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Exosomes in Renal Cell Cancer: Diagnostic and Therapeutic Nanovehicles

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Abstract

Early diagnosis is crucial for enhancing the survival rate of renal cell cancer patients, and exosomes present potential advantages in this area. Their small size, high mobility, and lipid bilayer structure enable exosomes to cross biological membranes easily, protecting the bioactive cargo within from degradation. Exosomes significantly influence the invasion and metastasis of RCC, and they also contribute to tumor drug resistance and immune evasion.

Keywords

Exosomes, renal cancer, tumor microenvironment, extracellular vesicles, biomarker

Abbreviations

ANGPT1, angiopoietin-1; CAFs, cancer-associatedfibroblasts; DC-TEX, dendritic cell vaccineloaded with tumour exosomes; EFNA3, ephrin-A3; EVs, extracellular vesicles; FGF2, fibroblast growthfactor-2; GGT, gamma-glutamyl transferase; lncRNAs, long non-coding RNAs; miRNAs, microRNAs; *MLH1*, *human mutL homolog 1*; MMP, metalloproteinases; mRNAs, messenger RNAs; PD-L1, programmed cell deathprotein 1; RCC, renal cell cancer; THP, Tamm-Horsfall protein; TKIs, tyrosine kinaseinhibitors; TME, tumourmicroenvironment; uEVs, urinary extracellularvesicles; VEGF, vascular endothelial growthfactor; *VHL*, *Von Hippel-Lindau*

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Renal cell cancer (RCC) is the third most common cancer of the urinary system and is among the most commonly diagnosed and deadly cancers worldwide. Approximately 40% of RCC patients experience tumor recurrence after surgical resection. Patients with metastatic RCC or those who relapse after local therapy require systemic treatment. Current systemic therapies include small-molecule tyrosine kinase inhibitors (TKIs), cytokines, and monoclonal antibodies, including checkpoint inhibitors, used as first-line and second-line treatment options.[1] Drug resistance in RCC remains a significant challenge, contributing to high mortality.[2] There is a pressing need for specific biomarkers and predictive models for metastatic and advanced RCC to improve diagnostic and personalized treatment options.

Extracellular vesicles (EVs) in the tumor microenvironment (TME) are increasingly recognized as critical players in carcinogenesis, angiogenesis, premetastatic niche formation, immune system dysfunction, and the spread of drug resistance, thereby adding complexity to the TME. EVs include a

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heterogeneous group of vesicles released by cells, classified into exosomes, microvesicles, and apoptotic bodies. Exosomes originate from the inward budding of multivesicular bodies and release as small vesicles of 30 to 150 nm in diameter loaded with intracellular cargo, including proteins, messenger RNAs (mRNAs), microRNAs (miRNAs), and signaling molecules.[3] As research on tumor-derived exosomes in clear cell RCC progresses, their potential as a valuable source of molecular clues for diagnosing RCC becomes increasingly evident. Along with circulating neoplastic cells and DNA fragments in fluids, exosomes of liquid biopsies present a wealth of information about the molecular makeup of tumors. Their bilayer membrane protects the cargo from breakdown by RNase and protease, making exosomes highly reliable and instructive biomarker nano-carriers. Importantly, exosomes can encapsulate and transfer significant amounts of mRNA to recipient cells, influencing their functions and phenotype.[3] Grange *et al*[4] conducted a careful analysis of microvesicles, identifying specific mRNAs and proteins associated with tumor progression and metastasis, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF2), angiopoietin-1 (ANGPT1), ephrin-A3 (EFNA3), and metalloproteinases (MMP)-2 and MMP-9. Exosomal miRNAs exhibit distinct expression patterns between RCC patients and healthy individuals. In CD105 + microvesicles, for instance, 24 miRNAs, including miR-200c and miR-650, were upregulated, while 33 miRNAs, including miR-100 and miR-29, were downregulated, with miR-29a, miR-650, and miR-151 promoting tumor invasion and metastasis.

