Review | Received 2 September 2026; Revised 21 October 2026; Accepted 23 October 2026; Published 25 November 2026
https://doi.org/10.55092/exrna20260018

Urinary extracellular vesicle miRNAs are novel liquid biopsy biomarkers of urological malignancies

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Highlights:

- Urinary extracellular vesicle (uEV)-derived miRNAs provide a stable and non-invasive source of liquid biopsy biomarkers for urological malignancies.
- Distinct uEV-miRNA signatures show promise for early diagnosis, risk stratification, and treatment monitoring in renal, prostate, and bladder cancers.
- uEV-miRNAs are not only biomarkers but also active regulators of tumor proliferation, metastasis, immune escape, and therapy resistance.
- The developments of technologies for EV isolation, multi-omics research, and artificial intelligence are accelerating the translation of uEV-miRNA-based diagnostics from bench side to clinic.

Abstract: Urological malignancies, including renal cell carcinoma (RCC), bladder cancer (BC), and prostate cancer (PC), remain major clinical challenges because of tumor heterogeneity, insufficient early-stage detection, and limited non-invasive precision diagnostic methods. With the rapid development of precision medicine, there is an urgent need for sensitive and specific non-invasive biomarkers that can enable early detection, risk assessment, and real-time therapeutic response monitoring of urological malignancies. In recent years, microRNAs (miRNAs) encapsulated within urinary extracellular vesicles (uEVs) are emerging biomarkers and modulators in urological malignancies. uEVs are secreted by urinary tract cells and function as natural carriers and protectors of their miRNA cargos, thereby representing a class of remarkably stable and readily accessible biomarkers. Mounting evidence demonstrates that aberrant uEV-miRNA patterns are closely associated with the occurrence, progression, and prognosis of urological tumors. Moreover, altered specific uEV miRNAs are also reported to be involved in oncogenesis, including cell proliferation, metastasis, immune evasion, angiogenesis, and radiotherapeutic and chemotherapeutic resistance. In this review, we summarize recent research updates

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in the clinical significance of altered uEV miRNAs in RCC, PC, and BC. Furthermore, we also discuss the molecular mechanisms through which these miRNAs shape tumor microenvironment and behavior, as well as their potential for novel therapeutic applications. Additionally, we outline major technical and clinical challenges and discuss future directions that may facilitate the translation of uEV-miRNA biomarkers from bench to bedside.

Keywords: extracellular vesicles; microRNA; urological malignancies; liquid biopsy; biomarker; urine

1. Introduction

The global oncology landscape is undergoing a profound transition toward precision medicine, an approach that seeks to precise screening and diagnosis, personalized treatment, and real-time dynamic surveillance to the unique molecular and clinical features of each patient's disease. In practical terms, precision medicine is implemented by profiling tumor-associated molecular alterations including gene mutations, expression changes, and non-coding RNA signatures. Clinicians and molecular tumor boards increasingly rely on publicly accessible resources such as The Cancer Genome Atlas (TCGA), cBioPortal, the Catalogue of Somatic Mutations in Cancer (COSMIC), and OncoKB to contextualize tumor-derived molecular findings. Of the constantly emerging discoveries in precision medicine, extracellular vesicles (EVs) encapsulated RNA and vesicle repositories, including the exRNA Atlas, Vesiclepedia, ExoCarta, and miRBase, which provide reference frameworks for annotating RNA signatures obtained from biofluids. In the setting of liquid biopsy, these databases enable a clinician to match a patient's biofluid-derived molecular profile against validated biomarker–disease associations, thereby supporting decisions on early detection, risk evaluation, treatment decision-making, and longitudinal monitoring. Within this evolving framework, urological malignancies, including renal cell carcinoma (RCC), prostate cancer (PCa), and bladder cancer (BCa), remain major cause of cancer morbidity and mortality worldwide [1]. Despite rapid progress in medical imaging, and molecular profiling, many urological cancers occur among high-risk individuals who are not eligible for imaging screening, conventional diagnostic and prognostic tools, limiting the impact of screening and leading worse clinical management.

For instance, the prostate-specific antigen (PSA) test is widely used for PCa screening but far from ideal. Its limited specificity results in a high false-positive rate, and which leads to an estimated 75% of men with elevated PSA levels undergo unnecessary invasive biopsies [2,3]. Similarly, surveillance for BCa mainly depends on invasive, uncomfortable, and costly cystoscopy [4]. Currently, tissue biopsy, remains the golden standard for urological cancers' diagnosis, however, it offers only a static snapshot of a small portion of the tumor and cannot fully reflect the spatial and temporal heterogeneity that drives progression, metastasis, and treatment resistance. Together, these limitations underscore an urgent clinical need for reliable, non-invasive biomarkers of urological cancers that capable of enabling early detection, accurate risk stratification, and dynamic treatment monitoring.

Liquid biopsy is a non-invasive detection technology that collects body fluid samples such as blood and urine to detect biomarkers released by tumors to obtain tumor information, which has emerged as one of the most promising solutions to this unmet need. Notably, liquid biopsy can reflect tumor molecular characteristics in real time, overcoming the temporal and spatial limitations of traditional tissue biopsy Although circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) have been

widely investigated as liquid biopsy [5–7], our present review focuses on extracellular vesicles, which offer several unique advantages for urological oncology. For example, EVs are secreted by both tumor and non-tumor cells, and their surface proteins retain information about the cell of origin, as well as their lipid bilayer protects nucleic acid cargo from degradation. Among biofluids, urine is especially attractive for EV-based liquid biopsy of urological cancers. It is easy to collect, available in large volume, and in direct contact with the kidneys, ureters, bladder, and prostate, making urinary EVs an exceptional source of disease-related biomarkers shed directly from the urogenital tract [8].

EVs cargo is highly diverse and includes proteins, lipids, and nucleic acids, especially microRNAs (miRNAs) [9–11]. MiRNAs are small non-coding RNAs, typically 21–23 nucleotides in length, that regulate gene expression by binding to target mRNAs at post-transcriptionally level. Evidence revealed that miRNAs influence nearly every aspect of cellular biology, from development and differentiation to oncogenesis [12]. Importantly, when miRNAs are encapsulated within urinary EVs (uEVs), they are resistant from degradation by urinary RNases, which greatly enhances their stability and suitability for clinical use [13,14]. Accordingly, dysregulated uEV-miRNA profiles are increasingly recognized as useful molecular reflections of pathological changes within the urinary system, offering insight into tumor initiation, progression, and metastasis [15–17].

The present review focuses on the emerging role of uEV miRNAs as molecular biomarkers in the liquid biopsy of urological malignancies. We provide a comprehensive overview of their biological basis, clinical utility, and mechanistic significance, and we also discuss the opportunities and barriers that must be addressed to bring these promising biomarkers into routine clinical practice.

2. The biology and technology of urinary extracellular vesicles

To comprehensively understand the usefulness of uEV-miRNAs as clinical translational application prospects biomarkers, it is essential to clarify both the biology of EVs and the technologies used for isolating and analyzing them.

2.1. Biogenesis and cargo of extracellular vesicles

EVs are a diverse group of membrane-enclosed vesicles that can be broadly classified basing on their size and biogenesis. The two main subtypes are exosomes and microvesicles (MVs). Specifically, exosomes are typically 30–150 nm in diameter, while MVs, also known as ectosomes, which generally range from 100–1000 nm [18]. Exosomes arise through the endosomal pathway. The plasma membrane first invaginates to form an early endosome, which matures into a late endosome. The membrane of the late endosome then buds inward to generate intraluminal vesicles (ILVs), thereby producing a multivesicular body (MVB) that contains cytosolic components, including miRNAs. The MVB may either fuse with lysosomes for degradation or merge with the plasma membrane, releasing its ILVs as exosomes into the extracellular environment [9]. By contrast, MVs are formed through direct outward budding and fission of the plasma membrane, a process that also incorporates cytoplasmic material [10]. Because these subtypes overlap in size and lack universally definitive markers, the International Society for Extracellular Vesicles (ISEV) recommends the use of the umbrella term “extracellular vesicles” unless their biogenic origin can be clearly established [10] (Figure 1).

The molecular cargo of EVs is highly complex and mirrors the physiological or pathological state of the parent cell. EVs carry a rich assortment of nucleic acids, including miRNAs, long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), messenger RNAs (mRNAs), and both single- and double-stranded DNA fragments [19,20]. In addition, they are enriched in proteins involved in vesicle trafficking and fusion, such as the tetraspanins CD9, CD63, and CD81, heat shock proteins including HSP70 and HSP90, and components of the endosomal sorting complex required for transport (ESCRT), such as Alix and TSG101. EVs may also display cell-type-specific surface proteins, such as prostate-specific membrane antigen (PSMA) on EVs derived from prostate cells, which offers opportunities for targeted capture and analysis [21]. Their lipid bilayer is also enriched in cholesterol, sphingolipids, and ceramides, which contribute to structural stability and functional resilience [22] (Figure 1).

