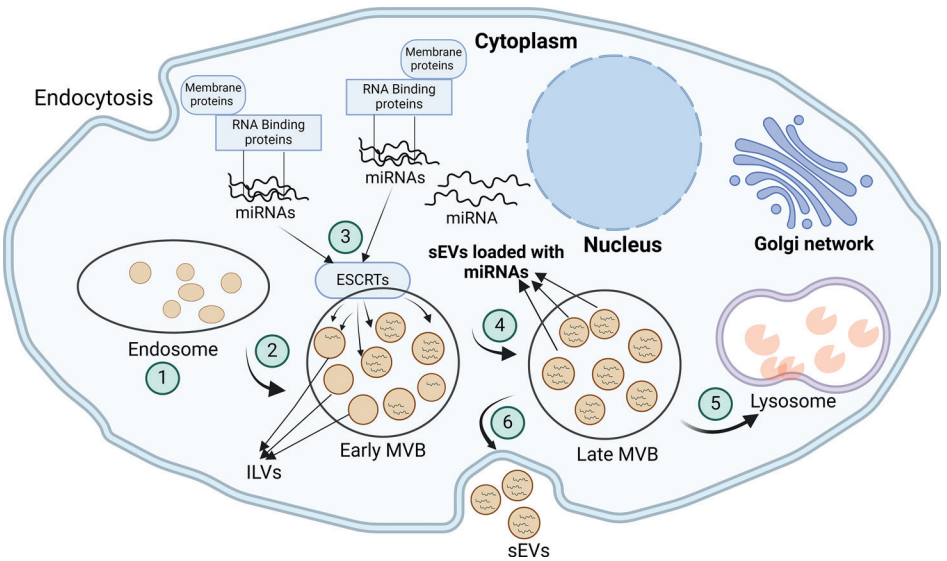

Figure 1.
Mechanism of sEV biogenesis and miRNA sorting. sEV biogenesis starts with formation of endosomes from the plasma membrane and is followed by development of MVB. Then, target miRNA molecules approach the MVB membrane for packing into sEVs. The sorting of miRNA into sEVs could be mediated by ESCRTs, RNA-binding proteins, and some of the membrane proteins. Afterwards, sEVs will be released into extracellular space by merging with lysosomes or fusing with plasma membrane.

Membrane proteins also contribute significantly to miRNA sorting. Caveolin-1 (Cav-1), for example, is essential in directing RNA-binding proteins to sEVs, especially under oxidative stress conditions. In such scenarios, Cav-1 influences the post-translational modifications of hnRNPA2B1, ensuring the selective incorporation of miRNAs into budding epithelial MVs [24]. Furthermore, the overexpression of Neural Sphingomyelinase 2 (nSMase2) has been shown to increase the expression of miRNAs in sEVs without altering the miRNA levels within cells [25]. Additionally, the overexpression of Vacuolar protein sorting-associated protein 4 (Vps4A) boosts the levels of sEV miR-27b-3p and miR-92a-3p, whereas inhibiting Vps4A decreases the levels of sEV-derived miR-92a and miR-150, as demonstrated in studies by Jin et al. and Charles et al., respectively [26, 27]. The complex process of directing miRNAs into sEVs showcases the synchronised actions of numerous proteins. Each of these proteins plays a specific role in accurately and methodically incorporating miRNAs into sEVs. This coordinated mechanism ensures that miRNAs can effectively participate in cellular communication and influence disease-related pathways [19].

In the context of cancer microenvironments, miRNA packaging into sEVs is influenced by additional, yet not fully understood, mechanisms, especially related to specific cancer conditions. Research by Diana et al. demonstrated that miR-10b is predominantly found in sEVs from wild-type cells, whereas miR-100 is more common in sEVs from cells with KRAS mutations [28]. Moreover, inhibiting NSMase resulted in an accumulation of miR-100 exclusively in the mutant cells, suggesting that the export of miRNAs can depend on the KRAS status. Another study by Sonia et al. showed that cancer-derived sEVs tend to accumulate the Dicer enzyme, facilitated by CD43, which is crucial for processing precursor miRNAs into their mature forms [29]. These discoveries highlight the intricate nature of miRNA sorting and processing within sEVs in cancer, pointing to novel diagnostic and therapeutic targets. Despite these insights, the exact reasons why miRNAs are sorted into sEVs in cancer remain debatable. The potential roles of this sorting range from simple elimination of cellular waste to participation in more complex biological processes [19, 30]. This uncertainty underscores the need for further research in sEV studies to fully understand the implications of miRNA sorting and its impact on tumour biology.

