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Research Article

НИРКИ KIDNEYS

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Urinary Extracellular Vesicles as Molecular Fingerprints of Kidney Injury and Disease Progression

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ABSTRACT

Urinary extracellular vesicles (uEVs) have emerged as promising non-invasive indicators of kidney injury and disease progression because they carry proteins, nucleic acids, lipids, metabolites, and signaling molecules derived from renal and urinary tract cells. Their molecular composition can reflect pathological alterations across glomerular, tubular, inflammatory, metabolic, and fibrotic pathways, supporting their potential use as dynamic molecular fingerprints of renal disease. This review examines the biological origin, molecular cargo, disease-associated alterations, and translational relevance of uEVs across acute kidney injury, chronic kidney disease, diabetic kidney disease, glomerular disorders, rare renal diseases, and kidney transplantation. Current evidence suggests that uEV-based signatures may support early detection, disease stratification, prognostic assessment, and longitudinal monitoring of renal dysfunction. Advances in proteomics, transcriptomics, metabolomics, mass spectrometry, Raman spectroscopy, and machine learning have further improved the characterization of multidimensional uEV profiles. However, variability in urine collection, vesicle isolation, normalization, analytical platforms, and biomarker validation continues to limit clinical implementation. Standardized workflows, larger multicenter cohorts, longitudinal validation, and integration of multi-omics data with clinical and histopathological findings are therefore required. Overall, uEV molecular fingerprinting represents a promising strategy for precision nephrology and for improving non-invasive assessment of kidney injury and disease progression.

Keywords: Urinary vesicles; Kidney injury; chronic kidney disease; Molecular biomarkers; Precision nephrology

1. Introduction

Kidney disease is a large and increasing clinical problem, as renal damage can occur insidiously, and often disease may be progressing without significant evidence detectable in traditional laboratory tests. Acute kidney injury and chronic kidney disease have been linked with gradual decrease in renal function, heart and blood vessel complications, hospital stay and kidney failure. Although nephrology has progressed in many respects, the early detection of cellular damage and the ability to predict disease course are still significant challenges in the clinical setting. The standard diagnostic modalities used today are primarily serum creatinine, estimated glomerular filtration rate, albuminuria, proteinuria, imaging and rarely, kidney biopsy. These parameters are clinically useful but do not give much information concerning molecular events within particular renal cell populations. EVs are therefore gaining more and more interest as potential biological markers reflecting pathological processes in the kidney 1.

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Extracellular vesicles are vesicles whose membranes are formed by different cell types and which are released into the biological fluids, where they mediate intercellular communication by transferring proteins, lipids, nucleic acids, and other bioactive molecules. One of the most appealing sources of extracellular vesicles is the urine itself, as it can be obtained without surgery or any invasive procedures and may contain vesicles from various parts of the urinary system. An important part of urinary extracellular vesicles come from cells of the nephron that are accessible to molecular information that is not necessarily captured by circulating biomarkers. They can have different molecular composition depending on physiological conditions, aging, renal injury, and progression of disease, which is why they have the potential to be markers of dynamic changes within the kidney 2.

Diagnostic value of urinary EVs is highly dependent on source and molecular composition of EVs. Proteins, messenger RNAs, microRNAs, lipids, metabolites, and other molecules specific to the cells that are originally in the vesicles can be found in vesicles from podocytes, glomerular endothelial cells, tubular epithelial cells, and collecting duct cells. Pathological changes of these renal compartments may then be reflected in changes in the number, type and function of urinary EVs. Their presence in urine and their ability to carry biologically relevant payloads have boosted their interest as non-invasive diagnostic, prognostic and monitoring tools of kidney diseases 3.

Most importantly, urinary EVs are not only viewed as passive carriers of markers but are also believed to play an active role in renal pathophysiology. In hypoxia, oxidative stress, metabolic dysfunction, inflammation, and/or tissue injury can cause changes in the molecular composition of vesicles and/or vesicle release by renal cells. These alterations can affect adjacent cells by the transfer of signaling molecules, and can also play a role in inflammatory processes, fibrotic remodeling, vascular dysfunction, maladaptive repair, and renal function deterioration. These processes indicate that EVs could provide mechanistic insight into the relation between the structural and functional changes of the kidney in the later phase of its damage and the earlier stage of cellular damage 4.

The use of urinary EVs as a molecular fingerprint is especially applicable to the context of chronic kidney disease, as there is a lot of variability in disease etiology, severity, progression, therapeutic response, and clinical outcome. Patients with identical estimated glomerular filtration rates or comparable degree of albuminuria may have very different underlying disease processes and disease course. Monitoring molecular signatures of specific injury pathways or progressive disease states in urine may thus be possible via study of the urinary EVs. These signatures may be able to distinguish between relatively stable renal dysfunction and rapidly progressive renal disease and offer insight beyond traditional clinical markers 5.

One of the biggest benefits of urine-based molecular testing is that it could be repeated with minimal bother to the patient. Although kidney biopsy is a useful diagnostic tool as it gives a direct history, it is an invasive technique that cannot be repeated for follow-up. Cellular and extracellular components in the urine may provide additional information on the molecular changes taking place and may aid in the detection of pathological changes earlier. The analysis of urinary kidney material has been thus suggested as a potential approach to better early diagnosis and prognosis of chronic kidney disease (CKD) 6. The lipid bilayer of urinary EVs makes them particularly attractive in this context as it shields the molecular cargo from degradation, and allows for repeated characterization of changes in molecular components that occur over the course of renal injury, repair, fibrosis and progression.

