

Extracellular RNA in diabetic kidney disease: mechanisms, biomarkers and paths to clinical translation



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Highlights:

- This review systematically establishes a biological framework for understanding extracellular RNA (exRNA) in diabetic kidney disease (DKD).
- From the pathological perspectives of glomerular, mesangial, tubular, immune and endothelial cells, it dissects exRNA governed molecular circuits underlying podocyte injury, mesangial expansion, tubulointerstitial fibrosis, inflammation and microvascular rarefaction.
- It compares the strengths and limitations of urine derived *versus* blood derived exRNAs as liquid-biopsy biomarkers.
- Four exRNA-oriented therapeutic strategies for DKD are summarized.
- A staged translational framework for exRNA research in DKD is proposed.

Abstract: Diabetic kidney disease (DKD) develops through maladaptive communication among glomerular, tubular, vascular and immune cells, but current clinical markers capture accumulated injury more readily than active molecular processes. Extracellular RNAs (exRNAs), including microRNAs, long noncoding RNAs, circular RNAs and messenger RNAs, are released in extracellular vesicles (EVs) or associated with ribonucleoproteins and lipoproteins. These carriers protect RNA against degradation and convey regulatory signals between cells, producing measurable molecular traces in urine and blood. In DKD models, exRNA pathways have been linked to podocyte loss, endothelial dysfunction, mesangial expansion, tubular stress, inflammation and fibrosis. Urinary EV-associated microRNAs have the most developed evidence from patient studies, with several varying alongside albuminuria or estimated glomerular filtration rate. Most of these studies are small and cross-sectional. Their findings are strongly affected by biofluid collection, EV enrichment, RNA normalization, treatment exposure and disease definition. Therapeutic studies remain preclinical, and effects of a nominated RNA must be separated from those of its carrier and co-packaged cargo. We critically assess biomarker and therapeutic evidence in relation to exRNA biology and renal cell-state transitions. The proposed staged translational framework addresses analytical validity, biological provenance, prospective clinical utility and scalable manufacturing. exRNA is therefore best



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viewed not as a single biomarker class, but as a platform whose value will depend on resolving cellular origin, context and causality.

Keywords: extracellular RNAs; diabetic kidney disease; biomarker; therapeutic

1. Introduction

Diabetic kidney disease (DKD) is a heterogeneous complication of diabetes in which metabolic, hemodynamic, inflammatory and fibrotic processes converge on the glomerulus, tubulointerstitium and renal microvasculature. Albuminuria and estimated glomerular filtration rate (eGFR) remain indispensable for diagnosis, staging and risk stratification, and contemporary therapies substantially reduce kidney and cardiovascular risk. Nevertheless, these measures do not fully resolve early cellular injury, nonalbuminuric disease, short-term molecular activity or the biological pathway that is dominant in an individual patient [1–4]. A repeatedly accessible molecular readout closely related to renal pathology could add information to established clinical markers without replacing them.

The biological effects of extracellular RNAs (exRNAs) and its use as a measurable signal are closely related. RNA released by viable or injured cells can be stabilized in RNase-rich fluids by enclosure in extracellular vesicles (EVs) or association with Argonaute proteins or lipoproteins, which may also carry it between renal and extrarenal cells. The same molecules can therefore be studied as mediators of cell and cell communication, as urinary or circulating biomarkers and as therapeutic cargo. Linking these roles is conceptually attractive, but the observations used to study them are not interchangeable. A change in kidney-tissue RNA does not establish extracellular release, detection in an EV fraction leaves intravesicular localization unresolved, and EV uptake does not identify the RNA responsible for a biological effect [5–8].

Extracellular RNA in DKD is considered here separately from evidence limited to intracellular RNA. EV is used as the generic term in accordance with current reporting recommendations unless an endosomal origin has been established experimentally [5,6]. Studies of urinary and circulating exRNA are examined alongside renal cell and animal models, with diagnostic association, mechanistic causality and therapeutic efficacy assessed as distinct forms of evidence. The central argument is that exRNA can become clinically informative only when molecular identity is linked to cellular provenance, technically reproducible measurement and a defined decision in the DKD care pathway.