3. Isolation and characterisation of RNA-containing sEVs

Isolating and analysing RNA-containing sEVs are critical steps in unravelling their roles in biological processes and assessing their potential in cancer diagnostics. Researchers use various techniques to extract sEVs enriched with RNA from bodily fluids such as blood, saliva, and urine. Standard methods include ultracentrifugation, size exclusion chromatography, precipitation, microfluidics, immunoprecipitation targeting EV surface markers, and commercial isolation kits. As detailed in our previous review [31], each method varied with respect to the yield and purity of sEVs produced. In addition, every technique has advantages and limitations.

Ultracentrifugation is a technique that can handle large sample volumes but requires costly equipment. Precipitation methods are more cost-effective and can be used with various biological fluids and sample sizes; however, the risk of co-precipitating contaminants is a concern. Size exclusion chromatography (SEC) can be optimised for different sample volumes, but it requires more standardisation and can produce varying results depending on the material and sample being used. Immunoaffinity methods can capture sEVs with high purity and potential for

Cancer type	Molecules	Exosome origin	sEV isolation method	Expression status (increased/ decreased)	Application	miRNA detection method	Ref
Lung	miR-7977	Serum	Commercial kit	Increased	Diagnosis	qRT-PCR	[32]
Lung	miR-98-3p	Serum	Commercial kit	Decreased	Diagnosis	qRT-PCR	[32]
Lung	miR-21-5p, -126-3p, and -140-5p	Serum	Ultracentrifugation	Increased	Early diagnosis	qRT-PCR	[33]
Lung	miR-146a-5p and miR-486-5p	Serum	Commercial kit	Increased	Early diagnosis	qRT-PCR	[34]
Lung	miR-620	Serum	Ultracentrifugation	Decreased	Diagnosis and prognosis	qRT-PCR	[35]
Lung	miR-20b-5p and miR-3187-5p	Serum	Ultracentrifugation	Decreased	Diagnosis	qRT-PCR	[36]
Lung	miR-17-5p	Serum	Commercial kit	Increased	Diagnosis	qRT-PCR	[37]
Lung	miR-21/Let-7a ratio	Serum	Commercial kit	Increased	Diagnosis	qRT-PCR	[38]
Lung	miR-451a	Plasma	Ultracentrifugation	Increased	Recurrence and prognosis	Microarray	[39]
Lung	miR-126	Serum	Ultracentrifugation	Decreased	Diagnosis and personalised therapeutic modality	qRT-PCR	[40]
Breast	miR-373	Serum	Commercial kit	Increased	Diagnosis	qRT-PCR	[41]
Breast	miR-1246	Plasma	Differential centrifugation	Increased	Diagnosis	Nucleic acid functionalised Au nanoflare probe	[42]
Breast	miR-223-3p	Plasma	Ultracentrifugation	Increased	Early diagnosis	qRT-PCR	[43]
Breast	miRNA-21-5p and miRNA-10b-5p	Serum	Commercial kit	Increased	Diagnosis and disease monitoring	qRT-PCR	[44]
Breast	miR-21	Plasma	Ultracentrifugation and commercial kit	Increased	Diagnosis	Dual-cycling nanoprobe	[45]