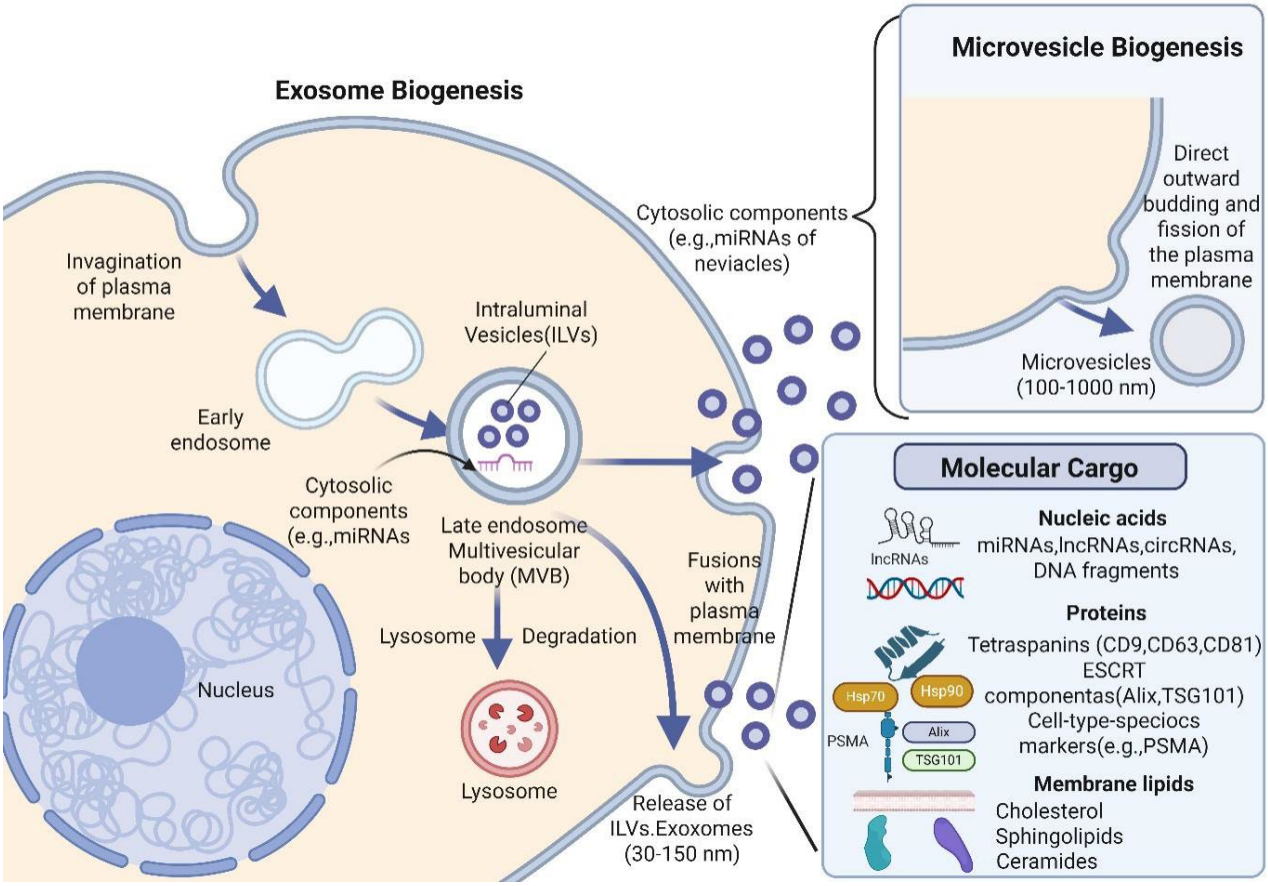

Figure 1. Biogenesis and molecular cargo of extracellular vesicles. Exosomes (30–150 nm) originate from the endosomal pathway: invagination of the plasma membrane generates an early endosome that matures into a late endosome; inward budding of the late endosomal membrane produces ILVs within a MVB, which packages cytosolic components including miRNAs. The MVB is either trafficked to lysosomes for degradation or fuses with the plasma membrane to release ILVs as exosomes. Microvesicles (100–1000 nm) are generated by direct outward budding and fission of the plasma membrane. Both vesicle classes carry a complex cargo of nucleic acids (miRNAs, lncRNAs, circRNAs, mRNAs, DNA fragments), proteins (tetraspanins CD9/CD63/CD81; HSP70/HSP90; ESCRT components Alix and TSG101; cell-type-specific markers such as PSMA), and membrane lipids (cholesterol, sphingolipids, ceramides). Because exosomes and microvesicles overlap in size and lack definitive markers, the umbrella term “extracellular vesicles” is recommended by ISEV [10,23].

2.2. Methodologies for isolation and analysis of uEV-miRNAs

The reliability of uEV-based research depends heavily on the quality of EV isolation. Urine is a biologically complex fluid with high content of Tamm-Horsfall protein, and which presents a major technical challenge because it can aggregate and co-precipitate with EVs, thereby affecting the purity of uEVs and interfering with downstream analyses. Thus, for urine specifically, the Urine Task Force of ISEV has issued a dedicated position paper that complements the MISEV guidelines, providing consensus recommendations on urine collection, pre-analytical handling, normalization strategies (e.g., to urinary creatinine or EV particle number), and reporting standards for uEV research [24]. A wide range of isolation strategies has therefore been developed, each with distinct advantages and limitations.

Ultracentrifugation (UC) is the most widely used method for EV separation and has long been regarded as the "gold standard" for EV isolation that recommended by ISEV. In differential UC, urine is subjected to a series of centrifugation steps at increasing speeds to remove cells, debris, and eventually EVs. Density gradient UC further improves purity by separating vesicles according to buoyant density, with exosomes typically banding at 1.13–1.19 g/mL [25,26]. Although UC can produce relatively pure preparations, it also exhibits several deficiencies including time-consuming, requires specialized equipment, and may damage vesicles or promote aggregation because of the high centrifugal forces involved.

By contrast, polymer-based precipitation methods use substances such as polyethylene glycol (PEG) to reduce EV solubility and drive vesicle precipitation from solution. This approach is simple, rapid, and does not require specialized instrument, making it widely used for high-throughput studies. However, its major limitation is the co-precipitation of non-vesicular proteins, particularly uromodulin, which reduces sample purity and limits its use in proteomic applications, although it remains acceptable for nucleic acid-based studies [27].

Size-exclusion chromatography (SEC) is another widely used method. In SEC, urine is passed through a column packed with porous beads. Larger particles such as EVs are excluded from the pores and elute earlier, whereas smaller soluble proteins enter the pores and are retained longer. SEC preserves EV integrity and biological function well and efficiently removes many soluble contaminants, but it can dilute the sample and offers lower throughput than precipitation-based approaches [28].

Immunoaffinity capture is often used in individual sample analysis with limited volume or when highly specific EV subpopulations are desired. This method relies on antibodies directed against EV surface markers such as CD9, CD63, or tumor-associated antigens like PSMA, which are immobilized on magnetic beads or solid supports. Although this strategy produces highly enriched EV fractions, it is costly and inherently selective, meaning that it may overlook relevant vesicle populations that do not express the chosen marker [29].

Lectin-based isolation represents a further affinity strategy with particular appeal for clinical translation. Lectins such as wheat germ agglutinin (WGA) and Concanavalin A bind glycan moieties enriched on the uEV surface, inducing vesicle agglutination that permits recovery by simple low-speed centrifugation. This approach is rapid, inexpensive, requires no specialized instrumentation, and has already been applied successfully to urinary exosomal miRNA profiling in prostate cancer [30]. In parallel, numerous commercially available isolation kits which most based on polymer precipitation, membrane affinity, or spin-column chemistry, providing standardized workflows with short processing times and small required sample volumes, features that are highly advantageous for routine clinical

laboratories [31]. However, kit performance varies considerably, and systematic comparisons have shown that the choice of kit can measurably influence downstream exosomal miRNA profiles, underscoring the need for method validation and consistent use of a single platform within any given clinical study [32].

In recent years, the rapid progress of bioengineering and microfluidics have accelerated new methods for EV isolation. For instance, microfluidic "lab-on-a-chip" platforms integrate EV capture and analysis into a single device, enabling automation, reducing sample requirements, and offering the potential for rapid point-of-care diagnostics [33]. These systems may rely on immunoaffinity, acoustic trapping, or size-based filtration such as deterministic lateral displacement [34]. Notably, to overcome the challenge of isolating EVs from dilute urine, researchers developed a microfluidic device incorporating anchored nanowires, which enables highly efficient EV capture and in situ extraction of a substantially greater number of miRNA species than conventional methods. This platform provides a powerful foundation for discovering urinary miRNA biomarkers across both urological and non-urological cancers, and it may help pave the way toward non-invasive early cancer detection [35].