A report by Zhang *et al*[5] showed that serum samples from clear cell RCC patients had elevated exosomal miR-210 and miR-1233 compared to healthy controls, with their levels decreasing after nephrectomy. The authors concluded that exosomal miR-210 and miR-1233 could be valuable indicators for low-invasive diagnosing and monitoring clear cell RCC patients. Serum exosomes of advanced RCC patients with distant metastases and microvascular invasion also exhibit increased gamma-glutamyltransferase (GGT) activity. Exosomal miR-21-5p from M2 macrophages is associated with RCC pro-metastatic effects by activating the PTEN/Akt pathway, with its inhibition in M2 exosomes lowering the spreading potential of RCC cells. Conversely, a study has shown that administering mesenchymal stem cell-derived exosomes can reduce metastatic spread and tumor growth in an orthotopic mouse model of clear cell RCC. These effects were attributed to exosomal miR-182 of stem cells, which enhanced T-cell-mediated immune response and decreased VEGF-A expression, inhibiting overall tumor progression.[6] Recent data indicate that the enrichment of cancer-associated fibroblasts (CAFs) is positively associated with cancer stemness and RCC progression. In a study, exosomal miR-181d-5p delivered by CAFs directly targeted RNF43, activateed Wnt/β-catenin signaling, enhanced cancer stemness, and promoted RCC progression.[7] These findings suggest a novel and promising strategy for RCC treatment based on CAF-derived exosomal miRNAs. Last but not least, increasing evidence suggests that exosomes can contain a wide variety of long non-coding RNAs (lncRNAs), which play crucial roles in cancer cell growth, proliferation, invasion, and metastasis.[8] Critically, exosome-mediated transfer of lncARSR enhances the expression of AXL and c-MET in RCC cells by competitively binding to miR-34/miR-449, thereby contributing to developing resistance to sunitinib.

Urinary extracellular vesicles (uEVs) have been extensively studied because they reflect kidney pathology.[3] Tamm-Horsfall protein (THP), abundant in urine, can trap uEVs. Research indicates that urinary miRNAs provide a stronger signature for acute kidney injury due to oxalic acid poisoning compared to serum miRNAs. uEVs are similar to those from various immortalized human kidney cell lines, such as podocytes, glomerular endothelial cells, mesangial cells, and proximal tubular cells. This similarity suggests that *in vitro* experiments can mimic *in vivo* conditions. Exosomal miRNAs from urine can distinguish RCC patients from healthy individuals. In particular, miR-126-3p levels in uEVs are significantly lower in clear cell RCC patients compared to healthy controls. The reduced expression of miR-126, influenced by lncRNA DUXAP8, is strongly associated with poor survival rates and metastatic RCC. Notably, the combination of urinary miR-126-3p and miR-449a effectively distinguishes clear cell RCC from healthy individuals. After nephrectomy, the levels of these miRNAs return to levels comparable to those in healthy samples. miR-224-5p is significantly upregulated in both uEVs and tissue from RCC patients compared to healthy controls. This miRNA stabilizes the expression of programmed cell death protein 1 (PD-L1) by directly suppressing the gene encoding cyclin D1 (CCND1). Small RNA sequencing of uEVs from clear cell RCC patients shows significantly lower levels of miR-30c-5p compared to healthy individuals. miR-30c-5p is a specific biomarker for RCC due to its distinct expression in RCC patients versus healthy controls, but it does not differentiate between bladder and prostate cancer. Studies have demonstrated that the phosphorylation of proteins derived from uEVs can classify the grade differences in RCC. A panel of uEV-derived proteins, including CAIX, CP, MMP9, PODXL, DKK4, CD10, DPEP1, EMMPRIN, Syntenin 1, and AQP1, are potential biomarkers for the early stages of clear cell RCC.

The inactivation of the *Von Hippel-Lindau* (*VHL*) gene in clear cell RCC is known to enhance the activity of several receptor tyrosine kinases, driving angiogenesis and shaping TME homeostasis. This mechanism is the primary target of TKIs approved as a first-line treatment for RCC.[1] However, the development of drug resistance presents an existential challenge for patients with advanced RCC. The kidneys play a unique role in the expulsion and reabsorption of drugs from the circulatory system, aided by an array of transporters and carriers of the proximal tubules. Therefore, failure in renal transporters can significantly impact the pharmacokinetics and disposition of drugs and their metabolites. lncARSR, for instance, contributes to the induction of resistance to the multi-targeted TKI sunitinib in initially sensitive RCC cells, a process

facilitated by exosomes. By competitively binding to miR-34/miR-449, lncARSR increases the expression of AXL and c-MET and activates the STAT3, AKT, and ERK signaling pathways. The activation of AKT further enhances the lncARSR expression by inhibiting the transcription factors FOXO1 and FOXO3a, setting off a positive feedback loop. In a similar vein, Rab27b, an essential protein in exosome secretion, contributes to RCC drug resistance through the MAPK and VEGF signaling pathways. This mechanism is independent of the role of this important GTPase in biogenesis and release of exosomes. Mechanistic analysis of therapy-resistance of RCC showed that cell-derived exosomes could transfer drug resistance from sorafenib-resistant clear cells to non-resistant RCC cells by delivering miR-31-5p, which targets the 3'-UTR region of the *human mutL homolog 1* (*MLH1*) gene. Xuan *et al*[9] found that TKI-resistant RCC cells and their exosomes had lower expression of miR-549a than TKI-sensitive cells. Delivering miR-549a to TKI-resistant renal cancer cells, therefore, is anticipated to reverse the resistance of RCC cells to TKIs. Several compounds, including ketoconazole and tipifarnib, have been explored for their potential to inhibit exosome biogenesis and prevent the transformation of chemotherapy-susceptible cells into resistant variants. Ketoconazole, for instance, has demonstrated the ability to decrease tumor-specific exosomes by inhibiting the expression of Alix, nSMase, and Rab27a proteins. Such an effect reduces the delivery of exosome substances, with favorable effects on sunitinib activity.[2]