2. The biological architecture of extracellular RNA

2.1. RNA species and extracellular carriers

The extracellular transcriptome includes short RNAs, long noncoding RNAs (lncRNAs), circular RNAs (circRNAs), messenger RNAs (mRNAs), transfer RNA derived fragments and other small RNA species. MicroRNAs (miRNAs) are the most extensively studied because their short length, sequence defined targets and compatibility with quantitative polymerase chain reaction (PCR) facilitate measurement. lncRNAs and circRNAs offer potentially richer cell-state information, although their lower abundance, isoform complexity and incomplete annotation create additional analytical challenges. CircRNAs are unusually resistant to exonuclease degradation owing to their covalently closed structure and can be enriched in EVs, but most kidney studies remain exploratory [7,9].

Y-RNAs, also called RNYs, are such a class of non-coding small RNA molecules, 83–112 nt in length. In the human genome, four RNY members have been identified: RNY1, RNY3, RNY4, and RNY5. These RNY gene clusters are located on chromosome 7, full-length RNYs (FL RNYs) and their derived small fragments bind to various carriers to exert functions within cells or are transported to the extracellular environment [10–12]. Zhao *et al.* study investigated whether circulating RNYs associate with DKD that may facilitate early detection and intervention of DKD. Levels of RNYs were determined in three main non-coding RNA carriers (EVs, RNA binding protein Argonaute-2 (AGO2), and high-density lipoprotein (HDL)). Y-RNAs are a type of highly abundant noncoding RNA that could potentially serve as DKD biomarkers [13].

Both miR-21 and miR-126 increased in EVs of DKD patients, whereas miR-660 increased in the Ago-2 fraction and miR-132 decreased in the HDL fractions. Moreover, *in vitro*, differentially expressed miRs improved endothelial cells (ECs) barrier formation (EV-miR-21) and rescued the angiogenic potential (HDL-miR-132) of ECs cultured in serum from DKD patients [14]. FcγRIII was regulated negatively by miR-326-3p in mouse glomerular mesangial cells (GMCs) under the condition of oxidized low density lipoprotein immune complex (ox-LDL-IC) [15].

EVs comprise heterogeneous membrane-bound particles released by essentially all renal cell types. EVs overlap operationally with particles often called exosomes, whereas larger exosomes (microvesicles) bud from the plasma membrane. Size alone cannot establish biogenesis. Lipoprotein particles and soluble ribonucleoprotein complexes overlap with EVs in density and size, and they can be co-isolated by precipitation, ultracentrifugation or size exclusion procedures. Consequently, an apparent EV associated RNA signal can reflect a mixture of vesicular RNA, non-vesicular RNA and contaminating macromolecules [5,16–19].

Selective RNA loading means that EV cargo need not reproduce cellular RNA abundance. RNA-binding proteins and sequence motifs, subcellular localization, membrane lipid composition and cell stress can influence cargo selection. RNA released abundantly during cell death can dominate biofluid profiles without participating in regulated communication. A mechanistic study should confirm extracellular localization experimentally and establish association with a defined carrier. Evidence is also needed for delivery to a recipient cell at a biologically plausible dose, along with loss or gain of the proposed phenotype when the nominated RNA is specifically depleted or restored.

2.2. Renal sources and routes of transfer

Urine contains exRNA released by podocytes, glomerular endothelial cells, tubular epithelial cells and cells lining the urinary tract. The relative contribution of these sources depends on urine flow, filtration, cell injury and EV release; membrane proteins and RNA cargo retained in urinary EVs can help identify the cells of origin. Circulating EVs can reach the kidney via the renal vasculature. The far larger plasma background of platelet, blood cell, endothelial and metabolic tissue particles means that a plasma signal might reflect systemic diabetes, vascular inflammation or reduced renal clearance rather than kidney specific pathology (Figure 1).

Directionality also matters. Glomerular endothelial cells, mesangial cells and podocytes exchange signals across a compact filtration unit, whereas proximal tubular cells encounter filtered proteins, glucose and metabolites and can transmit stress signals to interstitial fibroblasts and macrophages. In experimental DKD, EV mediated communication has been reported across several of these cellular pairs [20–23]. Yet

the effective concentration of a cargo *in vivo* is rarely quantified, and supraphysiological EV or mimic doses are common. The most persuasive models combine cell specific EV labeling or genetic manipulation of cargo loading with recipient cell target engagement and functional rescue.