Cancer type	Molecules	Exosome origin	sEV isolation method	Expression status (increased/ decreased)	Application	miRNA detection method	Ref
Brain	miR-21	CSF	Ultracentrifugation	Increased	Diagnosis and tumour recurrence	qRT-PCR	[46]
Brain	miR-301a	Serum	Commercial kit	Increased	Diagnosis and prognosis	qRT-PCR	[47]
Brain	miR-210	Serum	Ultracentrifugation	Increased	Diagnosis and prognosis	qRT-PCR	[48]
Brain	miR-2276-5p	Plasma	Ultracentrifugation	Decreased	Diagnosis and prognosis	qRT-PCR	[49]
Colon	miR-139-3p	Plasma	Commercial kit	Decreased	Early diagnosis and metastasis	qRT-PCR	[50]
Colon	miR-377-3p and miR-381-3p	Serum	Ultracentrifugation	Decreased	Early diagnosis	qRT-PCR	[51]
Colon	miR-196b-5p	Serum	Commercial kit	Increased	Diagnosis	qRT-PCR	[52]
Prostate	miR-141	Serum	Commercial kit	Increased	Diagnosis	qRT-PCR	[53]
Prostate	MiR-30b-3p and miR-126-3p	Urine	Ultracentrifugation	Increased	Diagnosis	Microarray	[54]
Prostate	miR-20b-5p	Prostatic fluid	Commercial kit	Increased	Early diagnosis	qRT-PCR	[55]
Prostate	miR-181a-5p	Serum	Commercial kit	Increased	Diagnosis	Deep sequencing and Chip array	[56]
Ovarian	miR-200a-3p, miR-766-3p, miR-26a-5p, miR-142-3p, let-7d-5p, and miR-328-3p	Serum	Ultracentrifugation	Increased	Early diagnosis	qRT-PCR	[57]
Ovarian	miR-145 and miR-200c	Serum	Commercial kit	Increased	Preoperative diagnosis	qRT-PCR	[58]
Gastric	miR-21	Plasma	Ultracentrifugation	Increased	Recurrence	Microarray	[59]
Gastric	miR-92a	Plasma	Ultracentrifugation	Decreased	Recurrence and prognosis	Microarray	[59]

Cancer type	Molecules	Exosome origin	sEV isolation method	Expression status (increased/ decreased)	Application	miRNA detection method	Ref
HCC	miR-4661-5p	Serum	Commercial kit	Increased	Early diagnosis	qRT-PCR	[60]
HCC	miR-21	Serum	Commercial kit	Increased	Diagnosis	qRT-PCR	[61]
Thyroid	miR-29a	Serum	Commercial kit	Decreased	Diagnosis and prognosis	qRT-PCR	[62]
Bladder	miR-96-5p and miR-183-5p	Urine	Commercial kit	Increased	Diagnosis	qRT-PCR	[63]
Oral	miR-130a	Plasma	Commercial kit	Increased	Diagnosis and prognosis	qRT-PCR	[64]

Table 1.
sEV miRNA expression patterns across different cancer types.

subtyping, but they are only suitable for small sample volumes. Microfluidics-based isolation can yield high-purity sEVs, but it has limited sample capacity and the conditions may affect the stability of the vesicles. Commercial kits can be used for simplicity and to preserve the integrity of sEVs, but they are expensive and less suitable for diluted samples like urine. Therefore, choosing the appropriate method for efficient sEV isolation is essential based on the sample volume and type [31]. Previous studies have mainly used commercial kits and ultracentrifugation to isolate sEVs from serum, plasma, and urine (**Table 1**).

Following isolation, the characterisation of these RNA-containing sEVs employs a range of techniques. Nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM) are utilised to assess the EVs size, concentration, and structural details. Researchers estimate ratios such as proteins to particles, lipids to particles, or lipids to proteins for broader quantification. Techniques like Western blotting, ELISA, and RT-qPCR detect specific proteins and genes associated with EVs, serving as markers. Mass spectrometry further complements these methods by profiling the protein content of the EVs, offering more profound insights into their molecular composition [31]. Together, these characterisation techniques verify the quality and quantify the isolated RNA-containing sEVs, providing a thorough understanding of their potential for diagnostic applications.