2.3. Characterization of uEVs

EV morphology, size distribution, and purity is curial for EV-based biomarker and function study, which highlight the importance of EV characterization. In line with the MISEV2023 guidelines and the ISEV Urine Task Force recommendations, no perfect single technique is sufficient to fully characterize EVs, and different methods should be combinedly used to finish the pre-analysis appraisal [23,24]. The most commonly used combination including nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and Western blotting. Of this combination, NTA quantifies particle concentration and size distribution by tracking the Brownian motion of individual vesicles, providing a rapid first assessment of yield; however, NTA cannot distinguish EVs from similarly sized non-vesicular particles such as protein aggregates or lipoproteins. TEM with negative staining remains the reference method for direct morphological confirmation, revealing the characteristic membrane-delimited, cup-shaped (an artifact of drying) or round vesicular structures, while cryo-electron microscopy preserves native morphology. Western blotting is used to demonstrate enrichment of EV-positive protein markers, including the CD9, CD63, and CD81, together with Alix and TSG101—and, equally important, depletion of negative markers indicative of cellular or urinary contamination, such as calnexin (endoplasmic reticulum), albumin, and uromodulin.

Other methods, such as flow cytometry, in bead-assisted format for bulk phenotyping or as high-resolution nano-flow cytometry for single-vesicle analysis, enables multiparametric surface-marker profiling and can resolve EV subpopulations, including tumor-associated subsets (e.g., PSMA-positive prostate-derived vesicles). Used together, these techniques establish the identity and purity of uEV preparations and are a prerequisite for reproducible miRNA biomarker studies, particularly in urine, where uromodulin contamination and highly variable sample dilution demand careful quality control [23,24].

2.4. uEVs miRNA quantification

After EV isolation, miRNA expression is typically quantified by quantitative real-time PCR (qRT-PCR) for targeted validation of known miRNAs or by high-throughput methods such as miRNA

microarrays and next-generation sequencing (NGS) for discovery-driven profiling of novel biomarker signatures [31,32,36].

3. uEV-miRNAs as clinical biomarkers in urological malignancies

Accumulating evidence has revealed that urinary extracellular vesicle miRNAs exhibit disease-specific expression patterns across major urological malignancies. These signatures are associated not only with tumor presence but also with disease stage, biological aggressiveness, recurrence risk, and therapeutic response. As such, uEV-miRNAs are emerging as a highly promising class of non-invasive biomarkers for renal cell carcinoma, prostate cancer, and bladder cancer (Figure 2 and Table 1).

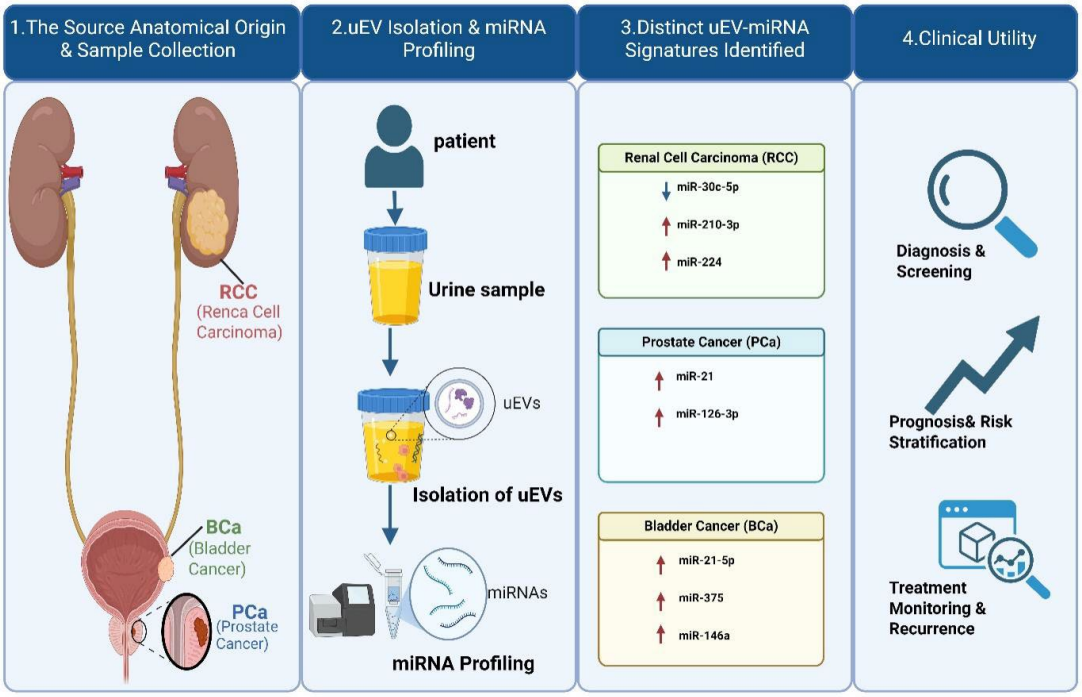

Figure 2. uEV-miRNAs as non-invasive biomarkers for urological malignancies. This schematic illustrates the clinical workflow and potential applications of uEV microRNAs in the management of urological cancers. The left panel depicts the anatomy of the urogenital tract with illustrative representations of RCC, PCa, and BCa, each secreting EVs directly into the urine. The second panel process begins with non-invasive urine collection, followed by uEV isolation and miRNA analyzing using methods such as qRT-PCR or next-generation sequencing. Distinct uEV-miRNA expression signatures are observed across different tumor types. The third panel represents representative uEVs miRNAs in urological cancers. In RCC, characteristic alterations include the downregulation of tumor-suppressive miRNAs such as miR-30c-5p and the upregulation of oncogenic miRNAs such as miR-210-3p and miR-224. In PCa, increased levels of miR-21 and miR-126-3p are frequently detected and may assist in diagnosis and risk stratification. In BCa, upregulation of miR-21-5p represents a robust diagnostic signal, while other miRNAs, including miR-375 and miR-146a, may help distinguish high-grade from low-grade disease. The final panel collectively summarizes these uEV-miRNA signatures provide clinically actionable information for: **(a)** Early diagnosis and differentiation from benign conditions; **(b)** Prognosis and risk stratification; **(c)** Real-time monitoring of therapeutic response and disease recurrence.

Table 1. Selected uEV-miRNAs as biomarkers in urological malignancies.

Cancer type	uEV-miRNA(s)	Sample/source	Clinical application	Key findings	Performance/remarks	Reference
RCC	miR-30c-5p	Urinary exosomes	Diagnosis	Significantly reduced in ccRCC compared with healthy controls	AUC 0.86; sensitivity 78.8%; specificity 86.1%	[37]
RCC	miR-126-3p-based panel	Urinary exosomes	Diagnosis; postoperative monitoring	Panel distinguished ccRCC, including small tumors, from healthy and benign cases; levels normalized after surgery	Strong non-invasive diagnostic value	[38]
RCC	miR-135b-5p, miR-196b-5p, miR-200c-3p, miR-203a-3p	Urinary-derived EVs	Early diagnosis	Four-miRNA signature showed stable upregulation in RCC cell models and tumor tissues	Proposed as an auxiliary diagnostic panel for early RCC	[39]
RCC	miR-204-5p	Urine from transgenic tRCC mouse model	Early detection	Elevated before histological tumor development	Potential marker for ultra-early diagnosis of Xp11 tRCC	[40]
RCC	miR-542-5p	Urinary exosomes in VHL syndrome	Early detection; monitoring	Higher in patients with renal tumors and decreased after tumor removal	Potential marker for VHL-associated RCC	[41]
RCC	miR-224	Extracellular miR	Prognosis	High levels correlated with poorer outcomes in ccRCC	Independent prognostic marker	[42]
RCC	miR-15a	Urinary EVs	Diagnosis / biology-linked biomarker	Upregulated and functionally linked to RCC progression via BTG2/PI3K/AKT axis	Biomarker with mechanistic relevance	[43]
PCa	miR-21	Urinary exosomes	Diagnosis	Frequently upregulated in PCa and able to distinguish PCa from BPH	One of the most consistently reported urinary biomarkers	[44,45]
PCa	miR-21, miR-451, miR-636	Urinary exosomal miRNAs	Prognosis; metastasis prediction	Combined signature predicted metastasis and prognosis when integrated with PSA	Multimarker prognostic panel	[44]
PCa/CRPC	miR-21-5p, miR-574-3p, miR-6880-5p	Urinary EVs	CRPC prognosis	Proposed as biomarkers linked to castration-resistant progression	Promising non-invasive prognostic markers	[46]