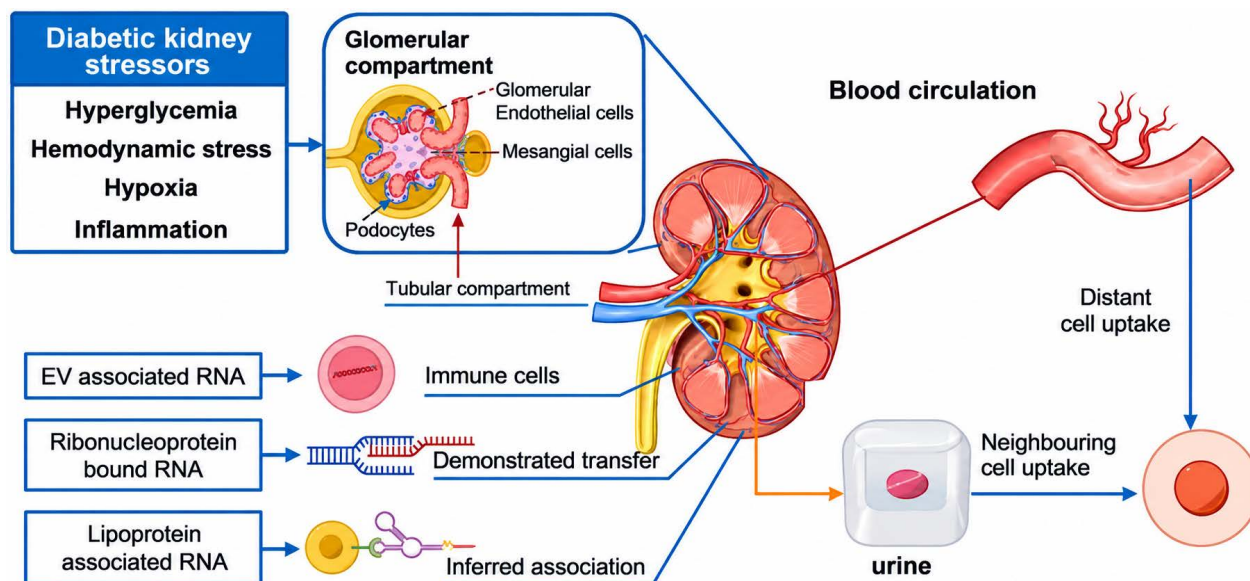


Figure 1. exRNA routes across the diabetic kidney. Hyperglycemia, hemodynamic stress, hypoxia and inflammation change RNA expression and extracellular release in glomerular endothelial cells, mesangial cells, podocytes, tubular epithelial cells and immune cells. RNA associated with EVs, ribonucleoproteins or lipoproteins can enter urine or blood and may be taken up by neighboring or distant cells. Solid arrows indicate transfer demonstrated experimentally; dashed arrows indicate inferred associations. Measured abundance can also vary with cell injury, filtration and clearance.

2.3. exRNA circuits in DKD pathogenesis

(1) Glomerular filtration barrier and podocyte injury

Podocyte depletion and foot process effacement are closely linked to proteinuria and progressive glomerulosclerosis. Multiple miRNAs regulate cytoskeletal organization, survival, autophagy and slit diaphragm proteins, but only a subset has been studied in an extracellular context. miR-21 illustrates both the promise and complexity of this field. Increased miR-21 activity has been linked to podocyte apoptosis, inflammation and fibrosis in diabetic models, and systemic miR-21 silencing ameliorated kidney injury in mice [24,25]. However, miR-21 is stress responsive in many cell types, is abundant outside the kidney and can have protective roles in other injury settings. Its detection in urine or EVs should therefore not be equated with a podocyte specific pathogenic signal.

Members of the miR-30 family are enriched in podocytes and help preserve differentiated structure. Their loss promotes cytoskeletal and calcium /calcineurin abnormalities, apoptosis and epithelial like phenotypic change. Experimental restoration of miR-30c reduced injury and fibrotic responses in diabetic models [26,27], whereas altered urinary EV miR-30 family members have been reported in human DKD cohorts [28–30]. The direction of urinary change is not always concordant with tissue abundance, which is biologically plausible: increased urinary export can coexist with intracellular depletion, and the measured

signal can shift as the cellular source is lost. Urinary abundance cannot be interpreted as a surrogate for intrarenal activity without paired tissue and urine measurements that also examine cellular origin.