4. Detection of sEV miRNA

Quantitative real-time PCR (qRT-PCR) is widely used to identify and quantify sEV miRNA expression (**Table 1**). Still, ddPCR enhances this quantification with superior sensitivity, reproducibility, and accuracy [65]. Additionally, innovative methods like electrochemical sensing and surface-enhanced Raman scattering (SERS) are showing promising results in terms of consistency and selectivity. For instance, Lipei et al. crafted a biosensor that combines electrochemical detection, ratiometric readout, and DNA structural transformation to sensitively detect EV miR-21, achieving a detection limit as low as 2.3 femtomolar (fM) [66]. This method stands out for its enhanced stability and reliability. Similarly, Xinyu et al. unveiled an ultra-sensitive electrochemical biosensor employing cascade catalytic hairpin assembly (CHA) and multilayered enzymes for detecting trace amounts of miRNA-21 in actual samples [67]. Yue et al. designed a microfluidic SERS sensor capable of detecting EV miRNA as low as 1 pmol/L, employing rolling circle amplification (RCA) and tyramine signal amplification (TSA) to boost sensitivity significantly [68]. In the realm of colorimetric detection, Yaokun et al. developed a copper-mediated strategy that uses DNAzyme signal amplification and visible light-triggered reactions for pinpointing miR-21 presence [69].

On the device front, Takao et al. presented a nanowire-anchored microfluidic device that efficiently isolates urine sEV-encapsulated miRNAs [70]. This device promises to enhance cancer diagnostics by facilitating rapid miRNA extraction from minimal urine volumes and is applicable to a wide range of cancers beyond just urological ones. Building on this, Xue et al. combined nanoflare technology with CHA amplification for in situ, extraction-free, and highly sensitive sEV miRNA analysis. Their clinical tests show potential for accurately distinguishing different cancer types with 99% accuracy from plasma samples [71]. Furthermore, Xuting et al. introduced a novel method for detecting sEV miR-1246 with high sensitivity by creating hybrids between sEVs and cationic liposomes, allowing for precise quantification of sEV

miR-1246 [72]. Together, these advancements highlight significant progress in the field of sEV miRNA detection, offering improved sensitivity and potential for clinical application in disease diagnostics.

5. sEV miRNA as diagnostic tools in cancer

miRNAs are crucial for gene regulation in eukaryotic organisms, affecting a wide range of developmental and disease processes. These miRNAs can be found in body fluids like saliva, urine, and blood, maintaining their stability within EVs. The miRNAs within sEVs, which reflect those of the tumour cells they originate from, are emerging as valuable biomarkers for cancer due to their durability and unique expression profiles [18, 19]. Initial research, such as the study conducted by Guilherme in 2009, found that miRNA profiles from sEVs in peripheral circulation and those from tumour-derived sEVs were similar, but the average concentration of miRNAs was significantly different in control groups [73]. This was followed by studies demonstrating the feasibility of detecting sensitive prostate cancer markers in sEV RNA from small urine samples [74]. In 2010, Keiichi and colleagues spotlighted the significance of the let-7 miRNA family in identifying metastatic gastric cancer through sEVs [75]. These early findings laid the groundwork for using sEV RNA as a snapshot of the tumour's genetic landscape, facilitating miRNA analysis without the need for tissue samples. This approach is promising for screening people without symptoms and tracking disease recurrence, enhancing cancer diagnostics in recent years. The streamlined workflow for identifying sEV miRNA-based biomarkers in cancer diagnosis is illustrated in **Figure 2**.

In lung adenocarcinoma (LUAD) patients, increased levels of serum sEV miRNA ExomiR-7977 and reduced miR-98-3p highlight their potential as markers for diagnosis and staging accuracy [32]. Min and colleagues discovered elevated sEV miRNAs, such as ex-miR-21-5p, -126-3p, and -140-5p, suggesting these are precise, sensitive, and reliable markers for LUAD diagnosis [33]. Additionally, early-stage non-small cell lung cancer (NSCLC) showed significantly higher levels of serum sEV miR-146a-5p and miR-486-5p compared to benign conditions and healthy individuals, according to qRT-PCR results. The combination of serum and sEV miRNAs increased diagnostic sensitivity and specificity [34]. Recent breakthroughs in technology have brought about a novel biosensor that leverages nanoflare technology and catalysed hairpin assembly (CHA) amplification. This tool allows for direct, highly sensitive analysis of miRNAs within sEVs without the need for sample extraction. In clinical cohort, this method distinguished between breast, lung, liver, cervical, and colon cancers in 64 patients with an astonishing 99% accuracy, showcasing its precision in cancer diagnosis [71].