Table 1. *Cont.*

Cancer type	uEV-miRNA(s)	Sample/source	Clinical application	Key findings	Performance/remarks	Reference
PCa	miR-21-5p, miR-375, miR-205-5p	Urinary EVs	Diagnosis	Improved discrimination of PCa from BPH and healthy controls	Joint model improved accuracy in PSA gray zone	[47]
PCa	miR-574-3p, miR-141-5p, miR-21-5p	Urinary exosomes	Diagnosis	Significantly upregulated in PCa using lectin-induced aggregation method	Low-cost isolation strategy with biomarker utility	[30]
PCa	miR-148a-5p, miR-21-5p, miR-181a-5p, miR-222-3p, miR-100-5p	Urinary EVs	Diagnosis	Five-miRNA panel distinguished PCa from BPH	Sensitivity 89%; specificity 72%	[48]
PCa	miR-126-3p	uEVs	High-risk disease prediction	Predicted lymph node invasion in high-risk PCa	Clinically relevant for treatment planning	[49]
PCa	miR-30b-3p, miR-126-3p	Urinary EVs after DRE	Diagnosis	Significantly overexpressed in PCa *vs* biopsy-negative men	Better specificity than serum PSA	[50]
PCa	miR-16-5p	Urinary exosomes	Screening	Expression differed significantly across PCa stages and between cancerous and non-cancerous cases	Useful particularly for intermediate-stage PCa	[51]
PCa	miR-122-5p, miR-125-5p, miR-16-5p, miR-532-5p	Urinary exosomes	Diagnosis/ aggressiveness assessment	Distinct urinary profile in PCa; miR-532-5p associated with aggressive features	Some overlap with kidney cancer signatures	[52]
PCa	hsa-miR-532-3p, hsa-miR-6749-5p	Urinary exosomal small RNAs	Diagnosis	Differentially expressed in PCa patients	Candidate diagnostic biomarkers	[53]
PCa	Seven-mRNA + two-miRNA panel including PCA3	Affinity-captured urinary EVs	Diagnosis	Distinguished cancer from benign biopsy-negative cases	AUC 0.843; improved to 0.955 with PCA3 and clinical variables	[54]

Table 1. *Cont.*

Cancer type	uEV-miRNA(s)	Sample/source	Clinical application	Key findings	Performance/remarks	Reference
PCa	Six validated miRNAs + PSA	Urinary EVs	AI-assisted diagnosis	Deep learning-based model integrated miRNAs and PSA	AUC 0.9556; sensitivity/specificity/accuracy 93.33%	[55]
PCa	miR-145	Urinary EVs	Diagnosis	Elevated in PCa and associated with higher Gleason score	Improved discrimination when combined with PSA	[56]
PCa	miR-335:miR-501 ratio, miR-501-3p	Urinary exosomes	Biopsy prediction	Distinguished PCa from biopsy-negative men	AUC 0.75; PPV 0.85; NPV 0.51	[57]
PCa	isomiRs of miR-21, miR-375, miR-204	Urinary EVs	Diagnosis	Mature miRNAs were less informative, but 3′-modified isomiRs showed strong discrimination	Three-isomiR signature outperformed PSA	[58]
PCa	49 differentially expressed miRNA ratios	Urinary EVs before/after therapy	Treatment monitoring	miRNA ratios changed after maximal androgen blockade, radical prostatectomy, or combined therapy	Supports longitudinal monitoring	[59]
PCa	miR-1290, miR-320a-3p, miR-155-5p, miR-186-5p, miR-30e-5p	Small urinary EVs	ISUP grade stratification	Multi-miRNA combinations discriminated disease grades	Potential tool for active surveillance	[60]
PCa	miR-532-5p, miR-26a-5p, miR-99b-3p	Urinary exosomes	Biochemical recurrence prediction	Significantly upregulated in recurrent disease	miR-532-5p showed strongest prognostic association	[61]
PCa	Prostate-derived EV RNAs in urine vs plasma	Urinary and plasma EVs	Biofluid selection for monitoring	Urinary EVs, not plasma EVs, carried prostate cancer-derived RNAs that decreased after surgery	Supports urine as preferred biofluid for EV RNA biomarkers	[62]
BCa	miR-21	BCa-cell-derived exosomes	Mechanistic biomarker/ progression	Exosomal miR-21 educated macrophages toward a pro-tumor phenotype	Links biomarker status with tumor-promoting function	[63]
BCa	miR-21-5p	Urinary EVs	Diagnosis	Novel biomarker of urothelial carcinoma	Sensitivity 75.0%; specificity 95.8%	[64]

Table 1. *Cont.*

Cancer type	uEV-miRNA(s)	Sample/source	Clinical application	Key findings	Performance/remarks	Reference
BCa	miR-21	Urinary exosomes	Diagnosis; prognosis	Highly expressed in BCa and correlated with stage and grade	Useful for early and advanced disease	[65]
BCa	miR-375, miR-146a	Urinary EVs	Grade stratification	miR-375 associated with high-grade BCa; miR-146a with low-grade disease	Useful for treatment decision-making	[66]
BCa	miR-96-5p, miR-183-5p	Urinary exosomes	Diagnosis; postoperative monitoring	Combined use outperformed cytology; levels correlated with stage and grade and declined after surgery	Sensitivity 88.2%	[67]
BCa	miR-19b1-5p, miR-136-3p, miR-139-5p, APE1/Ref1, BLCA-4, CRK	Urinary biomarker panel	Diagnosis	Combined exosomal miRNAs and protein markers differentiated BCa from controls	High accuracy, especially for low-risk disease	[68]
BCa	miR-6724-5p, miR-10400-5p, miR-7704	Urine	Diagnosis	Three-miRNA panel derived from high-throughput sequencing	> 90% sensitivity; promising non-invasive assay	[69]
BCa	miR-17-5p	Urinary exosomes	Invasiveness assessment	Upregulated in muscle-invasive BCa compared with non-muscle-invasive disease	Potential marker of invasive behavior	[70]
BCa	miR-451a, miR-486-5p	Urinary EVs	Monitoring; recurrence surveillance	Upregulated in presurgical stage T1 samples compared with postsurgical follow-up	Potential biomarkers of recurrence-free survival	[71]
BCa	miR-146b-5p, miR-155-5p, miR-138-5p, miR-200a-3p	Tumor tissue and urinary EVs	Invasiveness stratification	miR-146b-5p and miR-155-5p elevated in MIBC urinary EVs; tissue panel linked to high grade	Helps distinguish MIBC from NMIBC	[72]
BCa	uEV transcriptomic signature (AI-based)	Urinary EVs	Molecular subtyping; prognosis	AI-driven pipeline integrating standardized EV isolation, transcriptomic profiling, and prognostic scoring	High-precision subtyping and prognosis from urine alone	[73]

3.1. Renal cell carcinoma

Renal cell carcinoma is the third most common urological malignancy, with clear cell renal cell carcinoma (ccRCC) accounting for approximately 80% of cases [1,37]. Although more than 60% of RCCs are now detected incidentally during imaging for unrelated conditions, symptomatic disease is often already advanced at presentation. This clinical silence makes the development of non-invasive biomarkers particularly important, both for early detection and for postoperative surveillance after nephrectomy.

A growing body of work supports the diagnostic potential of uEV-miRNAs in RCC. Song *et al*. found that the levels of miR-30c-5p were significantly declined in urinary exosome of patients with ccRCC as compared with control subjects. Receiver operating characteristic analysis demonstrated an area under the curve (AUC) of 0.86, with 78.8% sensitivity and 86.1% specificity for discriminating ccRCC from controls, revealing that urinary exosomal miR-30c-5p may have the potential as a non-invasive diagnostic biomarker for ccRCC [38]. Beyond individual urinary exosomal miRNAs, miRNA panels have also shown substantial diagnostic value. For instance, uEVs miRNAs' combinations involving miR-126-3phave been reported to distinguish ccRCC patients even those with small tumors from healthy individuals and from patients with benign renal lesions. Notably, the miRNAs' expression levels were normalizing after surgery [39]. These findings demonstrated that uEV-miRNA profiles may reflect tumor size and could be utilized for both diagnosis and postoperative monitoring.

Our group also identified four uEV miRNAs, consisting of miR-135b-5p, miR-196b-5p, miR-200c-3p, and miR-203a-3p, that were markedly and stablly upregulated in RCC patients as compared with control subjects. Systematic analysis indicated that this combined uEV-miRNA panel may have value as an auxiliary, non-invasive diagnostic indicator for early-stage renal cancer [40]. Such multi-marker approaches are particularly attractive because they may overcome the limited sensitivity and specificity associated with single biomarkers.

Importantly, dysregulated uEV-miRNAs have also been identified in less common RCC subtypes and hereditary cancer syndromes. In a transgenic mouse model of Xp11 translocation RCC, a rare pediatric kidney cancer, urinary exosomal miR-204-5p was significantly elevated even before histological tumor development, highlighting its potential as a biomarker for very early disease detection [41]. Similarly, in patients with Von Hippel-Lindau syndrome, who are predisposed to RCC, urinary exosomal miR-542-5p was elevated in those with renal tumors and returned toward normal levels after surgical tumor removal, supporting its possible utility for early detection and longitudinal monitoring [42].