This review aims to critically assess urinary EVs as a non-invasive molecular fingerprint of kidney injury and progression of disease. They include their renal origin, biological properties, molecular composition, alteration upon injury, disease specific profile, prognostic value, analytical approaches, and clinical application. The goals are to summarize existing knowledge on the relationship between urinary EVs and glomerular, tubular, inflammatory, fibrotic, and progressive renal damage; discuss their utility in early detection, disease classification, prognostic risk stratification, and longitudinal assessment; and highlight methodologic challenges, standardization needs and translation priorities that will need to be overcome before EVs can be used in routine precisions nephrology.

2. Biology, Origin, and Molecular Composition of Urinary Extracellular Vesicles

2.1 Biogenesis and Renal Origin of Urinary Extracellular Vesicles

The urinary extracellular vesicles represent a diverse and heterogeneous group of membrane-bound vesicles secreted into the urine by epithelial and non-epithelial cells of the urinary tract. The biological functions of these vesicles are closely associated with their cellular origin, for the contents of the vesicles can maintain molecular properties of the parent cell. In the kidney, the cells that can give rise to urinary extracellular vesicles include the podocytes, glomerular endothelial cells, proximal and distal tubular epithelial cells, thick ascending limb cells, and collecting duct cells. Experimental studies have suggested that cells within the kidney make a significant contribution to the urinary EV pool, and that various kidney cell types secrete EVs that have molecular profiles that are different 7.

There are multiple pathways of intracellular and plasma membrane associated processes that lead to the formation of extracellular vesicles. The endosomal system can generally be the source of small extracellular vesicles, which result from the inward budding of the endosomal membrane to form intraluminal vesicles within multivesicular bodies. These fuse with the plasma membrane and result in vesicles being secreted to the extracellular milieu. Larger vesicles, on the other hand, can result from outward budding and from direct shedding from the plasma membrane. These vesicles then fuse with recipient cells, interact with them via receptors, or internalize them, thus allowing the transfer of biologically active cargo. This intercellular communication allows extracellular vesicles to function as mediators of intercellular communication, influencing cellular function in both normal and disease

states 8.

The urinary extracellular vesicles are thus a mixture of molecular products from various parts of the nephron and urinary tract. This diversity is of a biological value, allowing to evaluate several compartments of the kidneys by a single urinalysis. It also increases analytical complexity because increases or decreases in the number of vesicles or the nature of vesicles contents can reflect changes in cell types, urine concentration, nephron activity, systemic physiology or injury associated with the disease.

2.2 Molecular Cargo of Urinary Extracellular Vesicles

The molecular content of extracellular vesicles in the urine greatly determines the biological and diagnostic potential of these vesicles. They are filled with a variety of proteins, messenger RNAs, microRNAs, long non-coding RNAs, lipids, metabolites, enzymes, receptors, transport proteins and other factors inside the cell. These molecules are selectively incorporated, which does not result in a random distribution of the cellular content within EVs. Rather, cargo sorting could be actively controlled, depending on the cellular state, environmental factors, metabolism and trauma. Importantly, the presence of miRNAs in EVs is thought to be reflective of kidney-specific regulatory mechanisms and can yield disease-associated molecular signatures with relative immunity to extracellular degradation 9.

Coordinated intracellular mechanisms are involved in the sorting of microRNAs into extracellular vesicles, suggesting that they regulate the intracellular selection of regulatory RNAs. These mechanisms are relevant to the understanding why specific microRNAs are found in higher or lower concentrations in extracellular vesicles in disease. Protected lipid bilayer of EVs also provides enhanced stability of encapsulated RNA molecules, which is more advantageous for molecular profiling compared to free circulating RNA. Extracellular vesicle-associated microRNAs can regulate gene expression in recipient cells and may play a role in the regulation of inflammatory, apoptotic, fibrotic and metabolic pathways 10.

The proteomics and transcriptomics of urinary EVs have revealed that they carry many molecules associated with membrane transport, cytoskeletal organization, cell adhesion, metabolism, intracellular signaling, and renal epithelial function. Proteins found in particular nephron segments can be helpful to determine the origin of the vesicles and renal processes represented in urine. Urinary extracellular vesicles are an appealing option for the study of diseases that have varying involvement of the various compartments of the kidney.

Another exciting aspect of vesicles are those containing metabolic components, which are another form of biological information that is being discovered. Metabolomics characterization can provide insight into the alterations in small molecular changes related to energy metabolism, lipid pathways, oxidative stress, and cellular homeostasis. Metabolites associated with EVs may complement proteomic and transcriptomic markers of kidney injury and chronic renal dysfunction, and may be part of a more comprehensive molecular phenotyping 11.

In Figure 1, the sources, biogenesis and main molecules of urinary EVs are shown.

