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REVIEW

How Advanced Are Extracellular Vesicles in Renal Cell Carcinoma? For Diagnostic and Therapeutic Frontiers

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Abstract: Renal cell carcinoma (RCC), a prevalent malignant tumor of the urinary system, presents significant challenges in early diagnosis and treatment. The invasiveness of traditional tissue biopsies and the limitations of imaging techniques necessitate the exploration of novel biomarkers through liquid biopsy. Extracellular vesicles (EVs), functioning as natural nanoscale carriers, encapsulate a variety of tumor-derived molecules and exhibit distinctive potential in non-invasive diagnosis, regulation of the tumor microenvironment (TME), and resistance to treatment in RCC. Serum- and urine-derived EVs can effectively differentiate RCC patients from healthy individuals by utilizing specific biomarkers, such as miRNAs, proteins, and snoRNAs. They can predict tumor staging, metastasis, and prognosis. TME is reshaped by EVs through the transmission of immunosuppressive factors and pro-angiogenic molecules, facilitating immune escape and the formation of pre-metastatic niches. Furthermore, the drug resistance mechanisms mediated by EVs provide new insights for targeted therapy, and their application as drug carriers demonstrates therapeutic potential. Nonetheless, the clinical translation of EVs faces several technical challenges, including the standardization of isolation techniques, inadequate validation of biomarkers, and the lack of large-scale clinical studies. Future efforts are focused on integrating multi-omics analysis, AI-assisted diagnosis, and novel isolation techniques to facilitate the transition of EVs from the laboratory to clinical application. Overall, EVs hold significant promise for the precision diagnosis and treatment of RCC; however, their widespread application necessitates systematic validation and technological innovation.

Keywords: liquid biopsies, miRNAs, tumor microenvironment, immunosuppressive, drug resistance mechanisms

Introduction

Renal cell carcinoma (RCC) ranked as the 14th most commonly diagnosed cancer worldwide in 2020. Approximately 430,000 new cases are identified annually, representing about 2.2% of all cancer diagnoses, with an estimated 180,000 deaths attributed to kidney cancer each year.[1] The average age at diagnosis is 64 years. The most prevalent histopathological subtype is clear cell RCC (ccRCC), comprising approximately 75% of cases, followed by papillary RCC types 1 and 2 at 15%, and chromophobe RCC at 5%. Several rare RCC subtypes and unclassified RCCs are also identified.[2] Most RCC patients are asymptomatic at the time of diagnosis, even those with larger tumors.[3] The increasing use of imaging modalities such as CT, MRI, and ultrasound for non-clinical purposes results in a rise in the incidental detection of RCC during early clinical stages.[4] However, existing diagnostic tools often lack accuracy, particularly for small renal masses (SRMs), which can lead to potential overtreatment.[5] Retrospective study results indicate that among patients undergoing surgery for SRMs less than 4 cm, approximately 19–20% of the final histopathological diagnoses are benign.[6] Percutaneous tumor biopsy can reduce the likelihood of overtreatment; however, the risk of adverse events following biopsy is estimated at 8.1%.[7] Consequently, the European Association of Urology (EAU) guidelines recommend

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percutaneous tumor biopsy only for specific patients, as performing kidney biopsies is more challenging for SRMs than for larger tumors.[8] The current state of RCC epidemiology indicates that precision oncology has yet to be fully integrated into clinical practice. RCC encompasses a variety of tumors that differ in morphology, behavior, and gene expression. The inability to accurately diagnose RCC, specifically in distinguishing between benign and malignant renal masses, hinders efforts toward personalized treatment.[9] Consequently, histopathological analysis of surgical or biopsy specimens remains the gold standard for RCC diagnosis. A reliable non-invasive diagnostic strategy is an unmet clinical need that could significantly enhance healthcare services.

Compared to other cancer types, liquid biopsy plays a relatively limited role in the clinical practice of renal cancer. Unlike tissue biopsy, liquid biopsy utilizes the patient's biological fluids, such as blood or urine (Table 1). These fluids are analyzed for molecular biomarkers, which provide insights into the characteristics of an individual patient's disease. The invasiveness and associated pain of tissue biopsies hinder their routine use in cancer diagnosis and monitoring procedures. Liquid biopsies offer the advantage of minimally invasive and non-invasive sampling through blood and urine. As the focus shifts towards the earlier diagnosis of RCC, new biomarkers are needed to address the challenges in SRM identification and RCC subtype characterization. The most promising liquid biomarkers for RCC diagnosis are circulating tumor cells (CTCs), extracellular vesicles (EVs), and cell-free DNA (cfDNA). CTCs detection offers the highest specificity, along with relatively low processing time and cost, but its sensitivity remains relatively low. Most CTC detection methods rely on the expression of epithelial cell adhesion molecule (EPCAM) for enrichment and detection. However, EPCAM is more frequently down-regulated in RCC compared to other cancers, which hinders the clinical application of CTCs as diagnostic biomarkers.[10] cfDNA can aid in the diagnosis of high-volume RCC; however, compared to other cancers, cfDNA content in the blood of RCC patients is relatively low and positively correlated with tumor volume. This correlation diminishes its potential as a diagnostic biomarker for all RCC cases, particularly those in early stages. EVs exhibit the highest biological abundance and sensitivity, with specificity comparable to that of cfDNA detection. They are widely applicable, present in all biological fluids, and found in extremely high concentrations in blood and urine, even in patients with small tumor volumes. EVs carry diverse molecular cargo, including proteins, RNA, DNA, and lipids, making them suitable for a wide range of analytical applications.[11] Therefore, EV detection holds the greatest potential for diagnostic applications in RCC, including the identification of SRMs. However, before liquid biopsy can be implemented in clinical practice, all experimental RCC biomarker tests must be further standardized and simplified.

