REVIEW

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Extracellular vesicles in renal physiology and clinical applications for renal disease

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Many cells in the nephron release extracellular vesicles (EVs). EVs envelop nucleic acids, proteins, and lipids. The surfaces of EVs express donor cell-specific markers, ligands, and major histocompatibility complex molecules. They are involved in cell-to-cell communication, immune modulation, and the removal of unwanted materials from cells. EVs have been studied as biomarkers of specific diseases and have potential therapeutic applications. Recent research has emphasized the functions of EVs in the kidney. This review provides an overview of recent findings related to the roles of EVs in the nephron, and their utility as biomarkers and therapeutic factors in renal disease.

Keywords: Extracellular vesicles; Cells; Communication; Kidney

INTRODUCTION

Extracellular vesicles (EVs) refer to all membrane-bound vesicles released from cells into the extracellular space [1,2] and include exosomes, microvesicles, microparticles, ectosomes, and oncosomes [3]. The general term EVs is used in this review due to the lack of methods to specifically identify vesicles. EVs were discovered over 30 years ago [4]. Their physiological role was not well understood at first. In the last two decades, the roles of cell-derived EVs in cell-to-cell communication after intercellular contact, and in the transfer of secreted molecules, have been identified [5,6]. EVs are released from almost all cell types, including mammalian, prokaryotic, and plant cells. Additionally, EVs can be purified from all types of biological fluids (e.g., serum, urine, breast milk, cerebrospinal fluid, malignant ascites, bronchoalveolar lavage fluid, and saliva) [7]. EV biogenesis and release from cells is controlled by precise mechanisms [8,9]. In this review, the roles of EVs in the nephron, their utility as biomarkers for kidney diseases, and their therapeutic potential are discussed.

Why has recent research focused on EVs?

The number of EV studies has increased significantly, with 361, 1,228, and 4,058 published articles found in PubMed in 2000, 2010, and 2018, respectively. EVs are potential biomarkers or therapeutic tools for several diseases. They reflect the conditions of source cells, which contributes to their utility as disease biomarkers

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[10]. EVs are involved in normal physiological processes and the pathogenesis of diseases, and contain a broad and heterogeneous range of molecules [11,12]. Research indicates that cells modulate the contents of EVs in response to extracellular stress, including infection, hypoxia, oxidative stress, and other cellular stresses that alter the composition of EVs [13-16]. Changes in EV contents affect neighboring cells and alter their phenotypes, affecting disease and repair status [17-20]. The enveloped membrane protects materials from enzymatic degradation, making EVs stable carriers of enclosed materials [21]. EVs are stable over long periods of time at room temperature and after thawing from frozen [22]. The selective transfer of cell cargo is now recognized as an essential pathway for intercellular communication in both healthy and disease states. These characteristics make EVs promising disease biomarkers [23]. EVs can be detected by non-invasive liquid biopsy techniques. A biopsy is a sample of tissues or cells obtained from almost any part of the body that is used to check for markers of disease, including cancer, autoimmune diseases, and hormonal diseases. Renal disease is detected using blood or urine samples. It may eventually be possible to use small sample volumes to screen for disease and monitor disease activity in clinical settings.

Methods have been developed to modulate EV biogenesis and release, emphasizing the usefulness of EVs as a therapeutic platform [24]. Recent studies have successfully altered the contents of EVs [25]. Techniques for the precise control of EVs will facilitate their therapeutic application.

THE ROLE OF EVs IN THE NEPHRON

The role of the EV pathway in selective transfer of cell cargo is increasingly recognized as an essential process for intercellular communication [23]. Cells in the nephron constitutively release EVs under healthy conditions. EVs contain nearly identical cell surface proteins to those of their cells of origin, and can fuse to target recipient cells [7]. When EVs are taken up by recipient cells, they transfer a variety of biological molecules [26]. These materials prompt a cellular response in recipient cells. The number and content of EVs changes according to disease status [7,27-29]. A change in disease state may yield different responses in neighboring and distant cells in the body.