Biomarker observations for miR-145-5p have begun to agree with evidence from mechanistic studies. Elevated urinary EV miR-145-5p has been reported in DKD. Patient derived urinary EVs induced podocyte injury in experimental systems, with evidence implicating the miR-145-5p-SRGAP2-RhoA/Rho-associated coiled-coil containing kinase (ROCK) axis [31,32]. Independent cohorts and cargo-specific depletion controls are still needed to validate these findings. For a disease-associated RNA discovered in biofluid, a mechanistic explanation depends on identifying the cells that release and receive it and testing its activity by targeted perturbation.

(2) Mesangial expansion and matrix remodeling

Mesangial cells increase extracellular matrix production in response to hyperglycemia, mechanical stress, angiotensin II and inflammatory mediators, with several miRNAs modulating the central transforming growth factor-beta (TGF- β) Smad dependent signaling pathway (SMAD) pathway. Reports linking miR-192 to increased collagen expression in diabetic glomeruli were followed by studies describing context-dependent or opposite changes. Inhibition of miR-192 reduced fibrosis in experimental diabetic nephropathy, with the response depending on cell type, model and disease stage [33]. Loss of miR-29 activity favors fibrosis by reducing its usual repression of collagen and matrix-gene expression. The intracellular actions of miR-192 and miR-29 support their investigation as exRNA candidates without establishing extracellular transfer.

In cell culture and diabetic animals, EVs from glucose-stressed mesangial cells or macrophages can stimulate matrix production and inflammatory signaling in recipient renal cells [20,22]. The proteins, lipids and multiple RNA species in these preparations complicate attribution of the effect to a single miRNA. Such attribution also depends on stringent controls that account for changes in cargo and donor physiology after donor-cell knockdown, contamination by transfection reagents and the requirement for membrane permeabilization when interpreting RNase treatment. An EV-level phenotype is generally better supported than a causal effect of one RNA cargo.

(3) Tubular stress, partial epithelial transition and fibrosis

Declining kidney function has a strong histological correlation with tubulointerstitial fibrosis. *In vivo*, injured tubular epithelial cells can adopt partial repair, inflammatory or profibrotic states and communicate with fibroblasts, endothelial cells and immune cells without necessarily completing a classical epithelial to mesenchymal transition. exRNA studies should therefore avoid treating marker changes in cultured epithelial cells as proof that tubular cells become matrix-producing myofibroblasts.

miR-26a and miR-30c suppress TGF- β induced matrix and phenotypic responses in podocytes and tubular models [26,27,34], whereas miR-21 can enhance profibrotic signaling through targets that include SMAD [35] and PTEN [24,35]. EV associated miR-126 and miR-145 have also been linked to cells undergoing endothelial or epithelial transition and to the urine of patients with DKD [36]. Circ_0008529 was reported to aggravate high glucose induced tubular dysfunction through a miR-185-5p-SMAD2 axis [37]. This circRNA study supports pathway plausibility, but independent replication, vesicle localization controls and human longitudinal data are required before clinical interpretation.

The tubular compartment is also a major producer of EVs under albumin, hypoxic and metabolic stress. Tubular EVs can activate fibroblasts or promote inflammatory macrophage phenotypes, providing

a route by which a localized epithelial stress response becomes interstitial disease [20]. RNA perturbation studies combined with proteomic and lipidomic analysis are needed to resolve whether exRNA drives the spread of injury or accompanies a broader EV response.

(4) Inflammation, oxidative stress and immune cell communication

In DKD, inflammation is sterile and occurs with metabolic stress rather than conventional inflammatory glomerulitis. The roles of miR-146a and miR-155 in Toll like receptor, nuclear factor- κ B (NF- κ B) and cytokine pathways depend on the biological setting. In diabetic models, miR-155 has been linked to podocyte injury and pro-inflammatory macrophage responses, while loss of miR-146a can exacerbate endothelial and renal inflammation [7,38]. Circulating or urinary increases can reflect compensatory release, pathogenic signaling, immune-cell abundance or impaired clearance, none of which can be distinguished from the direction of change alone.