Research has identified a significant increase in hsa-miR-21-5p levels within breast cancer (BC) sEVs, making it possible to differentiate BC patients from healthy individuals with a high degree of accuracy (sensitivity of 86.7% and specificity of 93.3%, as per ROC analysis) [76]. Pre-therapy miRNA profiling of 435 BC patients revealed variations in expression across different subtypes, with miR-155 and miR-301 linked to predicting responses to treatment. Notably, the miRNA profile varied significantly between the overall BC cohort, those with HER2-positive BC, and patients with triple-negative BC [17]. A particular study found that elevated levels of sEV miR-373 were associated with triple-negative and more aggressive forms of BC, indicating its potential for diagnostic use [41]. Continuing research efforts include developing

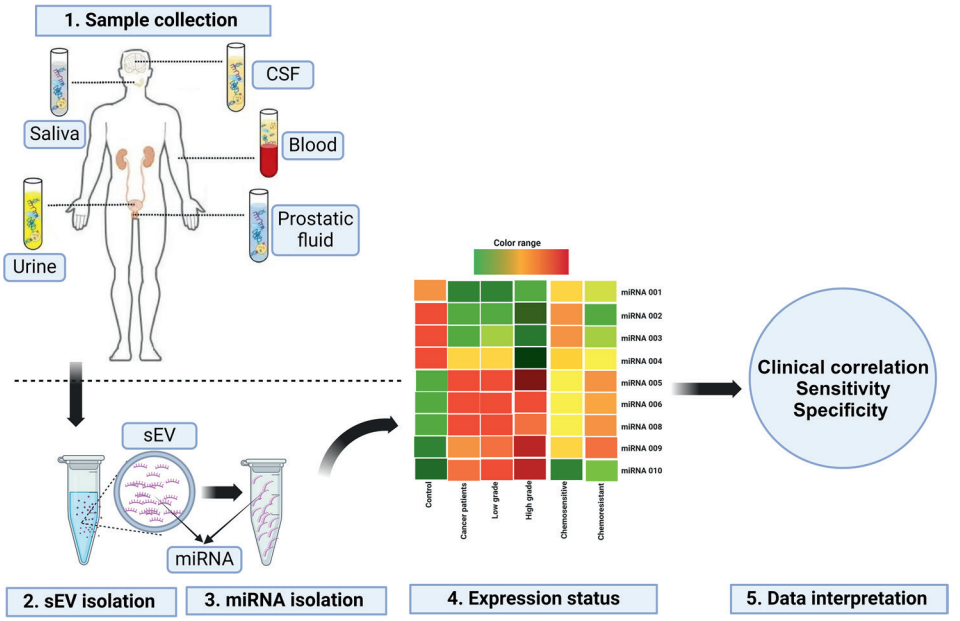

Figure 2.
Detection workflow of sEV miRNA-based biomarkers in cancer diagnosis. This figure presents a streamlined workflow for identifying miRNA biomarkers within sEVs for cancer diagnostics. The process begins with the collection of biofluid samples, including blood, urine, and saliva, which are rich sources of sEVs. Following collection, sEVs are meticulously isolated from these samples to retrieve their miRNA cargo. The extracted miRNAs are then subjected to qPCR for precise quantification and analysis of specific miRNA expression patterns associated with various cancer types. The final step involves evaluating the clinical relevance of these miRNA signatures by determining their correlation with the presence of cancer, its progression, and its response to treatment, alongside assessing the diagnostic sensitivity and specificity based on the gathered data.

methods for direct miRNA detection from exosomes in breast cancer cases, such as the introduction of an Au nanoflare probe by Leu et al. [77]. This probe, designed to target miR-1246 specifically, enters plasma sEVs and emits a fluorescent signal, offering a simple, accurate, and sensitive diagnostic approach.