Extracellular vesicles (EVs) were first identified in 1983, when researchers observed reticular cells releasing 50-nanometer vesicles containing transferrin receptors into the extracellular space.[12] According to the MISEV 2023 guidelines, "EVs" refer to particles naturally released from cells, encompassing multiple subtypes classified based on their synthesis and release mechanisms. These subtypes include exosomes (50–100 nm), microvesicles (100–1000 nm), apoptotic bodies (100–5000 nm), and exomeres (≤50 nm). Exosomes originate from multivesicular bodies (MVBs),

Table 1 Comparative Analysis of Tissue Biopsy and Liquid Biopsy

	Tissue Biopsy	**Liquid Biopsy**
Commonality	Diagnosis, staging, prognostic assessment and treatment monitoring of cancer	
	Detection of tumor-related biomarkers	
	Early cancer screening, dynamic monitoring of efficacy, early warning of recurrence and drug resistance analysis	
Difference	Direct access to diseased tissue by surgery, puncture	Access to circulating tumor components via body fluids
	Highly invasive and may cause pain, bleeding	Non-invasive and less traumatic.
	Heterogeneous tumors may miss local mutations	High sensitivity to reflect systemic tumor heterogeneity
	Higher costs	Relatively low-cost
	Precise Staging of Confirmed and Advanced Tumors	Early screening, micro residual disease (MRD) monitoring

Notes: Tissue biopsy and liquid biopsy are both widely used in tumor diagnosis and treatment. Tissue biopsy is often limited by its invasiveness and sampling bias. In contrast, liquid biopsy is non-invasive, convenient, and more suitable for dynamic tracking.

while calcium ion influx and cytoskeletal remodeling lead to the direct budding and fission of the cell membrane to form microvesicles. During programmed cell death, cytoplasmic fragmentation results in the formation of apoptotic bodies. Exosomal particles are also formed by the separation between the cell body and large cytoplasmic protrusions. EVs can be classified by the type of source cell, such as platelet-derived or endothelial cell-derived, or by the physiological state of the cell, such as "oncosomes" released by cancer cells and "prostasomes" produced by prostate cells.[13] Due to the lack of specific distinction between subcellular sources, it is recommended that these entities be collectively referred to as EVs.[14] The biogenesis of EVs begins with the invagination of the plasma membrane, resulting in the formation of early endosomes. These early endosomes undergo further invagination and mature into late endosomes, leading to the formation of MVBs, which contain numerous intraluminal vesicles (ILVs) ranging from 40 to 150 nm in diameter. Collectively, these vesicles are referred to as EVs.[15] Some MVBs are directed to lysosomes for degradation, while others fuse with the plasma membrane to facilitate EV secretion. This secretion process may be dependent on or independent of the ESCRT pathway (Figure 1).

EVs are nanoparticles, ranging in size from nanometers to micrometers, enclosed within lipid bilayer membranes. They protect their molecular cargo from enzymatic degradation and mechanical forces in the bloodstream. This cargo, including nucleotides, proteins, and lipids, originates from the parent cell and can be transferred to distant cells. This intercellular communication enables the originating cell to influence remote cellular environments.[16] EVs express a variety of cell-specific antigens, including fusion proteins, adhesion molecules, and integrins, which play crucial roles in targeting specific receptor cells.[17] EVs are involved in promoting the establishment of pre-metastatic niches, enhancing angiogenesis, and activating immune suppression. Additionally, they serve as channels for transporting various cellular components, facilitating complex cellular communication, and mediating numerous biological processes.[18] In addition, EVs hold significant potential in disease diagnosis, efficacy prediction, and prognosis assessment. Their specific functions depend on their cellular origin, reflecting the cell type of origin, cell state, differentiation stage, and environmental stimuli present during formation.[19] For example, EVs derived from tumor cells are essential for intercellular communication, particularly in processes related to migration and invasion.[20] As multifunctional information complexes, EVs mediate communication between innate and adaptive immunity,