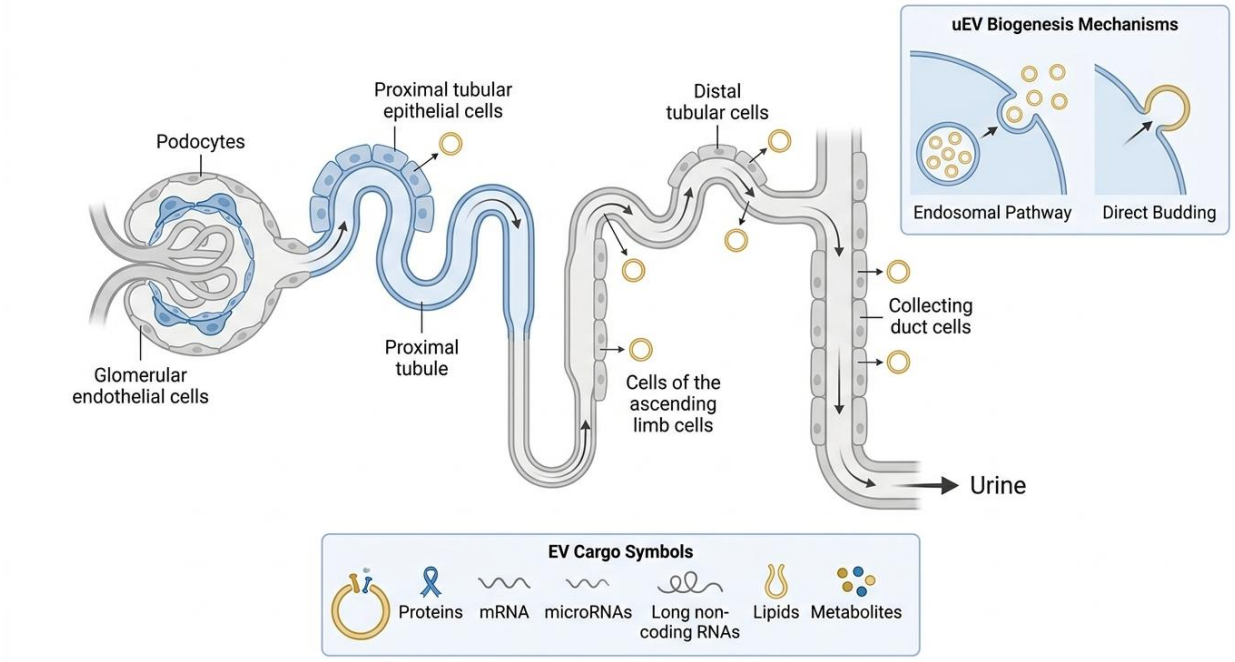

Figure 1. Renal Cellular Origin, Biogenesis, and Molecular Cargo of Urinary Extracellular Vesicles

2.3 Physiological Functions and Intercellular Communication

Beyond their use as biomarkers, Urinary EVs play significant roles in physiological functions. They are involved in communication between the cells of the kidney, transferring bioactive molecules from one segment to another. This process could play a role in coordinated regulation of transport systems, adaptation of cells, immune response and tissue homeostasis. Altered production or cargo within EVs can then alter

the microenvironment in the kidney and impact on the activities of adjacent or downstream cells.

Physiological conditions, other than kidney disease, can also alter the molecular makeup of urinary EVs. The surface-phenotype and RNA content of urinary vesicles could be affected by changes in circulating factors, physical stressors and systemic physiological changes. These observations highlight the importance of taking into account the biological context of each person's urine, and considering the urine to be a dynamic record of molecular events, not a mere molecular snapshot 12.

The role of extracellular vesicle mediated communication in kidney-distant organ interactions can also be involved. Advanced renal dysfunction may involve extracellular vesicles in the systemic signaling related to inflammation, endothelial dysfunction, uremia, and cardiovascular complications both in circulation and in urine. Their interpatient role in the heart-kidney axis, where local cardiac changes may contribute to systemic effects, highlights the possible association between local renal alterations and systemic effects, especially in patients with chronic kidney disease or renal replacement therapy 13.

Urinary EVs, overall, have biological relevance due to a combination of their cellular origin in the kidney, molecular content that is selectively packaged, relative stability in urine, and the ability for intercellular communication. These properties have rendered them ideal candidates as dynamic markers of renal physiology and also given a framework for how they are altered in kidney injury and progression of disease.

The most important renal sources, the typical molecular cargo and the biological significance of uEVs are summarized in Table 1.

Table 1. Major Renal Sources, Molecular Cargo, and Biological Relevance of Urinary Extracellular Vesicles

Renal/urinary source or feature	Representative uEV cargo	Major biological relevance	Reference
Podocytes and glomerular cells	Proteins, miRNAs, mRNAs, membrane-associated molecules	Maintenance of the glomerular filtration barrier and glomerular signaling	7
Proximal and distal tubular epithelial cells	Transport proteins, enzymes, RNAs, metabolites	Tubular transport, metabolism, and nephron segment communication	7
Multiple renal and urinary tract cells	Proteins, lipids, nucleic acids, signaling molecules	Intercellular communication and transfer of biologically active cargo	8
miRNA-containing uEVs	Kidney-associated miRNAs	Post-transcriptional regulation and generation of disease-related molecular fingerprints	9
Selectively packaged miRNAs	Regulatory miRNAs	Modulation of inflammatory, apoptotic, metabolic, and fibrotic pathways	10
Metabolite-containing EVs	Amino acids, lipids, and other metabolites	Reflection of metabolic activity and cellular homeostasis	11
Physiologically modified uEV populations	Surface markers and miRNAs	Dynamic changes in vesicle composition under systemic physiological influences	12
EV-mediated systemic communication	Proteins, RNAs, inflammatory mediators	Contribution to kidney-organ communication, uremic signaling, and systemic effects	13