From proximal cells to distal cells

Studies of the biological role of EVs in intercellular communication in the nephron indicate that they may act as messengers. EVs from parent cells specifically interact with recipient cells in the nephron [30]. Recipient cells take up EVs from donor cells using cilia (Fig. 1B) [30]. Electron microscopy analyses have shown that EVs adhere to cilia and emerge from an intracellular vesicle near the base of the cilia *in vivo*. The uptake of EVs may be concentration-dependent [31]. Cellular stress leads to an increase in the release of EVs from cells. Podocytes are highly specialized, terminally differentiated epithelial cells, and are key sites of injury in a variety of renal diseases. Stress induces podocyte apoptosis and triggers other types of cell injury [32,33]. High glucose levels induce podocytes to generate more EVs, which are released into urine (Fig. 1A) [34]. These EVs are taken up by tubular epithelial cells and promote tubular fibrotic changes via p38 phosphorylation (Fig. 1C) [35]. This podocyte-tubular cross-talk contributes to the development of tubulointerstitial fibrosis following podocyte injury and to a decline in renal function in glomerular disease. The proximal to distal signaling pathway in the nephron has been described previously [36]. Wu et al. [36]. demonstrated that EVs from endothelial cells exposed to high glucose levels cause podocyte dysfunction. EVs shuttle from proximal to distal cells in the tubule. Proximal tubular epithelial cells affect distal tubular epithelial cells via their EVs. EV glycealdehyde-3-phosphate dehydrogenase (GAPDH) released by proximal epithelial cells regulates the epithelial sodium channel (ENaC) in recipient distal cells and collecting duct cells (Fig. 1G) [37]. This indicates that proximal cells contribute to the adjustment of sodium reabsorption in the distal tubule and collecting duct via EVs. Additionally, EVs transfer aquaporin-2 between cells, which may be stimulated by physiological signals (Fig. 1H) [38]. Water reabsorption may also be regulated by intercellular communication via EVs. EVs from epithelial cells stimulated with a dopamine receptor agonist reduce reactive oxygen species (ROS) in distal tubule cells [31]; the mechanism underlying this decrease in ROS in recipient cells is unclear. The