In addition to diagnostic value, several uEV-miRNAs appear to carry prognostic information. Fujii *et al*. analyzed extracellular miR-224 in 108 patients with ccRCC and found that high levels were associated with poorer clinical outcomes, establishing miR-224 as an independent prognostic marker [43]. Similarly, exosomal miR-15a was found to participate in RCC progression through targeting the BTG2/PI3K/AKT axis, which directly linking its biomarker potential to an oncogenic function and prognostic indicator [44]. Moreover, Crentsil VC *et al.* investigated whether the miRNA content of exosomes from ccRCC cells reflects the miRNA levels within the cells themselves, and found that exosomal levels of miR-205 and miR-150 show consistently expression pattern with their cytoplasmic concentrations. These findings suggest that exosomal miR-205 is a promising biomarker as its expression in exosomes is significantly different between cancer and normal kidney cells, and which accurately reflects the cellular state [45].

Collectively, these results demonstrate that uEV-miRNAs may provide a diverse and biologically informative biomarker source for RCC. Future study will more likely construct multi-miRNA panels capable of integrating diagnostic, prognostic, and postoperative surveillance functions into a single non-invasive platform.

3.2. Prostate cancer

Prostate cancer is the second most common male urological cancer worldwide [46]. In clinical practice, the main challenge is not only diagnosing PCa but discriminating indolent tumors since aggressive PCa needs immediate treatment. In current, the detection of PCa mainly relies on PSA, however, PSA testing remains imperfect because of it lacks sufficient specificity and often leads to unnecessary biopsies. uEV-miRNAs may provide a promising strategy to refine PCa diagnosis, improve risk stratification, and guide individualized management.

Of the dysregulated uEV miRNAs in PCa , miR-21 is the most commonly and consistently reported uEV-miRNA. Multiple studies have shown that miR-21 is upregulated in urine samples from patients with PCa, and which also has the ability to distinguish PCa from benign prostatic hyperplasia (BPH) [47,48]. Shin *et al*. further profiled urinary exosomal miRNAs and identified a prognostic signature comprising upregulated miR-21 and miR-451 together with downregulated miR-636. When combined with preoperative PSA levels, this signature effectively predicted metastasis and clinical prognosis [47]. These findings indicate that uEV-miRNAs may add clinically meaningful information beyond PSA alone.

Several studies have extended this concept by identifying miRNA panels associated with advanced or treatment-resistant disease. Using size-exclusion chromatography combined with ultrafiltration to isolate low-contaminant urinary EVs, Lee *et al*. identified EV-associated miRNAs linked to castration-resistant prostate cancer progression. miR-21-5p, miR-574-3p, and miR-6880-5p were proposed as promising non-invasive urinary EV biomarkers for CRPC prognosis [49]. A multicenter study further showed that urinary EV-derived miRNAs, particularly elevated miR-21-5p and miR-375 together with reduced miR-205-5p, improved discrimination of PCa from BPH and healthy controls. Notably, the combination of these miRNAs, the free-to-total PSA ratio, PSA density, and neutrophil-to-lymphocyte ratio achieved high diagnostic accuracy, especially in patients within the PSA gray zone [50].

The development of EV isolation technologies has accelerated the uEV biomarkers discovery in PCa. For example, lectin-induced aggregation has been recognized as a simple and low-cost method for urinary exosome isolation using low-speed centrifugation. By analyzing the profile of exosomal miRNA that was isolated by this method, three miRNAs including miR-574-3p, miR-141-5p, and miR-21-5p showed significant upregulationin PCa, supporting their potential as non-invasive biomarkers for PCa diagnosis [30]. A narrative review further highlighted urinary EVs as a rich source of prostate-derived molecular cargo involved in PCa biology and increasingly used for non-invasive diagnostics and active surveillance. Current EV-based tests often rely on RNA and miRNA markers such as PCA3, ERG, miR-21, and miR-141, while future technological advances are expected to improve biomarker accuracy and strengthen precision uro-oncology [51].

A rapid, automatable magnetic bead-based pipeline has also been reported to improve uEV miRNA extraction efficiency. This method increased the uEV miRNA extraction efficiency by more than 10-fold compared with ultracentrifugation/TRIzol and enabled high-throughput biomarker analysis. Using this approach, a five-miRNA panel consisting of miR-148a-5p, miR-21-5p, miR-181a-5p, miR-222-3p, and

miR-100-5p was successfully constructed, which can distinguish PCa from BPH with 89% sensitivity and 72% specificity, demonstrating its translational potential for PCa non-invasive diagnosis [52].

Recent studies have uncovered the ability of uEV miRNA for identifying high-risk disease that related with PCa. For instance, in 2024, Dong *et al*. reported that uEV-derived miR-126-3p was a promising predictor of lymph node invasion in patients with high-risk PCa [53]. Similarly, uEV miRNA profiling after digital rectal examination identified miR-30b-3p and miR-126-3p were significantly elevated in PCa patients compared with those with biopsy-negative individuals. These EV miRNAs showed better specificity than serum PSA, suggesting their potential to improve non-invasive PCa detection [54].

Additional studies further confirmed the clinical value of urinary EV miRNAs. One recent study evaluated the discriminative ability of urinary exosomal miR-16 in PCa patients, BPH patient, and healthy controls. The results showed significant differences in urinary exosomal miR-16 expression between different PCa stages and between cancerous and non-cancerous individuals, confirming its diagnostic potential, particularly for intermediate-stage PCa [55]. Another study found a distinct urinary exosomal miRNA profile in PCa patients, including increased miR-122-5p and decreased miR-125-5p and miR-16-5p, while miR-532-5p correlated with aggressive disease features. Interestingly, some miRNA alterations overlapped between prostate and kidney cancers, suggesting that certain urinary miRNAs may function as broader indicators of urological malignancy rather than tumor-specific markers [56].

Using NGS, another study profiled small RNAs in urinary exosomes from 28 PCa patients and 25 BPH patients. In the training cohort, urinary exosomal miR-532-3p and miR-6749-5p showed significant differential expression in PCa patients, suggesting that these two miRNAs may serve as diagnostic biomarkers [57]. Vn96 peptide affinity-isolated urinary EVs have also been shown to provide a minimally invasive source of PCa biomarkers. A combined seven-mRNA and two-miRNA panel distinguished cancer from benign biopsy-negative cases with an AUC of 0.843, while incorporation of EV-derived PCA3 and clinical variables further improved diagnostic performance to an AUC of 0.955, with 86% sensitivity and 93% specificity [58].

Integration of artificial intelligence represents another important direction. The PruEV-AI system combines rapid uEV isolation using amine-modified zeolites/carbohydrazide with deep learning-based analysis of EV miRNAs and PSA. By combining six uEV miRNAs with PSA, the optimized AI model can achieve high diagnostic accuracy with an AUC of 0.9556, and 93.33% sensitivity and specificity [59]. This study highlights the advance interdisciplinary technology of engineering, molecular diagnostics, and computational modeling can accelerate to output clinically useful liquid biopsy systems.

Different uEV isolation approaches may also improve clinical feasibility of the encapsuled miRNA. As compare with classic ultracentrifugation method, hydrostatic filtration dialysis was shown to be a simple and economical approache for uEVs isolation and suitable for miRNA analysis. By applying this method, uEV miR-145 was found to be elevated in PCa, and was associated with higher Gleason score. Moreover, when combined with serum PSA, uEV miR-145 has the capacity for discrimination PCa from BPH with improving sensitivity and specificity [60].

Small RNA sequencing has further identified 12 miRNAs differentially expressed between urine samples from PCa patients and healthy controls. Independent validation showed that miR-501-3p expression and the miR-335:miR-501 expression ratio could distinguish PCa patients from those with negative prostate biopsy results. Incorporating the miR-335:miR-501 ratio, PSA, and total prostate volume into a logistic regression model improved diagnostic performance compared with individual

markers, achieving an AUC of 0.75, a positive predictive value of 0.85, and a negative predictive value of 0.51 [61]. This study also illustrates the usefulness of uEV miRNA expression ratio for PCa diagnosis in future. Notably, not all informative biomarkers are canonical mature miRNAs. One study showed that uEV miRNAs including miR-21, miR-375, and miR-204 did not reliably discriminate PCa patients from controls, nevertheless, their 3'-modified isomiRs exhibited much better discriminatory performance than the unmodified uEV miRNAs [62]. These findings suggest that miRNA isoforms may represent an underexplored but highly informative layer of uEV biomarker biology.

uEV-miRNAs may also assist in monitoring treatment response and guiding active surveillance. Shutko *et al*. profiled 13 PCa-associated miRNAs in urinary EVs from healthy donors and PCa patients before and after maximal androgen blockade, radical prostatectomy, or combined treatment. They identified 49 differentially expressed miRNA ratios affected by therapy, suggesting that urinary EV miRNA signatures may serve as non-invasive markers for diagnosis and treatment monitoring [63]. Another study showed that miRNAs in small urinary EVs could help stratify PCa patients by ISUP grade, identifying candidates such as miR-1290, miR-320a-3p, miR-155-5p, miR-186-5p, and miR-30e-5p. Multi-miRNA combinations achieved good discrimination between grades, supporting their potential for guiding active surveillance [64]. In intermediate-risk PCa, urinary exosomal miR-532-5p was identified as a potential predictor of biochemical recurrence. Together with miR-26a-5p and miR-99b-3p, miR-532-5p was significantly upregulated in patients who developed recurrence and showed the strongest association with poor prognosis [65].