3. Urinary Extracellular Vesicles as Molecular Fingerprints of Kidney Injury

3.1 Glomerular and Tubular Injury Signatures

Extracellular vesicles from the urine can reflect molecular changes from different compartments of the kidney and thus serve as a non-invasive snapshot of current kidney damage. Vesicles are secreted by podocytes, glomerular endothelial cells, proximal tubular cells, distal nephron segments, and collecting duct cells and alterations in their number or contents can help identify the site and extent of injury. During the acute phase of kidney injury, protein and nucleic acid content associated with tubular injury, repair and inflammatory signaling may change as a result of the change in vesicle release that occurs in the stressing of the epithelium during this period. Kidney epithelial-derived extracellular vesicles have therefore been studied as indicators of early tubular injury, and injury-related communication between renal cells 14.

The urinary extracellular vesicle composition can also be a sign of glomerular damage. Changes in the proteins and regulatory molecules present in urinary vesicles may occur with podocyte dysfunction, disruption of the filtration barrier, and altered glomerular signaling. These molecular differences could be used to differentiate clinically different glomerular disorders even if traditional markers like proteinuria have similar trends. For idiopathic nephrotic syndrome, urinary EVs have been shown to be suitable for proteomic profiling that can help differentiate clinical subgroups, suggesting that the cargo of EVs might carry disease-specific information linked to glomerular disease 15.

In progressive kidney disease, injury to the tubules and glomeruli is seldom a separate event. The ongoing reduction in glomerular function may lead to progressive exposure of tubular protein and metabolic stress, and tubular injury may lead to a progressive spread of inflammatory and fibrotic signals in the renal interstitium. Extracellular vesicles in urine could thus capture molecular signals from various parts of the nephron and hence provide a more complete picture of kidney injury than those measuring the filtration function.

3.2 Inflammation, Apoptosis, and Fibrotic Remodeling

Initial renal injury leading to sustained tissue injury and progressive renal loss of function is importantly linked to inflammation. When cells in the kidneys suffer damage, they release injured cells that change the production and composition of EVs that allow for the transfer of pro-inflammatory molecules between cells. Vesicular microRNAs are especially significant as they can modulate pathways involved with cytokine production, immune activation, apoptosis, and tissue repair. In diabetic chronic kidney disease, urinary extracellular vesicles contain microRNA signatures linked to inflammatory and apoptotic processes, indicating a way of gaining information on molecular events that may not be recognized by standard biochemical markers 16.

Maladaptive repair and renal fibrosis can then occur following chronic or unresolved injury. The fibrotic progression is characterised by the activation of fibroblasts, the accumulation of extracellular matrix, the disturbance of epithelial function, capillary rarefaction and continuous inflammatory signalling. These processes can affect the release of EVs and generate specific changes in the protein and RNA content of EVs. In the field of chronic kidney disease, molecular profiling of urinary extracellular vesicles has revealed changes linked to renal fibrosis, highlighting their potential as non-invasive markers of tissue remodeling and advancing structural damage 17.

The role of EVs in relation to fibrosis is also biologically relevant as EVs may be actively involved in intercellular communication. These regulatory RNA, signaling proteins and mediators of extracellular matrix turnover can affect recipient tubular cells, immune cells, endothelial cells and fibroblasts. Therefore, the urinary EVs are not only products of renal damage but can also be indicative and/or involved in the molecular networks that maintain inflammation and fibrogenesis.

3.3 Injury-Induced Alteration of uEV Cargo and Molecular Fingerprinting

The idea of a molecular fingerprint is based on the notion that renal injury causes consistent changes in the number, surface properties, and contents of vesicles. The rate of extracellular vesicle secretion and the molecules contained within the vesicles can change due to metabolic stress, hyperglycemia, oxidative injury, hypoxia, inflammation or toxic exposure. It has been reported that the levels of urinary extracellular vesicles and their contents change in diabetes-associated kidney injury, and that diabetes-related cellular stress reaches the urinary level of measurable molecular changes 18.

These changes can be more complex and may include patterns of integrated proteins, nucleic acids, lipids, metabolites and biophysical properties. These multidimensional signatures are appealing because kidney injury is a complex, biological process with multiple pathways interacting with each other. The biochemical and biophysical characteristics of urinary EVs can be altered by physiological stress, indicating that EVs are adaptable and reflective of alterations occurring in the cellular and systemic environment 19. This dynamic behavior makes them good molecular indicators but great care must be taken to interpret the significance of the change since it does not necessarily indicate renal pathological damage.

The idea of uEVs as ‘fingerprints of kidney injury’ has been further supported by use of advanced molecular profiling approaches. Vesicular signatures can be identified by spectroscopic analysis paired with computational classification, which is better at detecting more complex signatures that are not readily detected in conventional single analyte assays. Using the machine learning-based surface-enhanced Raman spectroscopy, the authors tracked the source of kidney damage according to multispectral molecular fingerprints to expand the possibilities for localization and classification of renal damage 20.