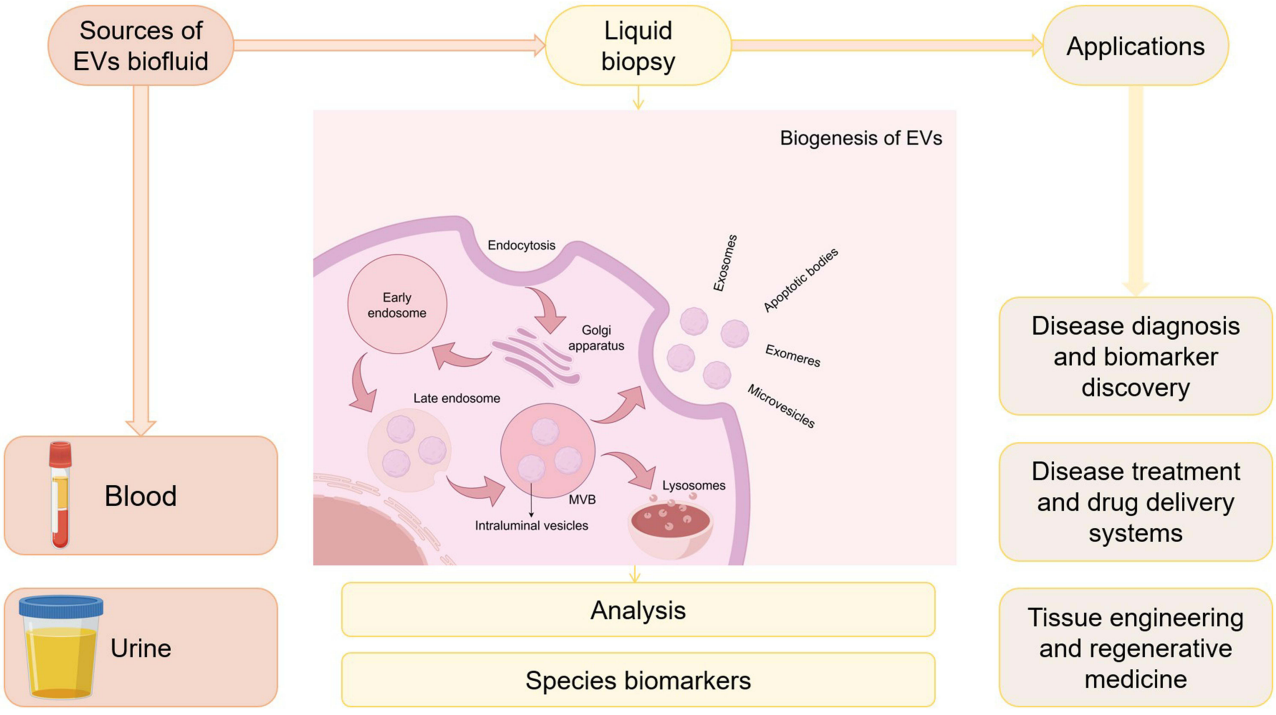

Figure 1 Extracellular vesicles in body fluids.
Notes: EVs are ubiquitous in various body fluids. Liquid biopsy, a cutting-edge technology in cancer diagnosis and prognosis prediction, enables the non-invasive capture and analysis of EVs in body fluids such as blood and urine. The clinical significance of EVs extends to disease diagnosis and biomarker discovery, disease treatment, drug delivery systems, tissue engineering, and regenerative medicine. Figure 1 was created by FigDraw.

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alter receptor cell genes, reshape the tumor immune microenvironment, and exert immune-enhancing or suppressive effects to regulate cancer progression and metastasis. This review systematically summarizes the latest research progress on EVs in RCC, with a focus on their application as liquid biopsy biomarkers for diagnosis and prognosis. Key mechanisms of EVs in TME remodeling and treatment resistance are explored, along with their therapeutic potential and current challenges in clinical translation. The aim is to provide a comprehensive perspective and reference for the future development of EVs in the field of precision medicine for RCC.

What Do We Understand About Key Extracellular Vesicles as Cancer Biomarkers?

Over the past decade, EVs have emerged as a potential source of biomarkers. EVs enriched from urine, serum, and plasma have become a focal point of research in RCC biomarkers (Figure 2), including the evaluation of their diagnostic potential. EVs carry a diverse array of molecular cargo, including proteins, microRNAs (miRNAs), and small nucleolar RNAs (snoRNAs). Human blood and urine are rich in proteins, including carcinoembryonic antigen (CEA), tissue-specific secretory proteins, and intracellular proteins released by tissue damage or cell death. Proteins associated with cancer often play crucial roles in the activation of specific tumorigenic pathways; therefore, their detection can reveal carcinogenic mechanisms or serve as therapeutic targets.[21] Raimondo et al first identified differences in protein content between urinary extracellular vesicles (uEVs) from RCC patients and healthy individuals, providing insights for the discovery of new RCC biomarkers.[22] In recent years, research has predominantly focused on the clinical application of miRNAs in liquid biopsy rather than proteins. Studies indicate that miRNAs play regulatory roles in RCC by promoting or inhibiting tumorigenesis and progression. However, no single miRNA has demonstrated sufficient sensitivity and specificity to serve as a biomarker for RCC. Consequently, panels composed of multiple miRNAs are frequently employed as diagnostic and assessment models for RCC.[21] In addition to proteins and miRNAs, snoRNAs are also anticipated to become biomarkers for various types of tumors, including ccRCC. SnoRNAs play a crucial role in various

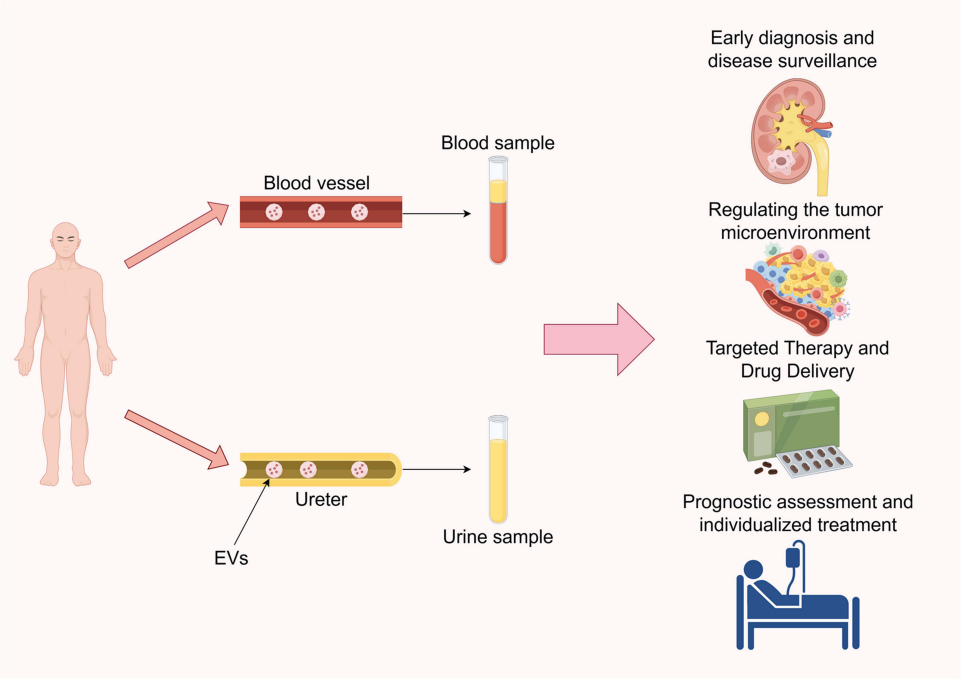

Figure 2 Applications of extracellular vesicles in body fluids.
Notes: Liquid biopsy for RCC primarily utilizes blood and urine samples. This non-invasive approach enables continuous collection of patient information and serves multiple roles at different disease stages. It can screen for RCC in healthy populations and assist in the differential diagnosis of urinary tract masses. Before treatment, liquid biopsy predicts the risk of disease progression, helping to identify high-risk patients, and forecasts patient responses to various treatment methods, aiding in the selection of appropriate treatment regimens. After treatment, liquid biopsy monitors treatment efficacy in real time, helping to prevent postoperative recurrence and metastasis. Figure 2 was created by FigDraw.