In glioma, analysis of cerebrospinal fluid (CSF) from patients with recurrent disease revealed higher levels of sEV miR-21, suggesting its utility as a marker for diagnosis and prognosis [46]. This increase in EV miR-21 was found to correlate with tumour metastasis and recurrence. Within glioma tissues, miR-21 concentrations were associated with the tumour's grade and inversely correlated with patient survival rates. Experiments showing the suppression of miR-21 in glioma cells led to the upregulation of target genes, implicating its role in the progression of glioma. Additionally, increases in serum sEV miR-301a and miR-210 were observed in glioma patients, correlating with the severity and recurrence of the tumour [47, 48]. On the other hand, a decrease in plasma sEV miR-2276-5p was linked to poorer survival outcomes in glioma patients, identifying RAB13 as its target [49]. These discoveries underscore the significant potential of sEV miRNAs as biomarkers for the diagnosis and prognosis of glioma.

In colorectal cancer (CRC), the discovery of sEV miR-320d as a non-invasive marker has been pivotal in differentiating metastatic from non-metastatic cases, offering a clearer path for diagnosis and treatment strategies [78]. Additionally, miR-125a-3p and miR-122 found in plasma sEVs have been identified to not only aid in the

early diagnosis of colon cancer but also serve as independent prognostic indicators, particularly for patients with liver metastasis [79, 80]. On the other hand, a decrease in sEV miR-139-3p levels in CRC patients, especially those with metastatic and submucosal involvement, has been noted as a potential marker for prognosis [50]. A group of sEV miRNAs, including miR-100 and miR-92a, has shown potential in distinguishing between chemotherapy-resistant and -sensitive CRC patients [81]. Bioinformatics analyses have further highlighted an sEV miRNA-mRNA network that plays a critical role in CRC, underlining the diagnostic importance of these circulating biomarkers [82].

In the realm of prostate cancer (PCa), serum sEV miR-141 has shown promise as a diagnostic biomarker, particularly noting its significant increase in cases of metastatic PCa compared to healthy individuals or those with benign prostatic hyperplasia (BPH) [53]. A study by Kyosuke et al. demonstrated the superiority of urine sEV samples in diagnosing PCa, with miR-30b-3p and miR-126-3p showing higher expression levels than traditional serum prostate-specific antigen (PSA) markers [54]. Further analysis by Manuel et al., using the International Society of Urological Pathology (ISUP) grading system, identified differentially expressed miRNAs that could help in the nuanced management of PCa under active surveillance, showing strong potential with AUCs ranging from 0.79 to 0.88 [83]. Expanding on this, Zhenquan et al. discovered plasma exosome-derived miRNAs, including hsa-miR-125a-3p, hsa-miR-330-3p, hsa-miR-339-5p, and has-miR-613, as potential markers for detecting bone metastasis in PCa patients, marking significant progress in the development of non-invasive diagnostic and prognostic tools for prostate cancer [84].

Research into sEV miRNAs as tools for diagnosing and prognosticating a wide array of cancers, including bladder, ovarian, gastric, hepatocellular, thyroid, oral, and oropharyngeal cancers, underscores their vast potential across a myriad of cancer types. This wide-ranging investigation highlights the critical role of sEV miRNAs in enhancing the accuracy of cancer diagnosis and improving patient prognoses. In the case of bladder cancer, the detection of elevated levels of specific urinary sEV miRNAs, such as miR-96-5p, miR-183-5p, miR-93-5p, and miR-516a-5p, has been linked to significant clinicopathological features, offering a new method for early detection [63, 85]. A study by Akira et al. revealed that a combination of six miRNAs isolated from serum sEVs of ovarian cancer patients, when used alongside the CA-125 marker, greatly improved the sensitivity and specificity of tests for distinguishing ovarian cancer patients from healthy individuals, and for identifying early-stage ovarian cancer from benign tumours [57]. Furthermore, Naruyoshi et al. identified plasma sEV miR-21 and miR-92a as independent markers for the early detection of gastric cancer [59]. Additionally, Yoshimasa et al. found that sEV miR-23b serves as a reliable indicator for the recurrence and prognosis of gastric cancer at various stages [86].