These findings together indicate a sequence of events that renal cellular stress/injury affects production, molecular packaging and release of EVs to the urine. These changes in proteins, microRNAs, metabolites and biophysical properties create a measurable urinary

"fingerprint" that could be used to identify the type, site, and severity of kidney damage. This molecular fingerprinting idea paves the way to take the urinary exV analysis from a mere discovery of the biomarkers to the disease stratification, assessment of progression, and precision monitoring of renal pathology.

Figure 2 shows the mechanistic cascade of events from renal insult to change in uEV cargo and clinically useful molecular signatures.

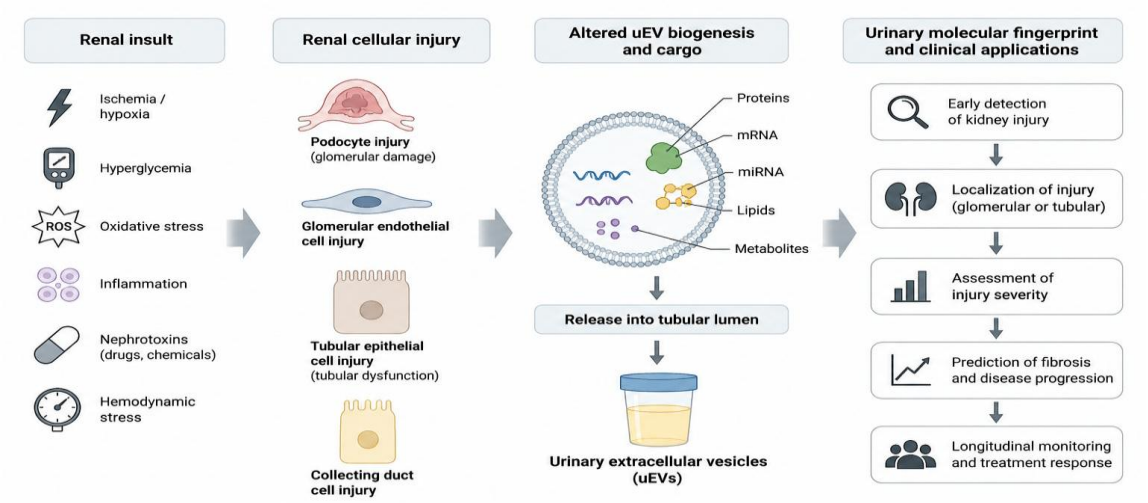

Figure 2. Mechanistic Basis of Urinary Extracellular Vesicles as Molecular Fingerprints of Kidney Injury

The principal mechanisms of kidney injury and their associated uEV molecular alterations are summarized in Table 2.

Table 2. uEV Molecular Alterations Associated With Major Mechanisms of Kidney Injury

Kidney injury mechanism	Predominant renal process	Characteristic uEV alteration	Potential molecular significance	Reference
Acute tubular injury	Epithelial stress and tubular dysfunction	Altered kidney epithelial-derived EV release and cargo	Early tubular injury fingerprint	14
Glomerular injury	Podocyte dysfunction and filtration-barrier disruption	Altered urinary EV protein profiles	Differentiation of glomerular disease phenotypes	15
Inflammation	Cytokine activation and immune signaling	Altered inflammatory miRNA signatures	Identification of inflammatory kidney injury	16
Apoptosis	Renal-cell death and stress	Changes in apoptosis-associated miRNAs	Assessment of cellular damage	16
Renal fibrosis	Fibroblast activation and matrix deposition	Fibrosis-associated proteins and molecular profiles	Detection of progressive structural remodeling	17
Diabetic metabolic injury	Hyperglycemia, oxidative stress, and metabolic dysfunction	Changes in uEV abundance and molecular cargo	Diabetes-associated renal injury fingerprint	18

Physiological/environmental stress	Cellular adaptation and altered metabolism	Changes in EV biophysical and biochemical characteristics	Demonstrates sensitivity of uEVs to cellular stress	19
Complex kidney injury	Multiple interacting injury pathways	Multispectral molecular fingerprints	Localization and classification of kidney injury	20

4. Disease-Specific uEV Profiles and Kidney Disease Progression

4.1 Acute Kidney Injury

Acute kidney injury is characterized by a rapid decline in renal function resulting from ischemic, septic, toxic, or hemodynamic insults. Conventional markers such as serum creatinine often increase only after substantial functional impairment has occurred, creating a need for molecular indicators that can detect injury earlier. Urinary extracellular vesicles are particularly relevant in this context because renal epithelial cells can rapidly alter vesicle release and cargo in response to acute cellular stress. These changes may provide information on tubular damage, inflammatory activation, mitochondrial dysfunction, and reparative responses before overt clinical deterioration becomes apparent.

Experimental and translational studies suggest that extracellular vesicle-based approaches may contribute both to understanding the mechanisms of acute kidney injury and to identifying molecular signatures associated with renal recovery. Vesicles derived from injured or therapeutically manipulated cells can carry proteins, nucleic acids, and signaling molecules that influence inflammatory, apoptotic, and regenerative pathways. Their application in acute kidney injury research has therefore expanded from biomarker discovery toward broader systems-based therapeutic and mechanistic investigation 21.

4.2 chronic kidney disease and Renal Fibrosis

Chronic kidney disease is a progressive disease that is characterized by progressive loss of renal functional reserve due to persistent nephron injury, inflammation, microvascular dysfunction and fibrosis. Disease activity is variable and standard measures like eGFR and albuminuria are not always enough to identify patients who are likely to have rapid disease progression. Therefore, the urinary EVs offer a potential to identify molecular changes that are directly linked to diseased renal tissue and could potentially aid in the evaluation of disease activity and prognosis.