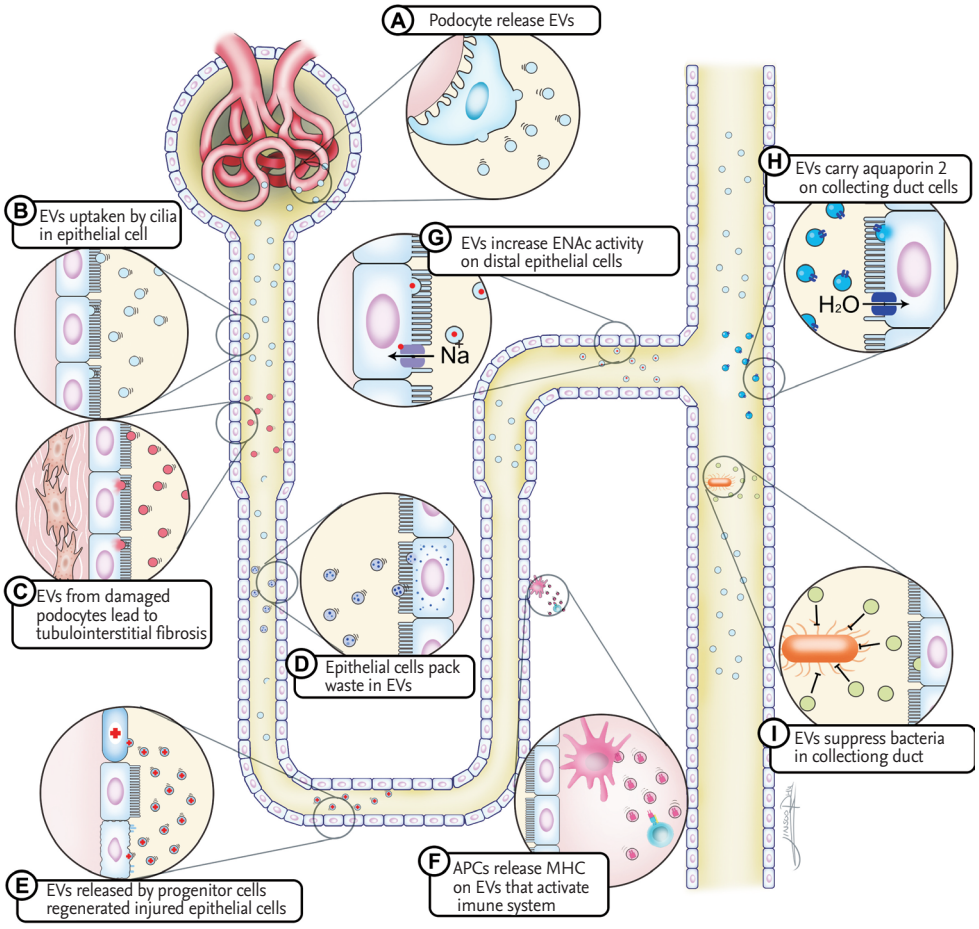

Figure 1. Extracellular vesicles (EVs) participate in cellular communication. Cells in the nephron release EVs under normal or stress conditions. EVs can amplify or limit renal damage, and may carry waste or aquaporin. APC, antigren presenting cell; MHC, major histocompatibility complex.

role and pathophysiology of nephron cell EVs require further investigation.

Renal regeneration capacity of EVs

Damage to tubular epithelial cells characterizes several kidney injuries. Tubular epithelial cell regeneration may involve paracrine, autocrine, or endocrine activity in reparative cells [39]. EVs play a role in kidney regeneration, mediating interactions between epithelial cells and stem cells via cell transition [40]. Scattered renal tubular cells undergo proliferation after injury, contributing to renal recovery (Fig. 1E) [39]. These cells confer protective effects in the ischemic kidney via the release of EVs [41]. This process can involve small RNAs and mitochondrial transfer between cells via EVs [41,42].

Maintenance of cellular homeostasis

Aging induces the accumulation of damaged organelles and protein aggregation. The kidney is particularly susceptible to age-related renal damage, such as glomerulosclerosis [43]. Higher eukaryotic cells are equipped with self-defense mechanisms to maintain cellular homeostasis. One of the most important functions of EV release is the removal of waste from cells. EV secretion by reticulocytes has been identified as a mechanism underlying the eradication of molecules [44]. EVs preserve cellular homeostasis by excreting harmful materials from cells (Fig. 1D). EV secretion from cells eliminates misfolded and prion proteins [45], as well as harmful chromosomal DNA fragments [46]. The inhibition of EV secretion results in cytoplasmic accumulation of

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nuclear DNA, which induces elevated intracellular levels of ROS. EV secretion also prevents aberrant innate immune responses. Autophagy is used by all cell types to recycle nutrients, remove unwanted or damaged intracellular constituents, and as a response to starvation. The selective removal and secretion of harmful proteins, by EVs or by the autophagy-lysosomal pathway, are coordinated processes involved in protein homeostasis and the maintenance of cellular fitness [47].

Immune response to urinary tract infection

The anatomy of the urinary tract results in its continuous exposure to large numbers of bacteria. However, the urinary tract is generally sterile above the urethral meatus, indicating that an effective system maintains urine sterility by antibacterial activity. Hiemstra et al. demonstrated that EVs from the urinary tract are significantly enriched for innate immune proteins, including antimicrobial proteins and peptides, as well as bacterial and viral receptors [48]. Urinary EVs inhibit the growth of pathogenic *Escherichia coli,* the primary cause of urinary tract infections (UTIs) (Fig. 1I). This indicates that EVs in the urinary tract are innate immune effectors that contribute to host defense, which is consistent with other results indicating that EVs in the respiratory tract are associated with innate defense [49]. Tissue factor, the primary initiator of coagulation *in vivo*, is thought to play an important role in sepsis caused by UTI [50]. EV-associated tissue factor activity is related to disease severity and bacteremia in patients with febrile UTI caused by *E. coli* [51]. Tissue factor in EVs may prevent bacteria in the urinary tract from spreading beyond the uroepithelial barrier.

EVs as carriers of native antigens

Activated dendritic cells (DCs) release EVs with enriched major histocompatibility complex T-cell co-stimulatory molecules and adhesion molecules on their surface (Fig. 1F) [52]. High concentrations of antigen-presenting cell-derived EVs can function as antigen-presenting vesicles for T-cell clones and primed T-cells [52,53]. EVs from activated donor DCs promote the activation of recipient DCs [54]. Additionally, EVs have been shown to induce auto-antibodies and provoke antibody-mediated rejection [55]. Suppressing the release of EVs in graft DC may prevent rejection in kidney transplantation.

EVs AS KIDNEY DISEASE BIOMARKERS

The majority of studies of exosomes in kidney disease have focused on biomarker discovery. The association of EVs with disease indicates that they may be candidate diagnostic or prognostic biomarkers.