biological processes, and their abnormal expression or deficiency is linked to a variety of diseases. Recently, the role of snoRNAs in hepatocellular carcinoma (HCC), colorectal cancer (CRC), and breast cancer (BRCA) has become a focus of research. Numerous studies demonstrate that snoRNA expression is abnormal in the cells and tissues of various cancers.[23] However, limited research currently exists on the mechanism of action of snoRNAs in ccRCC. Grützmann et al identify snoRNAs as the primary differentially expressed regions in patients with ccRCC and urolithiasis. Four snoRNAs (SNORD99, SNORD22, SNORD26, and SNORA50C) are found to be differentially expressed in EVs from ccRCC patients, with SNORD99 and SNORA50C achieving an accuracy rate of 0.811 (p=0.0091). This finding provides a novel snoRNA biomarker combination strategy for non-invasive ccRCC screening.[24]

UEVs are secreted into urine by kidney and urinary tract cells and constitute the most commonly assessed source for RCC diagnosis, followed by serum and plasma. RCC tumors may directly secrete EVs into urine, which serves as an easily collected source of biomarkers. The study of urinary biomarkers provides patients and doctors with comprehensive molecular profiling and is an important source of additional tumor genetic information (Table 2). Consequently, uEVs are evaluated as a tool for diagnosing RCC. Mallouk et al establish a specific protocol for detecting carbonic anhydrase IX (CAIX) in urine EVs from ccRCC patients, validating its feasibility as a liquid biopsy biomarker. They propose that this technology could be combined with antibodies to other EV biomarkers to enhance diagnostic performance.[25] Zhao et al find that SHC1 is overexpressed in high-grade ccRCC and is associated with poor prognosis. It regulates the expression of polymerase I and transcript release factor (PTRF) through the EGFR/PI3K-Akt signaling pathway. PTRF is specifically enriched in the urine EVs of 50–90% of ccRCC patients with abnormal EGFR activation, suggesting its value in both diagnosis and therapeutic monitoring.[26] The Kuczler team screens four mRNAs—ALOX5, RBL2, VEGFA, and TLK2—derived from uEVs as potential biomarkers for RCC. However, further validation of negative controls and enhancement of urine collection techniques are required.[27]

EVs-miRNAs demonstrate significant potential in defining disease specificity. Zhang et al identify four miRNAs in urinary EVs-miR-135b-5p, miR-196b-5p, miR-200c-3p, and miR-203a-3p-that effectively distinguish early-stage (stage I) RCC patients from controls.[28] Patients with Von Hippel-Lindau (VHL) syndrome, who are prone to developing multifocal

Table 2 Urine Extracellular Vesicles as Carriers of Renal Cancer Biomarkers

Biofluid	EV Enrichment Method	Molecular Biomarker Detection and Marker	Outcomes	AUC
Urine[22]	Differential centrifugation	LC-MS/MS analysis Western blotting	RCC UE protein content is substantially and reproducibly different from that of control UE	NA
Urine[25]	ExoQuick-TC	Immunogold staining	The detection of CAIX in small EVs from the urine of patients could constitute a liquid biopsy for CCRCC	NA
Urine[26]	Ultracentrifuge	qRT-PCR Western blot	PTRF was detected in the exosomes isolated from ccRCC patients' urine and ccRCC cancer cells' culture medium	NA
Urine[28]	Ultracentrifuge	RT-qPCR	Four uEVs miRNAs (miR-135b-5p, miR-196b-5p, miR-200c-3p, and miR-203a-3p) were significantly and stably upregulated in RCC in vitro and in vivo	0.785
Urine[27]	Ultracentrifuge	NanoString®	Four EV derived mRNA transcripts (ALOX5, RBL2, VEGFA, TLK2) were found specific to urine samples	NA
Urine[24]	Ultracentrifuge	RNA-seq qPCR	Four snoRNAs (SNORD99, SNORD22, SNORD26, and SNORA50C) are differentially expressed in ccRCC patient EVs	0.766
Urine[29]	Ultracentrifuge	Antibody-based array assays and Western blot techniques NGS	MiRNA profiling of exosomes from readily available biofluids to both distinguish VHL patient urine from normal control urine microRNAs and to provide biomarkers for the presence of VHL syndrome-associated ccRCC	NR

Abbreviations: AUC, area under the curve; NR, not reported; NA, not applicable.

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RCC and other tumors, provide an ideal model for studying the role of urinary EVs-miRNA in the early development of ccRCC. Walter et al find that miR-542-5p is downregulated in the urine of normal controls but upregulated in VHL-related ccRCC tissues, offering a biomarker for diagnosing VHL syndrome-related ccRCC.[29] Compared to EVs in serum and plasma, the concentration of EVs in human urine is lower, and samples often contain unrelated EVs, such as non-tumor EVs, along with co-enriched molecules not encapsulated by EVs. These molecules result from impure enrichment, necessitating further refinement of EV enrichment in RCC to improve the signal-to-noise ratio and enhance the distinction between RCC patients and healthy individuals.[30]