Interestingly, RNA sequencing results revealed that the RNA expression signatures of uEVs but not plasma EVs from pre- and post-prostatectomy were similar to those from matched prostate tissues. Moreover, the contents of prostate cancer-derived RNAs that were contained in uEVs were decreased after surgery. These findings reinforce the concept that urine is a particularly suitable biofluid for detecting and monitoring prostate-derived EV RNA biomarkers [66].

Collectively, these studies show that uEV-miRNA signatures can complement and, in certain contexts, outperform traditional PSA-based approaches. By integrating molecular specificity with non-invasive accessibility, uEV miRNAs may enable a more precise and clinically nuanced framework for PCa diagnosis, risk stratification, and treatment monitoring.

3.3. Bladder cancer

Bladder cancer ranks first in the incidence rate of urinary tumors in China and accounting for about 1.9% of all malignant tumors, which is characterized by high recurrence rates. Particularly, BCa patients with non-muscle-invasive requires lifelong surveillance. However, current follow-up depends heavily on repeated cystoscopy, which is an invasive and costly procedure that imposes a substantial burden on patients and healthcare systems. Thus, simple and reliable urinary biomarker test capable of detecting disease, monitoring recurrence, and informing tumor aggressiveness would therefore have major clinical impact.

Like in other urological cancers, miR-21 again emerged as a central biomarker and crucial regulator in BCa. For example, Lin *et al*. demonstrated exosomal miR-21 that was secrete by BCa cells can "educate" macrophages within the tumor microenvironment to adopt a pro-tumorigenic phenotype, thereby promoting cancer progression [67]. Clinically, the diagnostic utility of miR-21-5p is well clarified. Matsuzaki *et al*. identified uEV miR-21-5p as a novel biomarker for urothelial carcinoma, achieving 75.0% sensitivity and 95.8% specificity in their validation cohort [68]. Another study found

that urinary exosomal miR-21 was highly expressed in BCa patients and closely correlated with tumor stage and grade. It demonstrated strong diagnostic value for both early and advanced disease and showed potential for predicting poor prognosis [69].

Beyond miR-21, several studies have identified uEV-miRNAs capable of stratifying BCa by grade and invasiveness. Andreu *et al*. used microarray analysis of uEVs from BCa patients and identified a panel of 26 dysregulated miRNAs. Subsequent validation showed that miR-375 could serve as a biomarker for high-grade BCa, whereas miR-146a could identify patients with low-grade tumors, offering clinically relevant information for treatment decisions [70]. Another study identified urinary exosomal miR-96-5p and miR-183-5p as promising non-invasive biomarkers, with their combined use producing a diagnostic sensitivity of 88.2%, higher than that of traditional cytology. Their expression also correlated with tumor grade and stage and decreased significantly after surgery, supporting their potential value for both initial diagnosis and post-treatment monitoring [71].

Multi-analyte panels may further improve diagnostic performance. A urinary biomarker panel combining exosomal miRNAs and protein markers identified miR-19b1-5p, miR-136-3p, miR-139-5p, APE1/Ref1, BLCA-4, and CRK as promising candidates. This panel distinguished BCa patients from healthy controls with high accuracy, particularly in low-risk disease, although validation in larger cohorts remains necessary [72]. High-throughput sequencing has also enabled the discovery of new urinary miRNA signatures. One preliminary study identified several significantly upregulated mature miRNAs in BCa patients and developed a three-miRNA panel consisting of miR-6724-5p, miR-10400-5p, and miR-7704. This panel demonstrated superior diagnostic performance with more than 90% sensitivity, highlighting its potential as a highly sensitive and specific non-invasive detection method pending further validation [73].

Several uEV-miRNAs have been associated with muscle invasion, a key determinant of treatment strategy. miR-17-5p was reported to be significantly upregulated in urinary exosomes from patients with muscle-invasive bladder cancer compared with those with non-muscle-invasive disease [74]. In a NGS study of EV-contained miRNAs from urine and serum samples of patients with non-muscle-invasive BCa, urinary EV miR-451a and miR-486-5p were significantly upregulated in presurgical samples from stage T1 patients compared with postoperative follow-up samples [75]. These findings suggest that miR-451a and miR-486-5p may serve as non-invasive biomarkers for monitoring disease status and recurrence-free survival in T1 BCa.

Another study identified a miRNA panel associated with BCa invasiveness. miR-146b-5p, miR-155-5p, miR-138-5p, and miR-200a-3p were linked to high tumor grade in tissue samples. In uEVs, miR-146b-5p and miR-155-5p were significantly elevated in muscle-invasive BCa, suggesting their potential to distinguish MIBC from NMIBC and support clinical decision-making [76].

Most recently, the integration of standardized uEV workflows with artificial intelligence has forward urinary liquid biopsy in BCa beyond simple detection to molecular subtyping. Greenberg *et al*. developed an AI-driven uEV liquid biopsy pipeline that couples standardized EV isolation with transcriptomic profiling and a machine learning-based prognostic scoring algorithm, achieving high-precision BCa subtyping and prognosis prediction from urine alone [77]. This work illustrates how uEV analytes, when combined with computational classifiers, may ultimately deliver the kind of molecular stratification currently obtainable only from tumor tissue, and it provides a template for future clinically deployable uEV assays in BCa.

Overall, uEV-miRNAs offer far more than a simple detection signal in BCa. They provide molecular information on tumor grade, invasiveness, recurrence risk, and potentially therapeutic sensitivity. With further validation, uEV-miRNA-based assays could reduce reliance on invasive cystoscopy and usher in a more patient-centered model of BCa surveillance.

4. Mechanisms of action: how uEV-miRNAs orchestrate urological malignancies

Increasing evidence indicates uEV-miRNAs are not only passive molecular traces shed by tumors. that they are active participants in cancer progression. By transferring miRNA cargo from donor cells, such as tumor cells, to recipient cells, including neighboring cancer cells, endothelial cells, stromal cells, and immune cells, uEVs reprogram gene expression across the tumor microenvironment. In doing so, they coordinate key biological processes that sustain malignancy, including proliferation, invasion, angiogenesis, immune escape, and therapeutic resistance (Figure 3 and Table 2).

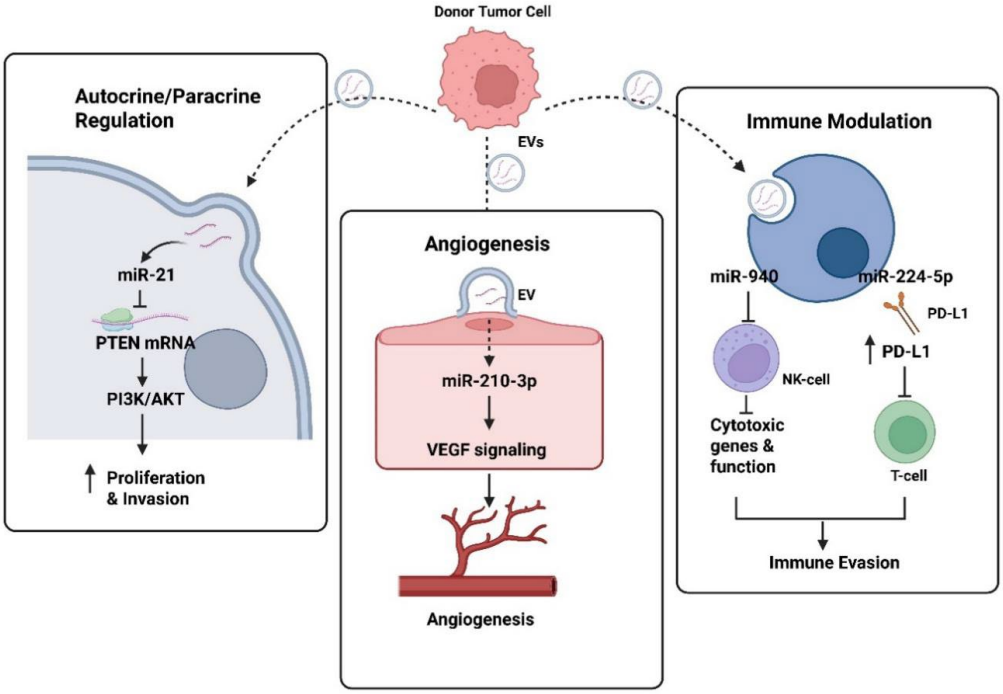

Figure 3. Mechanisms of action of uEV-miRNAs in the tumor microenvironment of urological malignancies. Tumor-derived extracellular vesicles act as intercellular messengers that shape cancer progression. Tumor cells release EVs carrying oncogenic or immunosuppressive miRNAs, which are taken up by diverse recipient cells within the tumor microenvironment. **(a)** In neighboring tumor cells, transferred miRNAs such as miR-21 may suppress tumor suppressor genes such as PTEN, activating pro-survival pathways including PI3K/AKT and promoting proliferation, invasion, and resistance to apoptosis; **(b)** In endothelial cells, pro-angiogenic miRNAs such as hypoxia-induced miR-210-3p stimulate pathways such as VEGF signaling, enhancing endothelial migration and new vessel formation; **(c)** In immune cells, uEV-miRNAs reshape anti-tumor immunity. For example, miR-940 can suppress cytotoxic gene expression in natural killer cells, whereas miR-224-5p can increase PD-L1 expression on tumor cells, thereby inhibiting T-cell activity and promoting immune evasion. These mechanisms demonstrate that uEV-miRNAs are not only biomarkers but also functional drivers of malignancy.