Urine contains EVs from kidney cells

Urinary EVs are secreted by almost all kidney cell types, including glomerular epithelial cells, podocytes, proximal/distal epithelial cells, and collecting duct cells [56]. Under physiological conditions, blood EVs cannot pass through the glomerular basement membrane [57]. Because circulating (blood) EVs can be eliminated by the kidney in the acute phase, EVs may also originate from systemic circulation, although they do not account for the majority of urinary EVs [58]. Therefore, urine EVs are generally derived from kidney cells or the urinary tract. It is possible to noninvasively collect samples from patients and obtain critical information related to diagnosis, prognosis, and treatment response. Table 1 summarizes human studies of EV biomarkers in renal disease [10,27-29,59-76].

Isolation and characterization of EVs

Ultracentrifugation is a conventional technique for EV isolation from biological fluids. This method is not suitable for clinical research due to its low yield. Commercial kits have been developed to improve EV yield and purity. Validated plasma and serum EV isolation kits for microRNA profiling are available [77]. Improved urinary EV isolation strategies have also been developed [78]. The EV isolation kits minimize the labor, time, and clinical sample volume required. We have successfully analyzed EVs using these commercial kits [28,29,41,79]. Each EV isolation method has advantages and limitations; these should be considered prior to their practical application. Isolation methods for EVs have been reviewed elsewhere [80,81].

Sorting of EV subpopulations

There is increasing evidence that the functional transfer of EV contents is highly selective and infrequent [82,83]. These findings indicate the existence of EV subpopulations with unique characteristics. Studies of specific EVs are required to increase our understating

Table 1. Extracellular vesicular biomarkers in renal disease

Disease	Source/method	Biomarker	Reference
Diabetic kidney disease	Human urine/microarray	Let-7i-3p, miR-24-3p, miR-27b-3p, miR-15b-5p	[59]
	Human urine/microarray	miR-320c, miR-6068	[60]
	Human urine/proteomic analysis	EV density	[61]
	Human urine/Western blotting	WT-1	[62]
IgAN and TBM	Human urine/proteomic analysis	Aminopeptidase N, vasorin precursor, α-1 antitrypsin, ceruloplasmin	[63] [64]
IgAN	Human urine/qRT-PCR	*CCL2* mRNA	
FSGS	Human or mouse urine/immune blot	WT-1	[65]
	Human urine/qRT-PCR	miR-193a	[66]
Lupus nephritis	Human urine/qRT-PCR	miR-26a	[27]
ADPKD	Human urine/proteomics	TMEM2	[67]
	Human urine/proteomics	Apolipoprotein A1, actin	[68]
Hypertension	Human urine/flow cytometry	Podocyte EV number	[29]
	Human urine/qRT-PCR	miR-21, miR-92a, miR-93, miR200b	[28]
Acute kidney injury	Human or rat urine/Western blot	ATF3	[69]
	Human or rat urine/proteomics	Fetuin-A	[10]
Kidney transplantation	Human urine/Western blotting	NGAL	[70]
	Human plasma/qRT-PCR	Gp130, CCL4, TNFα, SH2D1B	[71]
	Human urine/magnetic bead	CD3-positive exosome	[72]
	Human urine/qRT-PCR	Bkv-miR-B1-5p, bkv-miR-B1-5p/miR-16	[73]
Renal carcinoma	Human plasma/qPT-PCR	lncARSR	[74]
	Human urine/microarray	GSTA1, CEBPA, PCBD1	[75]
	Human urine/proteomics	MMP-9, PODXL, DKK4	[76]

EV, extracellular vesicle; WT-1, Wilms tumor-1; IgAN, immunoglobulin A nephropathy; TBM, thin basement membrane; qRT-PCR, quantitative real-time reverse transcriptase poly chain reaction; CCL2, chemokine ligand 2; FSGS, focal segmental glomerular sclerosis; ADPKD, autosomal dominant polycystic kidney disease; TMEM2, transmembrane protein 2; ATF3, activating transcription factor 3; NGAL, neutrophil gelatinase-associated lipocalin; Gp130, glycoprotein 130; TNFα, tumor necrosis factor α; SH2D1B, SH2 domain containing 1B; lncARSR, long non-coding RNA activated in RCC with sunitinib resistance; GSTA1, glutathione S-transferase alpha; CEBPA, CCAAT/enhancer-binding protein alpha; PCBD1; pterin-4 alpha-carbinolamine dehydratase; MMP-9, matrix metalloproteinase 9; PODXL, podocalyxin; DKK4, Dickkopf-related protein.

of their functions. Magneto-immunocapture methods could be utilized to obtain parent cell-specific EVs from pre-enriched EVs [84,85].