The diagnostic value of serum and plasma EVs is a prominent focus of many studies. EV membranes protect their cargo in the bloodstream, encapsulating miRNAs and proteins released from cells, which can be used for diagnosing RCC (Table 3). Qian et al compare the diagnostic efficacy of serum and serum EVs, finding that serum EVs achieve a diagnostic accuracy of 78.7% for RCC, with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.88, significantly outperforming serum alone, which has an AUC of 0.71.[31] In several studies, the Xiao team identifies that hsa-mir-149-3p and hsa-mir-424-3p, derived from RCC plasma EVs, are upregulated, while hsa-mir-92a-1-5p is significantly downregulated. The diagnostic performance of these three miRNAs shows AUC values of 0.8324, 0.7188, and 0.7727, respectively, with sensitivities of 0.875, 0.750, and 0.750, and specificities of 0.773, 0.727, and 0.818.[32] Muramatsu et al analyze miRNAs in serum EVs and find that miRNA-4525 is expressed at higher levels in RCC tissue compared to adjacent normal tissue.[33] Xue et al find that hsa-miR-320d, derived from serum EVs, is significantly upregulated in recurrent or metastatic ccRCC ($p<0.01$);[34] Similarly, Dias et al analyze plasma EVs from ccRCC patients and observe that elevated levels of hsa-miR-301a-3p and decreased levels of hsa-miR-1293 are associated with metastasis.[35] These miRNAs exhibit significant potential as liquid biomarkers for diagnosing RCC recurrence or metastasis.

Table 3 Serum and Plasma Extracellular Vesicles as Carriers of Renal Cancer Biomarkers

Biofluid	EV Enrichment Method	Molecular Biomarker Detection and Marker	Outcomes	AUC
Serum[31]	Ultracentrifuge	Western blot Label-free SERS	CNN models based on EV SERS data show significantly higher diagnostic capacities than matched serum CNN models	0.88
Plasma[32]	Ultracentrifuge	qPCR	In the plasma exosomes of RCC, the expression levels of hsa-mir-149-3p and hsa-mir-424-3p were upregulated; the expression levels of hsa-mir-92a-1-5p were significantly downregulated	hsa-mir-92a-1-5p:0.8324 hsa-mir-149-3p:0.7188 hsa-mir-424-3p:0.7727
Serum[34]	Ultracentrifuge	qRT-PCR	Serum EVs-derived hsa-miR-320d was significantly upregulated in recurrent/metastatic ccRCC ($p<0.01$)	NR
Serum[33]	T-cell immunoglobulin domain and mucin domain-containing protein 4 conjugated to magnetic beads	RT-PCR Western blot	miRNA-4525 expression was higher in RCC tissue than in the adjacent normal tissue	NA
Serum[36]	T-cell immunoglobulin domain and mucin domain-containing protein 4 (Tim4)	PSMA-EV sandwich ELISA	PSMA-EV levels were found to be elevated in patients with metastatic RCC in comparison to those without metastasis	NA
Serum[37]	Ultracentrifuge	Western Blot Immunohistochemical Staining	In RCC patients, serum exosomal GGT activity was elevated in those with advanced stages and those with microvascular invasion	NA

Abbreviations: AUC, area under the curve; NR, not reported; NA, not applicable.

In another study by Dias et al, the suitability of EV-derived tissue metalloproteinase inhibitors (TIMPs) as biomarkers for ccRCC is evaluated. It is found that metastatic ccRCC patients have higher levels of EV-derived TIMP-1 mRNA, and those with elevated levels exhibit lower overall survival rates.[38] The Kawakami team employs highly purified EVs isolated from serum using mucin domain protein 4 (Tim4) to develop a sandwich ELISA assay for detecting prostate-specific membrane antigen (PSMA) on EVs, revealing that PSMA-EV levels are significantly elevated in metastatic RCC patients compared to non-metastatic patients.[36] The Horie team confirms that serum EV gamma-glutamyltransferase (GGT) activity is associated with microvascular invasion in RCC, and that preoperative GGT activity can predict postoperative pathological risk.[37] These findings suggest that proteins in these EVs may serve as candidate diagnostic biomarkers for RCC.

Serum and plasma EVs demonstrate strong diagnostic accuracy, with high area under the curve AUC values reported across various studies. Most of these studies include early-stage RCC patients, with EV-derived miRNAs being the most commonly studied molecular biomarkers, alongside evaluations of EV-derived proteins. This suggests that EVs hold significant potential in RCC diagnostic biomarker research. However, challenges arise from the technical reproducibility of EV detection and the biological heterogeneity of RCC, necessitating improvements in the standardization of EV enrichment and downstream analysis protocols to enhance consistency. Addressing these issues is crucial to overcoming the diagnostic challenges of identifying RCC and distinguishing its subtypes in patients with SRMs. Overall, all EV-based liquid biomarkers require further clinical validation.

How Do Extracellular Vesicles Drive Tumor Microenvironment Remodeling?

How Do Extracellular Vesicles Orchestrate Immunosuppression in the Tumor Microenvironment?

EVs enable tumor cells to evade immune surveillance and attack by impairing the function of immune cells, such as T cells, natural killer (NK) cells, and dendritic cells, or by regulating immune suppression pathways. Liu et al have found that PD-L2 is expressed in EVs located on the surface of ccRCC cells, systemically suppressing T cell function.[39] EVs facilitate the establishment of an immunosuppressive and tumor-growth-promoting microenvironment, thereby advancing tumor development and metastasis. Research by Zhang et al reveals that EVs-C3 in RCC promote tumor metastasis by inducing the immunosuppressive polarization of tumor-associated macrophages (TAMs).[40] EVs mediate interactions between tumor cells and immune cells, such as macrophages, within TME. They transmit microRNAs and other carriers, playing a crucial role in communication between different cell types. Zhang et al find that the overexpression of lncARSR in vitro induces phenotypic and functional changes in macrophages and promotes tumor growth in vivo.[41]