Table 2. Mechanisms of action of key uEV-miRNAs in urological tumors.

Cancer type	miRNA(s)	Main target(s)/pathway(s)	Biological effect	Mechanistic significance	Reference
RCC	miR-200a	SIRT1	Promotes apoptosis and suppresses cell growth	Tumor-suppressive miRNA whose restoration inhibits RCC progression	[78]
RCC	miR-224-5p	CCND1; PD-L1 stability	Enhances invasion, metastasis, and immune evasion	Links oncogenic signaling with immune checkpoint regulation	[79]
RCC	miR-210-3p	HIF-1α-induced; VEGF-associated angiogenic signaling	Promotes endothelial migration and vessel formation	Hypoxia-driven pro-angiogenic EV miRNA	[80]
RCC	miR-378a-3p	TOB2	Promotes proliferation, migration, and invasion	Supports metastatic progression and tumor aggressiveness	[81]
RCC	miR-15a	BTG2/PI3K/AKT axis	Aggravates RCC progression	Biomarker with direct oncogenic function	[43]
RCC	miR-940	Immunosuppressive programs	Supports tumor growth and immune evasion	Included in early diagnostic model; functionally linked to immune suppression	[82]
PCa	miR-891a/ 892a/892b cluster	Not specified in manuscript	Promotes proliferation	Oncogenic EV-miRNA cluster that drives t umor growth	[83]
PCa	miR-21-5p	MARCKS; PI3K/AKT survival pathway	Promotes invasion and apoptosis resistance	Central mediator of aggressive behavior and treatment resistance	[84,85]
PCa	miR-196a-5p	TAM polarization pathways	Promotes M2-like immunosuppressive macrophage phenotype	Facilitates immune escape and tumor-supportive microenvironment	[86]
PCa	miR-532-5p	Not specified; recurrence-associated signature	Associated with biochemical recurrence	Predictive biomarker for poor prognosis	[61]
BCa	miR-30c-5p	PRC1; CDK1/cyclin B1 axis	Induces G2/M arrest and suppresses tumor progression	Tumor-suppressive miRNA with biomarker and therapeutic potential	[87]
BCa	miR-21	Macrophage PI3K/AKT pathway	Reprograms macrophages toward a pro-tumor phenotype	EV-mediated crosstalk between tumor cells and immune cells	[63]
BCa	miR-148b-3p	Wnt/β-catenin pathway; PTEN	Enhances chemosensitivity when restored	Candidate biomarker and therapeutic sensitizer	[88]
BCa	miR-146a-5p	TET2 inhibition; c-Myc activation; VEGF-related angiogenesis	Promotes endothelial proliferation and angiogenesis	Functional link to tumor aggressiveness and recurrence risk	[89]
BCa	miR-940	Natural killer cell cytotoxic gene programs	Suppresses NK-cell-mediated anti-tumor activity	Facilitates immune escape by disabling innate immunity	[82,90]
BCa	miR-17-5p	ARID4B	Enhances invasion and shapes immune microenvironment	Connects EV-miRNA signaling with invasive phenotypes	[70]
BCa	miR-155-5p/miR-146b-5p	Invasiveness-associated pathways	Associated with muscle-invasive phenotype	Supports distinction between MIBC and NMIBC	[72]

4.1. Driving core oncogenic behaviors

uEV-miRNAs participate several biological processes of urological cancers, including cell-cycle progression, proliferation, survival, metastasis, and angiogenesis.

4.1.1. Cell cycle and proliferation

miRNAs may function either as oncogenes or tumor suppressors depending on cellular context. In RCC, the expression of miR-200a is downregulated and it was regarded as a suppressor. Notably, artificial restoration of its' expression in RCC cells can inhibit cell growth by directly targeting the pro-survival protein SIRT1 [78]. Conversely, the oncogenic miR-891a/892a/892b cluster in uEVs derived from PCa patients can promote the proliferation of prostate cancer cells, and referring as a growth-driving miRN network [79].

In BCa, miR-30c-5p can directly target and downregulate the expression of PRC1, which involving in the CDK1/cyclin B1 signaling pathway. This suppresses BCa cell survival and arrests cells in the G2/M phase. Conversely, downregulation of miR-30c-5p promotes BCa development through PRC1 activation, suggesting that miR-30c-5p may function both as a prognostic biomarker and as a potential therapeutic target [80]. In RCC, urinary EV miR-224-5p is significantly upregulated and has been identified as a candidate non-invasive biomarker. Functionally, miR-224-5p targets CCND1, promoting invasive and metastatic behavior while stabilizing PD-L1, thereby contributing to immune evasion and RCC progression [81]. In addition, miR-940 has been incorporated into an early diagnostic model and linked to immunosuppressive functions that may support tumor growth [82].

4.1.2. Metastasis and microenvironment remodeling

Metastasis involving several key events including tumor cell detachment, local invasion, intravasation, survival in circulation, extravasation, and colonization of distant organs. There is increasing evidence that uEV-miRNAs are key regulators of this process. In RCC, miR-378a-3p is frequently dysregulated miRNA and which can promote RCC cell proliferation, migration, and invasion by regulating TOB2, suggesting its potential as a therapeutic target for RCC [83]. Similarly, in PCa, the most famous omcomiR, miR-21-5p, can facilitate tumor invasion by directly targeting MARCKS, a protein that involving in cell motility and adhesion [84]. In BCa, cancer cells secreted exosomal miR-21 can enter into macrophages, thus activating the PI3K/AKT signaling pathway and inducing a pro-migratory, tumor-supportive phenotype of macrophages [67]. This illustrates how tumor-derived uEV-miRNAs can remodel the tumor microenvironment by co-opting immune cells.

Moreover, mechanistic studies have also uncovered the therapeutic potential of dysregulated EVs miRNAs. For instance, Shan *et al*. found that miR-148b-3p was downregulated in EVs from cancer-associated fibroblasts, and restoration of miR-148b-3p enhanced the chemosensitivity of BCa cells by modulating the Wnt/β-catenin pathway and PTEN expression [85].

4.1.3. Angiogenesis

Angiogenesis is essential for tumor growth, invasion, and metastasis. uEV-miRNAs contribute to this process by transmitting pro-angiogenic signals to endothelial cells. In the hypoxic core of RCC tumors,

HIF-1α induces miR-210-3p expression. Moreover, miR-210-3p can be encapsulated into EVs and delivered to endothelial cells, where it promotes the expression of pro-angiogenic factors such as VEGF, thereby stimulating neovascularization and enhancing tumor growth [86].

In BCa, the expression of uEV miR-146a-5p is elevated in high-grade disease and is linked to tumor aggressiveness, recurrence risk, and angiogenesis. Mechanistically, BCa-derived EV miR-146a-5p promotes endothelial cell proliferation by inhibiting TET2 and activating c-Myc expression, further strengthen the viewpoint that EV miRNAs may function simultaneously as biomarkers and therapeutic targets [87].

4.2. Modulating the tumor immune microenvironment and therapeutic resistance

Immune evasion is a pivotal characteristic of cancer, and uEV-miRNAs are increasingly recognized as central regulators of this process. By reshaping immune checkpoint expression, suppressing effector immune cells, and activating survival pathways, these miRNAs can help tumors evade immune destruction and resist therapy.

4.2.1. Regulating immune checkpoints

The PD-1/PD-L1 axis is crucial for maintaining immune tolerance, nevertheless, this regulatory pathway is often hijacked by tumors to suppress anti-cancer immunity. Qin *et al*. found that uEV-derived miR-224-5p increases PD-L1 abundance in RCC cells through inhibiting the expression of Cyclin D1, which in turn prevents PD-L1 degradation. This creates an immunosuppressive shield that protects tumor cells from T-cell-mediated cytotoxicity and may contribute to resistance against PD-1/PD-L1 inhibitor therapies [81].