Proteomic analysis of EV urinary proteins identified several candidate proteins that are linked to CKD, suggesting their potential role in the identification of biomarkers and molecular subtyping. These proteins could represent abnormal transport of tubular proteins, metabolic regulation, immune signaling, cellular stress, or extracellular matrix remodeling, and provide a more comprehensive picture of renal disease than individual conventional markers 22.

In addition, surface proteins of EVs carry potentially valuable diagnostic information, which can include cell of origin and pathological state of the vesicle population. Extensive characterization of EVs surface proteins has identified candidate biomarkers with the potential to differentiate vesicle populations from chronic kidney disease and may lead to the development of more selective capture and detection protocols for future clinical assays 23.

4.3 Diabetic Kidney Disease

Diabetic kidney disease is one of the more significant factors of chronic kidney disease and kidney failure. These processes of development include glomerular hyperfiltration, podocyte injury, endothelial dysfunction, tubular stress and metabolic dysregulation, inflammation, and progressive fibrosis. An important aspect of this is that these molecular abnormalities occur prior to functional deterioration, and thus urinary extracellular vesicles are being investigated as a tool for early detection of pathological processes and stratification of the disease severity.

Spectroscopic analysis of extracellular vesicles in urine has been shown to be a promising tool for discriminating type 2 diabetes patients based on their CKD status. The molecular pattern of disease-associated substances can be obtained without depending on a single analyte by integrating biochemical information from lipids, proteins and nucleic acids, achieved by Raman-based profiling in vesicles. These strategies are consistent with the idea of vesicle-based molecular fingerprinting for clinical stratification in DKD 24.

Addition of proteomic analysis can also enhance early detection by recognizing changes in proteins within vesicles prior to the manifestation of clinically advanced nephropathy. Proteomic analysis of the urinary EVs has been linked to early development of diabetic nephropathy, indicating that the urinary EVs protein signature may offer more sensitivity than traditional markers when used together and reflect podocyte dysfunction, tubular damage, metabolic stress, and inflammatory signaling 25.

Metabolic changes also occur at various phases of the diabetic kidney disease. Metabolic signatures of EVs can change according to the stage of the disease and include aspects of energy metabolism, lipid pathways, oxidative stress, and systemic metabolic dysfunction. These patterns could help elucidate the link between early diabetic renal stress and chronic kidney disease and worsening renal structural injury 26.

4.4 Glomerular, Rare, and Other Kidney Disorders

Common types of chronic kidney disease are not the only diseases that can be detected by urinary extracellular vesicle profiling. Very rare and unusual kidney diseases can also produce unique vesicular signatures corresponding to their structural and cellular and molecular defects. Proteomic analysis of

extracellular vesicles in urine has identified a unique kidney disease associated protein signature in medullary sponge kidney disease, a relatively rare renal disease, showing that vesicle analysis can be used to identify disease associated molecular pathways, even in relatively rare renal diseases 27.

The application of urinary exosomes in the study of rare kidney diseases is another good example where traditional markers of kidney disease are lacking, and patient numbers are few. The vesicles contain molecules that are relevant to the disease conditions of the cells, and they can therefore offer extra information for discovering disease-specific pathways, understanding phenotypic variability and aid in the search for biomarkers in rare renal diseases 28.

4.5 Kidney Transplantation and Advanced Kidney Disease

Kidney transplantation is a promising environment for urinary extracellular vehicles analysis as graft derived EVs can offer direct molecular information about the transplanted organ. Urinary vesicles may change their cellular composition and molecular cargo due to rejection, viral infection, ischemic injury and chronic allograft dysfunction. Serial urine collection could then be a non-invasive approach to maintaining and/or monitoring the health of a graft and detecting complications before significant functional loss.

Kidney transplant recipients have been found to have molecular differences associated with the presence of BK polyomavirus viruria in urine and viremia. These results indicate that vesicle proteins might be useful to define the biological response of the renal allograft to viral insult and to the more precise monitoring of the complications after transplantation 27

The urinary extracellular vesicles profile can also vary between kidney failure patients and kidney transplant patients. The discovery of candidate biomarkers in all these clinical situations indicates that vesicles might be used not just for diagnosis, but also for longitudinal monitoring of the recovery of the graft and residual renal injury after transplantation, broadening their utility from diagnosis to advanced kidney disease 29.

4.6 uEVs as Indicators of Disease Progression

Perhaps the most important clinical role of urinary extracellular vesicles is that they can reflect the dynamic changes of the molecular composition throughout the progression of kidney disease. The evolution from early cellular stress to chronic inflammation to fibrosis, to nephron loss and to kidney failure is not adequately captured by a conventional laboratory parameter. Unlike uEV, uEV profiles can encompass several aspects of disease biology, such as proteins, RNAs, metabolites, surface markers and biophysical features.

In theory, a disease-progression signature may be used to distinguish early from advanced disease, as well as stable from rapidly progressive renal function and reversible injury from maladaptive repair. Longitudinal profiling can also detect changes that occur with treatment response, relapse, graft dysfunction or fibrotic deterioration. Whether this approach is clinically useful will depend on the reproducibility of disease-specific signatures; their independence from traditional biomarkers and their predictive utility in a variety of populations.