Macrophages are the most abundant infiltrating immune-related stromal cells within and surrounding tumors. They exhibit diverse phenotypes and functions, adopting two distinct polarization states, switching between M1 and M2 activation phenotypes in response to various external stimuli. M2 macrophages primarily exert immunosuppressive functions and promote tumor growth, serving as key drivers of cancer metastasis. Huang et al first discovered that circSAFB2 mediates M2 macrophage polarization through the miR-620/JAK1/STAT3 axis, thereby facilitating immune escape in RCC.[42] Zhang et al find that miR-21-5p, carried by M2-derived EVs, promotes metastatic characteristics of RCC cells through PTEN/Akt signaling transduction;[43] Similarly, Feng et al report that M2-derived EVs inhibit NEDD4L by transferring miR-342-3p, thereby enhancing the proliferation, migration, and invasion of RCC cells.[44] In another study, Gan et al identify that LDHA is highly expressed in ccRCC and drives macrophage M2 polarization by upregulating tumor EVs-EPHA2, which activates the PI3K/AKT/mTOR pathway, thus promoting tumor proliferation and invasion.[45] Conversely, EVs can transport and release immune regulatory factors, thereby stimulating the activation and regulation of the immune system and enhancing the body's ability to combat malignant diseases. This dual function offers promising avenues and targets for tumor immunotherapy. Given their immunogenicity, EVs can be explored as drug delivery vehicles, providing new treatment strategies for cancer patients.

How Do Extracellular Vesicles Remote-Control Angiogenesis and Metastatic Spread?

A characteristic feature of RCC is abnormal angiogenesis. This condition, coupled with its ability to evade the immune system and promote hypoxia, confers resistance to chemotherapy and radiotherapy. Targeting angiogenesis represents a critical therapeutic approach. A deeper understanding of the intrinsic mechanisms of tumor angiogenesis in RCC may pave the way for the development of novel therapeutic agents to control tumor progression. Liu et al find that TAM-derived EVs down-regulate TIMP2 expression in RCC cells by shuttling miR-193a-5p, thereby promoting vascular mimicry (VM) and invasion.[46] Other studies reveal that prostate-specific membrane antigen (PSMA)-positive vesicles, released by prostate cancer cell lines, enhance angiogenesis in vascular endothelial cells. Similarly, EVs secreted by RCC cells convert vascular endothelial cells into PSMA-positive cells, thereby promoting angiogenesis;[47] Cells overexpressing branched-chain ketoacid dehydrogenase kinase (BCKDK) enhance vascular permeability and formation through EVs;[48] Carbonic anhydrase 9 (CA9) is overexpressed in various tumors, including RCC. Hypoxic RCC promotes angiogenesis in the microenvironment via EVs-CA9, accelerating tumor progression.[49] Microbial genetic material carried by EVs may also play a role in pathological processes. Uemura et al first discovered that circulating bacterial-derived DNA serves as a biomarker for RCC. In subsequent studies,[50] Jingushi et al identify specific enrichment of Propionibacterium acnes DNA in serum EVs from RCC patients. Functional experiments demonstrate that Propionibacterium acnes EVs are taken up by renal cancer cells, promoting their proliferation and exhibiting tumor growth and angiogenesis activity in xenograft models.[51]

Epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET) enable cancer cells to detach from the primary tumor, migrate, and spread to distant sites. The regulatory role of EVs in metastasis is likely mediated by their influence on EMT. The involvement of EVs in the formation of pre-metastatic niches offers new targeting opportunities and holds prognostic and predictive value. Communication between VHL+ and VHL- cells is crucial for distant metastasis in RCC. Research indicates that VHL- cells produce significantly more EVs than VHL+ cells, and these EVs enhance EMT and the migration of VHL+ cells.[52] In several experiments, Wang et al find that cancer stem cell (CSC)-derived EVs from ccRCC patients suppress PTEN expression by delivering miR-19b-3p, thereby driving tumor cell proliferation and EMT.[53] Jin et al discover that RCC cell 786-O-derived EVs promote the binding of transcription factor ETS1 to the TFCP2L1 promoter by transporting MALAT1, thus enhancing RCC invasion and metastasis.[54] Song et al report that cells overexpressing RAB27A acquire metastatic potential by secreting miR-127-3p through EVs.[55]

How Do Extracellular Vesicles Mediate Treatment Resistance, and Can We Reverse It?

How Do Extracellular Vesicles Develop Resistance to Targeted Therapies?

EVs carry disease-related substances, including viral miRNAs and proteins, which act as immunosuppressive factors that enhance tumor metastasis or as growth signals that resist treatment. EV secretion also facilitates the transport of drugs out of cancer cells, thereby reducing drug efficacy and contributing to tumor resistance. Wang et al find that EVs secreted by RCC promote tumor cell resistance through the mTOR-ERK-STAT-NF-κB signaling pathway.[56] Since 2005, tyrosine kinase inhibitors (TKIs) targeting the vascular endothelial growth factor pathway have largely replaced interleukin (IL)-2 and interferon (IFN)-α in treatment regimens. Molecularly targeted drugs like sunitinib are widely used for treating metastatic or recurrent RCC.[57] However, during the 6- to 15-month treatment period, most patients develop drug resistance. Lim et al find that TKIs increase the number of EVs secreted by RCC cells in a dose-dependent manner and alter the metabolic cargo and activity of RCC-EVs;[58] In another study, his team reports that EVs transmit drug resistance information in the tumor microenvironment through the miR-31-5p/MLH1 signaling axis, thereby mediating sorafenib resistance in RCC.[59]

RCC resistance to TKIs is mediated by long non-coding RNAs (lncRNAs), and this resistance is transmitted between cells via EVs. High expression of lncRNAs is also associated with adverse reactions to sunitinib treatment in RCC patients. Qu et al find that lncARSR promotes the expression of AXL and c-MET in RCC cells by competitively binding to miR-34/miR-449, thereby enhancing sunitinib resistance;[60] In another study, Pan et al identify lncRNA IGFL2-AS1 as a driver of RCC drug resistance. IGFL2-AS1 is significantly upregulated in sunitinib-resistant RCC and is associated with poor prognosis in these patients.[61] Furthermore, bioactive lncRNAs are incorporated into EVs and disseminated to sensitive cells, propagating sunitinib resistance.