In hepatocellular carcinoma (HCC), the enrichment of miR-21 in serum sEVs offers a more sensitive detection method, as highlighted by Hongwei et al. [61]. The presence of elevated levels of miR-122, miR-148a, and miR-1246 in serum sEVs, in combination with alpha-fetoprotein, has been shown to enhance the diagnostic accuracy for early-stage HCC significantly [87]. Chen et al. reported that lower levels of serum sEV miR-34a are associated with poorer survival rates in HCC patients [88], suggesting that its combination with other biomarkers like alpha-fetoprotein could improve diagnostic effectiveness.

The role of serum sEV miRNAs in diagnosing and prognosticating papillary thyroid carcinoma (PTC) has also been elucidated in recent studies. For instance, decreased levels of miR-29a in PTC patients have been shown to differentiate them

from healthy controls, with this downregulation correlating with more aggressive disease characteristics and poorer patient outcomes [62]. Moreover, circulating sEV miR-146b-5p and miR-222-3p have emerged as significant markers for detecting lymph node metastasis in PTC [89]. In oral squamous cell carcinoma, the diagnostic and prognostic value of both plasma and saliva sEV miRNAs, including miR-130a and miR-486-5p, has been demonstrated, with miR-486-5p showing a robust correlation with stage II of the disease [64, 90]. These discoveries collectively affirm the crucial role of sEV miRNAs as biomarkers for the diagnosis and prognosis of various cancers, offering promising avenues for non-invasive testing and tailored patient care. A comprehensive table summarising the expression patterns of different sEV miRNAs across various cancer types further illustrates their diagnostic and prognostic utility (**Table 1**).

6. EV RNA databases

In the fast-evolving field of sEV RNA research, several vital databases have become critical for researchers. These platforms enable the collection, access, and analysis of data on EV RNA, playing a crucial role in advancing our understanding of these nano-vesicles. ExoCarta is a standout resource that provides detailed information on the molecular contents of EVs, including mRNAs, miRNAs, proteins, and lipids. It compiles data from 286 studies covering a wide range of organisms, making it a rich source of information for researchers. This web-based platform also supports detailed functional analysis and interaction studies, and it actively encourages the EV community to contribute by identifying publications that might have been overlooked, thereby continuously expanding its database [91, 92]. EVpedia is another comprehensive database that offers an integrated view of sEVs, compiling data from 503 high-throughput studies, 1114 datasets, and over 722,551 molecules. It is designed to enable comparisons across different studies and is particularly useful for exploring the RNA content of EVs. Since 2010, there has been a notable increase in the volume of articles, especially those related to eukaryotic organisms, aiding in discovering biomarkers and potential therapeutic targets [93]. VESICLEpedia consolidates a wide array of information on sEVs and related particles like microvesicles and apoptotic bodies. It comprehensively examines EVs' various cargos, including lipids, metabolites, nucleic acids, and proteins. This repository is invaluable for researchers focused on the diagnostic and therapeutic applications of EV RNA in various diseases [94]. Together, these databases represent vital tools for the EV RNA research community, offering extensive data repositories that support the exploration of EV functions, the identification of disease biomarkers, and the development of novel therapeutic strategies.