Overall, the evidence suggests that signatures of extracellular vesicles in the urine in various disease conditions are beginning to emerge in the context of acute kidney injury, chronic kidney disease, diabetic kidney disease, rare kidney diseases, and kidney transplantation. These vesicles can be used as "polymolecular fingerprints," in contrast to simple biomarkers, that give a comprehensive molecular profile of the biological activity of the kidney at various phases of injury and progression. This characteristic is especially attractive for prognostic stratification and longitudinal monitoring of kidney disease, as is uEV-based profiling.

The uEV signatures for each disease and their association with progressive renal injury are shown in Figure 3.

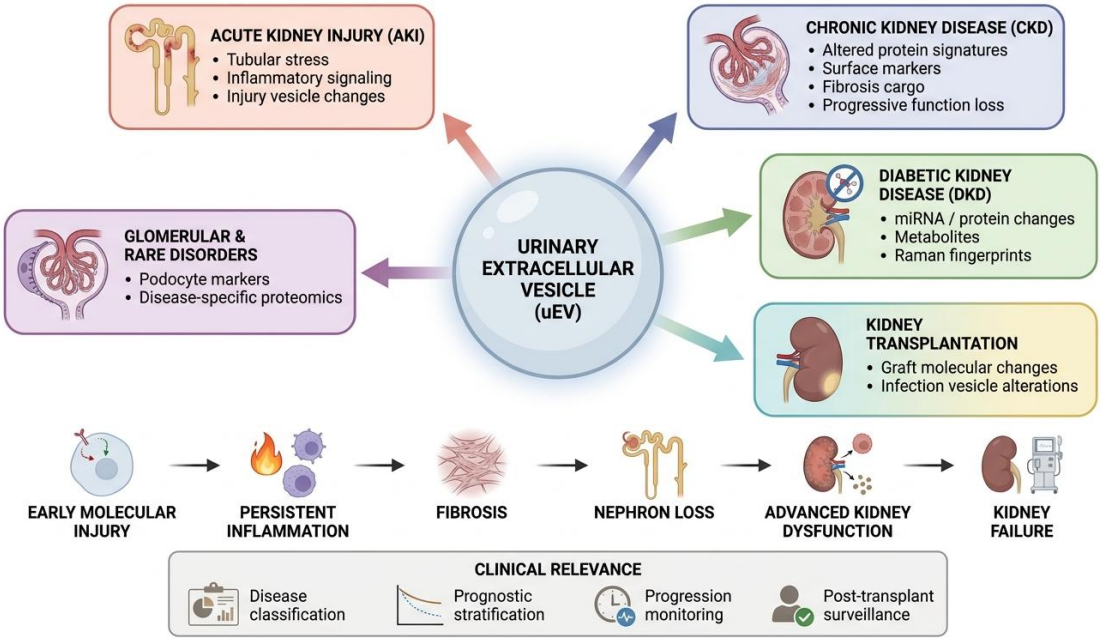

Figure 3. Disease-Specific Urinary Extracellular Vesicle Signatures Across Kidney Disorders and Disease

Progression

The major disease-specific applications of uEVs across kidney disorders are summarized in Table 3.

Table 3. Disease-Specific Applications of Urinary Extracellular Vesicles in Kidney Disorders

Kidney condition	Major uEV feature investigated	Potential application	Reference
Acute kidney injury	Injury-associated EV signaling and renal regenerative pathways	Mechanistic assessment and development of EV-based approaches to AKI	21
Chronic kidney disease	Urinary EV protein signatures	Biomarker discovery, diagnosis, and progression assessment	22
Chronic kidney disease	EV surface proteins	Identification of CKD-associated biomarker panels	23
Diabetic kidney disease	Raman spectroscopic uEV fingerprints	Stratification of patients with type 2 diabetes and CKD	24
Early diabetic nephropathy	Urinary EV proteomic alterations	Early detection of renal involvement in diabetes	25
Stage-dependent diabetic kidney disease	EV-associated metabolic alterations	Molecular characterization of disease stages	26
Medullary sponge kidney disease	Kinase-related urinary EV protein profile	Identification of disease-specific molecular hallmarks	27
Rare kidney disorders	Urinary exosomal molecular profiles	Biomarker discovery and molecular characterization	28
Kidney transplantation with BK polyomavirus infection	Urinary EV proteomic alterations	Monitoring viruria, viremia, and allograft-associated changes	27
End-stage renal disease and post-transplant state	Candidate uEV biomarkers	Monitoring advanced disease and post-transplant renal status	29

5. Clinical and Analytical Translation of uEV Biomarkers

5.1 Isolation, Detection, and Molecular Profiling

Isolation, characterization, and molecular profiling of urinary EVs are essential for their clinical utility. Ultracentrifugation, ultrafiltration, size-exclusion chromatography, precipitation, immunoaffinity capture and new microfluidic platforms are some of the common methods. These methods vary in the yield, purity and downstream repeatability of vesicles, which is why it is crucial to standardize methodologies for future biomarker development 30.

New detection platforms are being developed and advanced to detect biomolecules that associate with vesicles with increasing sensitivity. Detailed analysis of proteins and other molecular components can be carried out using mass spectrometry, which can aid in identification of disease-related vesicular signatures 31. Furthermore, quantitative proteomic and phosphoproteomic studies reveal the promise of urinary EVs for multidimensional biosignatures that are important for disease classification and biomarker identification 32.