Developing new therapeutic strategies to overcome TKI resistance is of great importance. RAB27B is a key protein involved in EV secretion. Tsuruda et al find that RAB27B expression is upregulated in RCC cell lines and sunitinib-resistant strains. Knocking out RAB27B inhibits cell proliferation, migration, and invasion, suggesting that RAB27B could serve as a prognostic marker and therapeutic target for both sunitinib-sensitive and sunitinib-resistant RCC.[62] Ketoconazole (KTZ), an antifungal drug, is shown to inhibit the biogenesis and secretion of EVs. The Greenberg team finds that adding KTZ to the 786-O cell line of sunitinib-resistant RCC inhibits EVs, reduces tumor proliferation, and enhances the efficacy of sunitinib.[63] In another study by Greenberg et al, the combination therapy of tipifarnib and sunitinib, which induces resistance to EVs, is analyzed. The study finds that tipifarnib inhibits both the endosomal sorting complex required for transport (ESCRT)-dependent and ESCRT-independent pathways required for EV transport, thereby blocking the biogenesis and secretion of EVs.[64]

How Advanced Are Extracellular Vesicles as Anti-Cancer Therapeutics?

Significant progress in RCC treatment has been made over the past decade, with regimens shifting from high-dose cytokine therapy combined with surgical resection to stage-dependent approaches based on targeted therapy. For early-stage RCC, partial or radical nephrectomy is preferred, achieving a 5-year cancer-specific survival rate exceeding 94%. However, a cancer diagnosis does not necessarily imply that RCC is life-threatening. The risks associated with CT imaging, linked to RCC treatment, make surgery a "risk" related to excessive cross-sectional imaging.[65] Furthermore, up to one-third of small tumors detected after nephrectomy are benign. The vast majority of patients with localized renal masses undergo treatment without histological confirmation, leading to adverse consequences, such as the widespread use of robotic surgery for SRMs and limited adoption of active surveillance.[66] For patients with metastatic RCC, new treatment options include immune checkpoint inhibitors (ICIs) and TKIs,[67] However, the effectiveness of these treatments is limited by patient resistance, and curative treatment for advanced RCC remains extremely rare. Advances in nanotechnology offer a promising new direction for addressing these challenges.

EVs represent a promising research direction in nanotherapy due to their unique double-membrane structure and various adhesion proteins, which facilitate uptake by recipient cells and enable them to serve as efficient drug carriers. This capability allows for the targeted delivery of therapeutic agents to specific cells or tissues, opening new avenues for cancer therapy. The active molecules loaded into EVs are crucial for their clinical applications, and the loading of specific cargo into EVs or onto their surface is essential for achieving therapeutic effects. This process, known as EV modification or engineering, enables the precise delivery of specific molecules to recipient cells.[68] Adem et al designed GE11+EVs, capable of carrying RNA-based drugs targeting dysregulated genes in RCC, as a novel delivery system for RCC treatment.[69] Shu et al find that nanomedicines composed of circSPIRE1 plasmids inhibit metastasis formation and may serve as predictors of metastasis and potential therapeutic targets for metastatic RCC.[70]

Natural EV-derived miRNAs hold significant potential as therapeutic targets for RCC, providing RCC patients with more effective personalized treatment options. Alves et al evaluate the biomarkers and therapeutic potential of phosphatase and tensin homolog (PTEN)-regulated miRNAs in two-dimensional and three-dimensional ccRCC models. Co-inhibition of these miRNAs significantly increases PTEN expression, reduces tumor cell proliferation and migration in two-dimensional models, and decreases the size and metabolic capacity of spheroid cells in three-dimensional models.[71] In several studies, Jiang et al confirm that EV-derived miR-1 significantly inhibits RCC cell proliferation, migration, and invasion.[72] Li et al find that miR-182 contained in mesenchymal stem cell-derived EVs promotes T cell immune responses by suppressing the expression of vascular endothelial growth factor A, thereby alleviating the progression of ccRCC.[73] Brossa et al's research demonstrates that EVs secreted by bone marrow mesenchymal stromal cells (MSCs) and mesenchymal-derived human liver stem cells (HLSCs) exert a dual regulatory effect on renal CSCs. HLSC-EVs deliver the miR-145/miR-200 family to tumor cells, inhibiting the malignant phenotype of CSCs by regulating these miRNAs, thus providing experimental evidence for the clinical application of EV therapy.[74] Other forms of non-coding RNA, such as lncRNA, are also significant compared to miRNA. Shen et al find that EVs from ccRCC promote ccRCC progression through the transmission of AP000439.2 via the STAT3 pathway, indicating that AP000439.2 serves as a potential therapeutic target for ccRCC.[75]

Although EVs exhibit diverse biological functions, they encounter several challenges, with low yield being a key issue limiting their clinical application. Additionally, precise and efficient targeting of EVs to specific tissues or cell types remains a significant challenge. Surface modification using ligands, peptides, or antibodies shows promise; however, these methods often face issues such as off-target effects, limited binding affinity, and potential immunogenicity of added targeting groups. Addressing these critical issues is essential for researchers to facilitate the translation of EVs from the laboratory to clinical applications.

What Are The Technological Innovations and Clinical Translation Challenges Associated With Extracellular Vesicles?