THERAPEUTIC APPLICATION OF EVs

The direct delivery of therapeutic materials, such as drugs, small molecules, and nucleic acids, to target sites would effectively minimize side effects and increase efficacy. Few synthetic platforms, including polymeric nanoparticles and liposomes, have been approved by the U.S. Food and Drug Administration [86]. Recent studies have focused on enhancing biological materials, rather than developing synthetic biological carriers; EVs have gained particular attention as a therapeutic tool [25]. The EV-mediated transfer of exogenous nucleic acids was first reported in 2010 [87]. EVs have many potential therapeutic applications for renal disease, e.g., to correct metabolic deficiency, promote kidney regeneration, and modulate kidney transplant rejection. EVs can be used to carry exogenous RNA or proteins to kidney cells *in vivo*. It is possible to increase the efficacy of EVs by modulating their contents or their cell or organ specificity. Favorable therapeutic application characteristics include very small size, high permeability, low immunogenicity, and low risk of tumor changes.

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EVs contribute to nephron repair

EVs from stem cells, and EVs engineered with loaded materials, could contribute to nephron repair. Mesenchymal stem cells (MSCs), endothelial progenitor cells, tubular scattered cells, antigen-presenting cells, and natural killer cells secrete EVs that induce nephron regeneration or inhibit the apoptosis of tubular epithelial cells [88]. Grange et al. [89] demonstrated that labeled MSC-derived EVs target the inured kidney after intravenous injection. In human studies, MSC-derived EVs improved the glomerular filtration rate and decreased albumin excretion in patients with stage 3 or 4 chronic kidney disease [90].

The safety of MSC-based therapy requires further investigation, as MSC therapy could exacerbate preexisting kidney damage in humans [91]. The therapeutic potential of EVs is limited by their low yield from cultured cells [2]. Isolating high-purity EVs remains a challenge [92]. The dosage, routes of injection, and cellular origin of EVs affect their distribution *in vivo*; these factors must be standardized for clinical trials [93]. The storability of EVs is also an important consideration. It may prove difficult to maintain the therapeutic activity of stem/progenitor cell-derived EVs *in vitro* prior to engraftment in the renal parenchyma [88]. It is not certain that cryopreserved stem/progenitor cell-derived EVs are as effective as freshly isolated stem/progenitor cell-derived EVs [94]. Finally, it is necessary to develop a tracking tool to determine the abundance of stem/progenitor cell-derived EVs following administration.

Loading of EVs with therapeutic materials

Methods for loading EVs include drug loading, for example through chemicals, proteins, or genetic materials, in purified EVs *ex vivo* [4], as well as pre-loading drugs or therapeutic factors to donor cells prior to EV purification [95].

Curcumin, doxorubicin, and paclitaxel have been successfully loaded into EVs [96]. EVs exhibit a higher loading efficiency and capacity for hydrophobic chemical drugs compared to liposomes [97]. Non-coding RNAs are attractive drug targets for treating renal disease [98]. Engineered anti-RNA oligonucleotides can prevent specific mRNAs from binding to miRNAs, thus inhibiting their function. Didiot et al. [99] developed a robust and scalable method for loading therapeutic RNA into EVs with co-incubation. Cholesterol conjugation and sonication are suitable alternatives for active loading of RNA with minimal aggregation and degradation [100,101].

Therapeutic agents can be incorporated into EVs from parent cells. Chemically treated MSCs release EVs with anti-proliferative activity against cancer cells *in vitro* [102]. MSCs engineered to overexpress miRNA-let7c were injected into mice with unilateral ureteral obstruction, thereby attenuating kidney injury [103].

CONCLUSIONS

EVs are promising biomarkers and active physiological agents with many possible therapeutic applications. Research has improved our understanding of EV characteristics but further investigation of the roles of EVs in the kidney is required. Host cell EVs can have beneficial or harmful effects on recipient cells. Despite the positive results of several EV studies, consistency has been lacking. Further research will improve our ability to modulate signaling mechanisms in the nephron and improve treatments for kidney diseases.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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