Spectroscopic methods are also potential for fast and label-free analysis. Surface enhanced Raman spectroscopy coupled with machine learning can be used to classify urinary extracellular vesicle signatures without depending on any individual biomarker, and can detect complex biochemical patterns 33. The combination of vibrational spectroscopy and computational analysis has also demonstrated that the molecular fingerprints of EVs can aid high-dimensional disease discrimination 33.

5.2 Clinical Utility and Translational Potential

The idea of molecular fingerprinting has been reinforced by experiments showing that the microRNAs carried by EVs could form unique disease-related 'fingerprints', as suitable for computing-based disease classification 34. Similarly, proteomic analysis of urinary extracellular vesicles (EVs) has demonstrated the presence of disease-specific protein signatures in urine, further substantiating the potential of vesicle-based liquid biopsy methods for disease diagnosis 35.

Extracellular vesicles also have translatable value since they mirror intercellular communication and changes in the molecular aspects of the disease. In the field of oncology, these have shown the potential for vesicles to carry biologically meaningful cargo that holds diagnostic and therapeutic promise, providing a conceptual model that can also be used for biomarker development in the kidney 36. A more comprehensive account of systemic disease reinforces the idea of eVs as mediators of organ–organ communication and as accessible sources of molecular information 37.

5.3 Challenges and Future Clinical Implementation

Although there is promising evidence, there are still significant challenges to implementing routine use of uEV-based diagnostics. They are related to urine collection, storage conditions, isolation of vesicles, urine normalization, analytical platforms, number of patients in the study, and validation of the biomarkers. Future research should focus on using standardized pre-analytical workflows, the use of reproducible isolation procedures, large multicenter cohorts, and independent validation of candidate signatures. Ultimately, the opportunity for integration of proteomics, transcriptomics, metabolomics, spectroscopy and machine learning can aid in the development of clinically useful uEV biomarker panels for early diagnosis, disease stratification, prognosis and longitudinal monitoring in the context of precision nephrology.

Table 4 provides a summary of the analytical and translational strategies for molecular fingerprinting of EVs.

Table 4. Analytical and Translational Approaches for Extracellular Vesicle Molecular Fingerprinting

Analytical approach	Main application	Major advantage	Key limitation/translational consideration	Reference
Ultracentrifugation, ultrafiltration, size-exclusion chromatography, and affinity-based isolation	EV isolation and enrichment	Enables recovery of vesicles for downstream analysis	Differences in yield, purity, and reproducibility require standardization	30
MALDI/mass spectrometry	Molecular characterization of EVs	Sensitive identification of vesicle-associated molecules	Requires specialized instrumentation and analytical expertise	31
Quantitative proteomics and phosphoproteomics	Identification of diagnostic biosignatures	Provides high-dimensional protein and phosphorylation profiles	Requires complex data processing and validation	32
Surface-enhanced Raman spectroscopy with machine learning	Label-free EV fingerprinting	Rapid detection of complex biochemical signatures	Requires standardized spectral preprocessing and independent validation	33
FTIR spectroscopy integrated with machine learning	Molecular pattern classification	Captures multidimensional EV biochemical information	Clinical reproducibility remains to be established	38
EV-associated miRNA fingerprinting	Molecular disease classification	Enables construction of multivariable RNA biomarker signatures	Requires robust normalization and external validation	34

Urinary EV proteomics	Identification of disease-associated protein signatures	Demonstrates feasibility of urine-based liquid biopsy	Disease specificity must be validated in larger cohorts	35
EV communication profiling	Interpretation of disease-associated intercellular signaling	Links EV biomarkers with biological mechanisms	Translation across different disease contexts requires caution	36
Systemic EV signaling analysis	Assessment of organ-to-organ communication	Demonstrates broader biological relevance of EV-mediated signaling	Mechanistic findings may not always translate directly to kidney disease	37

6. Conclusion

Proteins, nucleic acids, lipids, metabolites, and other bioactive molecules found in urine EVs are believed to serve as molecular markers for kidney injury and disease progression, making EVs an attractive, non-invasive, and new avenue to study kidney disease. The molecular makeup of the EVs in urine may represent the changes in glomerular, tubular, inflammatory, metabolic, and fibrotic pathways and can serve as dynamic molecular fingerprints of renal pathology. This quality gives it a clear benefit over traditional biomarkers which mainly indicate kidney function changes, but not cellular and molecular mechanisms. UEV-based signatures have the potential to be used for early detection, disease stratification, prognostic assessment, and longitudinal monitoring across all forms of acute kidney injury, chronic kidney disease, diabetic kidney disease, glomerular disorders, rare kidney diseases and kidney transplantation. The capacity to identify multidimensional vesicular patterns corresponding to different renal conditions and progression states is further enhanced by the advances in proteomics, transcriptomics, metabolomics, spectroscopy, and machine learning. However, significant challenges exist in the urine collection, isolation, normalization, analysis, and validation of urine biomarker data. To this end, future studies should focus on large multicenter cohorts, standardized protocols, long-term validation, and on combining multi-omics data with clinical and histopathological data. These developments make it possible that urinary EVs profiling could become an important marker in precision nephrology, and contribute to the development of more personalized diagnostic, prognostic and monitoring strategies for kidney diseases.

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