Given their multiple functions and clinical translational potential, obtaining high yields of high-quality EVs is of great significance. Currently, the characterization of EV biological activity primarily relies on various separation methods. Depending on size and affinity, different separation strategies are employed to isolate EVs from biological fluids or cell culture supernatants. However, significant challenges exist in maintaining biological activity while achieving large-scale production for clinical applications. Traditional separation techniques often struggle to enrich biologically active EVs on a large scale. In recent decades, an increasing number of EV separation techniques have been explored. Ultracentrifugation (UC) is the most commonly used method, widely applied due to its suitability for various biological fluids, and is considered the gold standard for EV separation. However, repeated UC can reduce EV yield, and the centrifugation process may damage EV membranes, which does not align with clinical application requirements.[76] Iliuk et al developed a rapid EV separation method based on chemical affinity, known as EVtrap, which identifies 2238 EV proteins from just 5 μL of plasma, thereby improving the capture efficiency over UC.[77] Himbert et al optimize an ultracentrifugation protocol that directly isolates high-purity, high-concentration EVs from human tissue samples.[78] Density gradient centrifugation, an improved UC technique, offers higher purity and separation efficiency than UC; however, it still presents challenges such as complex operation and high equipment costs.[79]

Size exclusion chromatography (SEC) is a widely recognized method for EV separation. Compared to UC, SEC preserves the physical structure and biological function of EVs more effectively and efficiently separates EVs from soluble impurities. This approach reduces sample damage and achieves a relatively high yield.[80] Ultrafiltration (UF) is a separation technology based on the size of EVs. It is easy to operate and does not require specialized equipment. Compared to UC, UF increases the yield and separation efficiency of EVs in a shorter processing time. Although UF has potential for large-scale enrichment, challenges such as membrane clogging and protein contamination can decrease enrichment efficiency and purity.[81] The Zieren team develops an EV separation protocol optimized for renal tumors and normal kidney tissue, significantly reducing impurity contamination and increasing particle yield.[82] Immunoaffinity capture technology primarily relies on membrane surface protein markers on EVs, such as CD9 and CD63. Various immunoaffinity capture methods are currently being developed.[83] The basic principle of label-based microfluidic technology is similar to that of immunoaffinity capture technology. Capture molecules, such as antibodies and aptamers, specifically bind to corresponding lipid components or proteins on the surface of EVs based on their chemical or physical properties.[84] For example, Kawakami et al developed a sandwich ELISA assay for PSMA-EVs using highly purified EVs isolated from serum with Tim4. This assay is applicable not only for the diagnosis and monitoring of prostate cancer (PC) but also for RCC and other potentially highly vascular solid tumors.[36]

Although EVs hold remarkable application potential, their heterogeneous physicochemical properties are not yet fully understood, which may indirectly influence the selection of different separation technologies. Numerous indicators, such as yield, purity, and separation efficiency, require measurement, complicating the determination of the optimal method.[85] Furthermore, validated screening protocols for EV biomarkers are currently lacking. Campi et al summarize the latest evidence on novel EV biomarkers and find that no biomarker or imaging modality has been validated or proven to have significant clinical value for RCC. Most studies are limited by retrospective design, small sample sizes, and a lack of external validation. EV biomarkers are not yet ready for clinical application, and further well-designed studies are needed to validate preliminary findings and explore their utility in clinical decision-making.[86]

The Future Prospective

Liquid biomarker detection methods for diagnosing RCC require large-scale clinical evaluation involving numerous RCC patients and healthy individuals. ccRCC is the most commonly diagnosed RCC subtype, yet research on using EVs to distinguish between different RCC subtypes remains insufficient, particularly in the SRM patient subgroup. To date, research on RCC-EVs is relatively limited compared to CTCs and cfDNA. However, EVs offer the highest sensitivity in detection, with specificity comparable to cfDNA detection methods. Unlike tissue aspiration biopsy, which only provides limited observation of a single tumor region at a specific time point, EVs overcome this limitation. Present in all biological fluids, EVs can be obtained from various bodily fluids, including urine, serum, and plasma, making them highly valuable for large-scale cancer screening. Furthermore, due to their capacity to carry various functional molecules, including proteins, RNA, DNA, and lipids, EVs have a wide range of technical applications. Even in patients with low tumor burden, EVs remain abundant in blood and urine.

EVs activate signaling pathways by mediating intercellular communication, promoting cancer cell proliferation, and participating in key metastatic processes such as epithelial–mesenchymal transition and tumor angiogenesis. They also enhance tumor resistance through various mechanisms, aiding cancer cells in evading immune surveillance and forming an immune-tolerant microenvironment. These functions position EVs as a critical foundation for developing new cancer diagnosis and treatment strategies. Additionally, loading specific drugs into extracellular vesicles (EVs) may enhance their specific functions. Endogenous modification allows nucleic acid or protein drugs to be loaded into EVs more cost-effectively and efficiently, whereas chemical drugs often require exogenous modification. EVs serve as natural targeted drug carriers, enabling precise targeting of tumor cells while maximizing protection of normal tissues. Based on existing research, EVs not only complement the current staging system for urological cancers but also demonstrate significant potential in predicting tumor recurrence and metastasis, assessing treatment responses, and providing critical information for clinical personalized treatment decisions.

EVs hold significant potential in supplementing RCC diagnosis and identifying RCC subtypes, with their role as liquid biomarkers in RCC assessment remaining promising. However, the urgent task at present is to standardize EV separation and detection technologies before these biomarkers can be applied clinically. Future efforts should focus on improving and developing simple, efficient, low-cost, and highly reproducible separation technologies. It is also essential to standardize separation strategies to ensure the accuracy of downstream experimental results and enhance the comparability of research findings. Secondly, artificial intelligence is expected to integrate multi-omics EV biomarkers, such as proteins and miRNAs, to construct more accurate predictive models that enhance the classification of RCC subtypes, assess recurrence risk, and predict treatment response. Finally, large-scale, multicenter, prospective clinical trials are essential for validating EV biomarkers for clinical application. These trials should include patients with various risk stratifications and RCC subtypes, with particular focus on the SRM patient population, and comprehensively compare the diagnostic efficacy of EVs with CTC, cfDNA, and other imaging or pathological indicators. Through technical optimization, bioinformatics integration, and rigorous clinical validation, EVs hold promise as a key component in advancing personalized medicine for RCC.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

We have no conflicts of interest to disclose.

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