Understanding the mechanisms underlying drug resistance mediated by EVs is crucial, as it may aid in identifying more effective therapies along with novel prognostic biomarkers. Several groups have explored the mechanism of exosome-based immunotherapy in oncology and its potential therapeutic use in cancer therapy, particularly emphasizing the potential for developing immunotherapeutic vaccines. A report by Zhang *et al*[10] demonstrated that exosomes derived from RCC cells under IL-12 stimulation induced the generation of cytotoxic T lymphocytes directed against RCC antigens, resulting in enhanced anti-tumor effects. Using a similar approach, the vaccine EXO-IL-12 was produced recently, tailored to facilitate the expression of kidney cancer-specific antigen G250, alongside immune-associated protein and GPIIL-12. This vaccine notably boosted the proliferation and activation of T lymphocytes *in vitro*. Finally, mice with RCC, immunized with a dendritic cell vaccine loaded with tumor exosomes (DC-TEX), showed prolonged survival compared to those immunized with a dendritic cell vaccine loaded with tumor cell lysates.[11]

From the brief critical overview and discussions, it emerges that in addition to their familiar and bright side as a biomarker and therapeutic nano-carries, exosomes pose a dark side, contributing to the pathobiology of RCC, promoting carcinogenesis, metastatic spread and resistance to therapy. Capitalizing on advantages and minimizing unfavorable effects merits further research in exosome biology to address emerging challenges and outstanding areas. Practically, urine typically contains epithelial cells, blood cells, bacteria, and viruses. Reducing microbial content requires careful attention to the entire uEV workflow. Additionally, an optimal workflow must address the presence of bacterial outer membrane vesicles in urine, which can originate from normal or pathogenic urinary tract microbiota. Another factor that may influence the EV secretion rate is urinary flow; kidney tubule cells have cilia that can be activated by flow and play a significant role in EV secretion. However, the *in vivo* implications of this mechanism have not been studied. The current strategies for cargo loading face limitations related to efficiency, cost, potential exosome damage, and the versatility of the loaded cargo. Many attempts have been made to load various therapeutic materials and naturally occurring substances into exosomes to enhance their therapeutic efficacy. There are two primary methods for loading drugs into exosomes—passive loading and active loading. The choice of method depends on the specific drug, its properties, and the desired release mechanism. Passive loading is simple and easy to perform but has low loading efficiency and limited control over the amount of drug loaded into the exosomes. It is generally most effective for lipophilic drugs that can easily cross cell and vesicle membranes. Active loading, which involves directly loading the drug into isolated exosomes, typically employs techniques that increase the permeability of the exosome membrane, such as sonication, heat shock, electroporation, incubation with detergents, or the use of saponins. This method achieves higher loading efficiency and allows for more precise control of the drug loading amount. However, it can be complex and time-consuming, with a risk of damaging the exosomes if not performed properly. Genetic engineering can also be used to load genetic substances into exosomes. Cells can be genetically modified to express a protein or RNA of interest, which is then incorporated into exosomes as they form. This approach allows for the loading of specific proteins or RNA molecules into exosomes but requires advanced molecular biology techniques and may not be suitable for all types of therapeutic agents. EVs exhibit diversity in size, content, morphology, and biological mechanisms, with single cells continuously producing a wide range of vesicles. This heterogeneity reflects their cellular origin and functional impact on recipient cells. Additionally, small extracellular non-EV particles, such as exomeres, can complicate the understanding of EV-related composition and functional properties. Each EV subtype may have distinct functions, necessitating alternative methods to distinguish between these subpopulations. Consequently, it is crucial to comprehend the diverse and heterogeneous nature of EVs. Given the rapid progress in research of exosomes and nanotechnologies for their optimization and use for cellular interrogation, biomarker research and delivery of therapeutics, we anticipate rapid progress towards harvesting their enormous potential to tackle the immediate and long-term needs of the field of renal cancers and beyond.

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