In the focused area of EV RNA research, three specialised databases have been developed to meet the varied needs of the EV community. These resources are crucial in advancing our understanding of extracellular RNA (exRNA), providing data storage, access, and analysis platforms. exRNA Atlas, created by the exRNA Communication Consortium (ERCC), is a dedicated repository for exRNA data. It contains a wealth of sequencing and RT-qPCR data from a wide range of human and mouse biofluids. The database enables users to search and analyse exRNA profiles based on the type of assay and specific biofluids, aiding in the identification and study of exRNA related to different health conditions and diseases [95]. exoRBase offers a focused look at long RNAs within EV, covering messenger RNA (mRNA),

Database	Focus	Website link
ExoCarta	Exosomal mRNA, miRNAs, proteins, and lipids	http://www.exocarta.org/
EVpedia	EV mRNA, miRNAs, proteins, and lipids	https://evpedia.info/evpedia2_xe/
VESICLEpedia	EV mRNA, miRNAs, proteins, and lipids	http://microvesicles.org/
exRNA Atlas	Exosomal RNA	http://exrna-atlas.org/
exoRBase	Exosomal long RNA species: mRNAs circRNAs, and lncRNAs	http://www.exorbase.org/
miRandola	Non-coding RNA	http://mirandola.iit.cnr.it/

Table 2.
EV RNA databases and their focused molecules.

long non-coding RNA (lncRNA), and circular RNA (circRNA) derived from various human body fluids. This database integrates and visualises RNA expression profiles, highlighting changes in functional pathways and the heterogeneity of circulating EVs. With a primary focus on blood-derived samples, exoRBase is a valuable tool for in-depth studies and comparative analysis of EV RNA [96]. miRandola is a niche database that centres on miRNAs band and includes information on lncRNAs and circRNAs. It features a network visualisation of RNA-disease associations, compiled from the scientific literature, to illuminate the connections between specific RNAs and different tumours. This functionality is particularly beneficial for researching the roles of potential RNA biomarkers in cancer, offering insights into how these molecules interact with various diseases [97]. These databases (**Table 2**) collectively advance EV RNA research by providing centralised data deposition, sharing, and analysis platforms. Researchers can leverage these resources to delve into the complexities of EV RNA content and its relevance in diverse biological contexts. These are expected to evolve as the field progresses, accommodating new findings and technologies in EV RNA research, further catalysing discoveries and innovations in this rapidly expanding field.

7. Conclusions

In conclusion, studies on sEV RNAs in cancer research have indeed revealed a wealth of potential diagnostic and prognostic biomarkers. These discoveries offer profound insights into the underlying mechanisms of various cancers and pave the way for innovative therapeutic interventions. In particular, sEV miRNAs have garnered attention for their remarkable stability, specificity, and detectability in a multitude of bodily fluids, making them excellent candidates for biomarker discovery in cancer. Researchers have identified numerous sEV miRNA signatures that correlate with different types of cancer, disease stages, and prognoses, significantly enhancing our capacity to diagnose, monitor, and potentially predict disease outcomes. However, it is imperative to recognise that much of the current evidence is derived from studies with relatively small sample sizes. There is a pressing need for further validation studies involving larger cohorts to determine the clinical utility of these biomarkers conclusively. Additionally, there has been limited research into the dynamic changes of circulating sEV miRNAs before and after cancer treatment, which could offer invaluable insights into the effectiveness of therapeutic interventions.

The potential of combining sEV miRNA profiles with existing cancer markers to improve diagnostic precision and sensitivity is particularly promising. Moreover, advancements in sEVs isolation and miRNA detection techniques are crucial for the application of these particles as novel biomarkers in clinical settings. Although the field is still developing, improving in situ detection methods for miRNAs represents a significant step forward. The creation and expansion of comprehensive databases such as ExoCarta, EVpedia, and exRNA Atlas have played a pivotal role in facilitating data sharing and analysis, accelerating research progress in this rapidly evolving field.

Overall, sEVs emanating from the tumour microenvironment possess unique miRNA cargoes that reflect the cellular origins and state of disease progression. The ability to capture these molecular signatures from biofluids and quantify them offers a promising avenue towards the realisation of minimally invasive or non-invasive cancer diagnostics. As we continue to explore the complexities of sEV miRNAs and their interactions within the tumour microenvironment, the prospect of harnessing these entities for the enhancement of cancer diagnosis and the development of personalised medicine approaches becomes increasingly tangible.

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Competing interests

None.

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