4.2.2. Suppressing immune cell function

uEV-miRNAs can also directly disrupt the immune function of immunocyte. In BCa, uEVs miR-940 can be transported to natural killer (NK) cells, a key component of innate anti-tumor immunity. Once enter into NK cells, miR-940 can suppress cytotoxic gene expression, and results in impairing their tumor-killing capacity [82,88]. Similarly in PCa, dysregulation of miR-196a-5p is recognized to induce the polarization of tumor-associated macrophages toward an M2-like, immunosuppressive phenotype that facilitates tumor growth rather than tumor elimination [89].

4.2.3. Mediating therapeutic resistance

Besides immune escape, uEV-miRNAs can contribute to resistance to chemotherapy, targeted therapies, and radiotherapy. In BCa, decreased levels of exosomal miR-148b-3p have been reported to relate with chemotherapy sensitivity [85]. In PCa, transfer of miR-21 activates the PI3K/AKT survival pathway, enabling cancer cells to resist apoptosis induced by therapeutic stress [90]. These findings emphasize that uEV-miRNAs are not only biomarkers of tumor therapeutical resistance but may also serve as actionable targets to overcome treatment failure.

5. Clinical translation: prospects, challenges, and the path forward

The remarkable stability, accessibility, feasibility and biological relevance of uEV-miRNAs highlight them as promising liquid biopsy candidates for next-generation diagnostic and therapeutic platforms. However, successful clinical translation will require coordinated progress in technology, standardization, validation, and computational integration.

5.1. Therapeutic anddiagnostic horizons

5.1.1. Advanced diagnostics

The future development of uEV-miRNA-based diagnostics need integrated panels for combining multiple miRNAs with clinical variables and complementary molecular features, which are expected to offer superior accuracy. Briefly, multi-miRNA signatures analyzed by machine learning algorithms may be combined with traditional urological cancer markers, routine laboratory indicators, ctDNA mutations, protein biomarkers, imaging findings, and clinicopathological parameters to create robust, comprehensive, and multi-modal diagnostic systems. Such platforms could support early detection, distinguish malignant from benign conditions, stratify disease aggressiveness, and monitor recurrence or therapeutic response in real time.

5.1.2. Therapeutic intervention

Because many uEV-miRNAs play functional roles in tumorigenesis and progression of urological tumors, they also have the potential as novel therapeutic targets and tools. One strategy involves inhibiting oncogenic miRNAs such as miR-21 or miR-224-5p using antisense oligonucleotides, including antagomirs. Conversely, tumor-suppressive miRNAs such as miR-200a may be restored using synthetic miRNA mimics. Moreover, EVs can be applied as natural nucleic acid drug carrier for delivering miRNA antagomirs or mimics during the course of treatment. These approaches could theoretically suppress tumor growth, reverse immune evasion, enhance chemosensitivity, and improve therapeutic outcomes. Although challenges remain in delivery, specificity, and safety, miRNA-based therapy represents a promising frontier in urological oncology.

5.1.3. EV-based theranostics

EVs themselves are increasingly being recognized as “theranostic” platforms that combine therapy and diagnosis. Their natural ability to cross biological barriers, low immunogenicity, and intrinsic capacity for molecular cargo delivery make them attractive pharmaceutical carrier for transporting chemotherapeutic agents, siRNAs, or miRNA-based therapeutics. EVs may also be engineered to carry imaging agents, enabling simultaneous tumor detection and treatment monitoring. The therapeutic reach of engineered EVs is expanding rapidly beyond cargo delivery of conventional drugs and RNA therapeutics. For example, in a recent study by Pan *et al*., EV-mediated delivery of gene-editing machinery in vivo successfully treating nonsyndromic progressive hearing loss in adult mice [91]. Although developed in a non-oncological context, this study provides proof of concept that EV platforms can deliver functional gene-editing payloads to therapeutically relevant tissues in adult animals—a capability with clear

implications for future genome-targeted interventions in urological malignancies. Such platforms align closely with the vision of personalized medicine, in which diagnosis, treatment selection, and therapeutic delivery are integrated into a single system [92–94].

5.2. Current challenges and future directions

Despite rapid progress in EVs and uEV-miRNA in recent years, several major obstacles must be solved before uEV-miRNA biomarkers can be moved towards into routine clinical practice.

5.2.1. Technical standardization and quality control

The lack of consensus on uEV isolation, purification, characterization, and miRNA quantification remains the most important obstacle to clinical application. Variability in sample collection, handling, storage conditions, normalization strategies, and analytical platforms can lead to inconsistent even the contrary results and poor reproducibility between different studies. The development of certified reference materials, standardized protocols, and inter-laboratory quality control systems will be critical for establishing clinically reliable assays [2,95]. Notably, guidelines from the International Society for Extracellular Vesicles—including the MISEV2023 recommendations and, for urine specifically, the ISEV Urine Task Force position paper, which addresses pre-analytical variables unique to urine such as collection timing, normalization to urinary creatinine or EV number, uromodulin removal, and storage conditions—provide an essential framework, but broader adoption is still needed [23,24].

5.2.2. Biological complexity and heterogeneity

EVs are a class of highly heterogeneous mixture of vesicles that were derived from different cells and biogenic pathways. Bulk analytical methods average signals from billions of vesicles, potentially masking clinically important subpopulations [96]. A central question remains: are tumor-derived EVs more diagnostically meaningful than EVs released from normal epithelial, immune, or stromal cells? Addressing this question will require single-vesicle analysis technologies and more precise strategies for isolating tumor-specific EVs, such as PSMA-based capture for prostate-derived vesicles. Dissecting this heterogeneity will be essential for improving biomarker specificity.

5.2.3. Rigorous clinical validation

Till date, most existing studies of uEV-miRNA are retrospective and based on relatively small, single-center cohorts. To obtain regulatory approval and clinical acceptance, candidate uEV-miRNA biomarkers must be validated in large, prospective, multicenter, and multiethnic trials. These studies should include well-defined patient populations, standardized analytical workflows, and clinically meaningful endpoints. Most importantly, they must demonstrate clinical utility—that is, evidence that the biomarker test improves decision-making, reduces unnecessary invasive procedures, detects clinically significant disease, or improves patient outcomes.

5.2.4. Bioinformatics and data integration

High-throughput miRNA profiling generates large and complex datasets. Advanced bioinformatic pipelines, machine learning, and artificial intelligence will be helpful for identifying reproducible multi-miRNA signatures as well as optimal miRNA-panel with true diagnostic and prognostic value. However, algorithmic performance depends on high-quality training datasets, rigorous validation, and transparent reporting. Publicly accessible, curated databases that integrate EV-miRNA profiles with clinical outcomes, pathology, imaging, and treatment response would accelerate discovery and improve reproducibility across the field.

6. Conclusion

uEV miRNAs represent a transformative frontier in the management of urological malignancies. By combining non-invasive accessibility, molecular stability, and close anatomical relevance to the urinary tract, uEV-miRNAs offer a powerful liquid biopsy platform for renal, prostate, and bladder cancers. Their value extends beyond detection to provide insight into tumor biology, prognosis, immune regulation, therapeutic resistance, and disease recurrence.

Importantly, uEV-miRNAs are not merely passive disease indicators. They are active molecular messengers with biological functions that participate in tumor progression by regulating proliferation, invasion, angiogenesis, immune escape, and treatment response. This dual identity as both biomarkers and biological effectors makes them uniquely attractive for precision oncology.

Nevertheless, the road of uEV-miRNAs from research laboratories to clinical translation remains challenging. Standardized methodologies, improved EV subpopulation resolution, robust multi-center validation, and advanced computational integration are urgently needed. Fortunately, the field is advancing rapidly. With continued technological innovation, interdisciplinary collaboration, and rigorous clinical evaluation, uEV-miRNAs are poised to become indispensable tools in urological oncology, enabling earlier diagnosis, more accurate risk stratification, and more personalized treatment for patients with urological malignancies.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used Gemini 2.5 pro only to improve language and readability. The authors take full responsibility for the content of the manuscript.

Acknowledgments

This work was funded by Basic Research Program of Jiangsu (BK20251737), Jiangsu Provincial Health Commission Key Scientific Research Project (No. K2023050), Natural Science Foundation Project of Nanjing University of Chinese Medicine (No. XZR2024099), the “Qingyun” High-end Talent Cultivation Program of Nanjing Hospital of Chinese Medicine (No. QY-2026-09), and Nanjing Hospital of Chinese Medicine High-level Talent Funding Project (No. YJRC-WC-2024).

Authors' contribution

Conceptualization, C.W., X.H., and G.W.; resources, C.W., X.H and G.W; writing—original draft preparation, R.T., J.S. and H.X.; writing—review and editing, D.N., and C.W.; visualization, X.D.; supervision, X.H.; funding acquisition, C.W. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no competing interests.

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