EVs may convey the effects of different macrophage inflammatory states to renal parenchymal cells. In preclinical systems, EVs from reparative macrophages can carry protective miRNAs, whereas those from glucose-exposed macrophages have been reported to promote mesangial activation, tubular phenotypic change and podocyte pyroptosis [20]. Single cell or spatially resolved characterization of donor macrophages would avoid reducing their tissue states to the oversimplified M1-M2 categories. In oxidative stress, exRNA can modify NOX4, Nrf2-KEAP1, mitochondrial dynamics and antioxidant pathways. Interpretation of these effects needs the metabolic and hemodynamic context of the diabetic kidney, rather than treating them as isolated RNA circuits.

(5) Endothelial dysfunction and microvascular rarefaction

Glomerular and peritubular endothelial cells are exposed to hyperglycemia, altered shear, oxidative stress and inflammatory mediators. Endothelial miR-126 supports vascular homeostasis and angiogenic signaling. EV associated miR-126 has also been reported in renal cell and urinary preparations from DKD [36]. Other EV cargoes can promote endothelial to mesenchymal like responses or impair angiogenesis. As with podocyte markers, urinary abundance cannot by itself distinguish increased release from viable stressed cells, cellular depletion or loss of the producing cell population.

Microvascular rarefaction links endothelial dysfunction to tissue hypoxia and fibrosis. A credible exRNA mechanism would therefore need to connect donor cell state, vascular uptake, target repression and a measurable change in capillary integrity or perfusion. Most current studies stop at target gene and culture level phenotypes (Table 1). This gap is important because vascular miRNAs are also altered in retinopathy and cardiovascular disease, which limits DKD specificity in blood based assays.

Table 1. Representative exRNA signals in DKD.

Candidate or class	Main sample or model	Reported association or function	Evidence boundary
miR-21 [24,25,30,35]	Kidney tissue, urinary EVs, diabetic models	Fibrosis, inflammation, podocyte injury; inhibition is protective in mice	Ubiquitous stress-responsive RNA; source and stage specificity are limited
miR-29 family [7,39,40]	Kidney models and stem-cell EVs	Restrains matrix programs; EV miR-29b has anti-fibrotic activity	Human urinary evidence is less mature than mechanistic evidence
miR-30 family [26,28,30,39]	Podocytes, kidney tissue and urinary EVs	Maintains podocyte and epithelial state; altered in several DKD cohorts	Tissue depletion and urinary abundance need not change in parallel

Table 1. Cont.

Candidate or class	Main sample or model	Reported association or function	Evidence boundary
miR-145-5p and miR-27a-3p [31,32]	Urinary EVs	Associated with DKD; miR-145-5p linked to podocyte injury	Requires independent validation and cargo-depletion experiments
miR-146a and miR-155 [38]	Kidney, immune and endothelial compartments	Regulate inflammatory and stress pathways	Direction and function vary by cell type and disease context
miR-4534, miR-615-3p, miR-3147, miR-663a [41–43]	Urinary EVs	Candidate diagnostic or staging markers	Mostly small, cross-sectional and single-center cohorts
lncRNA and circRNA panels [9,44]	Urinary EV transcriptomics	Candidate pathway and biomarker networks	Low abundance, annotation and ceRNA assumptions limit interpretation
circ_0008529 [37]	Tubular-cell models and DKD-associated material	Proposed miR-185-5p-SMAD2 regulation	Needs extracellular localization, replication and longitudinal human validation

(6) exRNA as a liquid-biopsy platform

Urine is noninvasive, repeatable and anatomically proximal to the kidney and urinary tract. EV association can protect low abundance RNA from degradation, whereas EV enrichment can partially reduce the background of free proteins. Urine concentration varies with hydration, diuretic use, glycosuria and kidney function. Particle recovery and RNA profiles can also vary with collection time, storage temperature, freeze-thaw cycles, centrifugation, bacterial contamination and Tamm Horsfall protein. Normalization to creatinine, urine volume, EV number, exogenous spike ins or endogenous RNAs addresses different sources of variation and can yield different conclusions [5,6,16,18] (Figure 2).

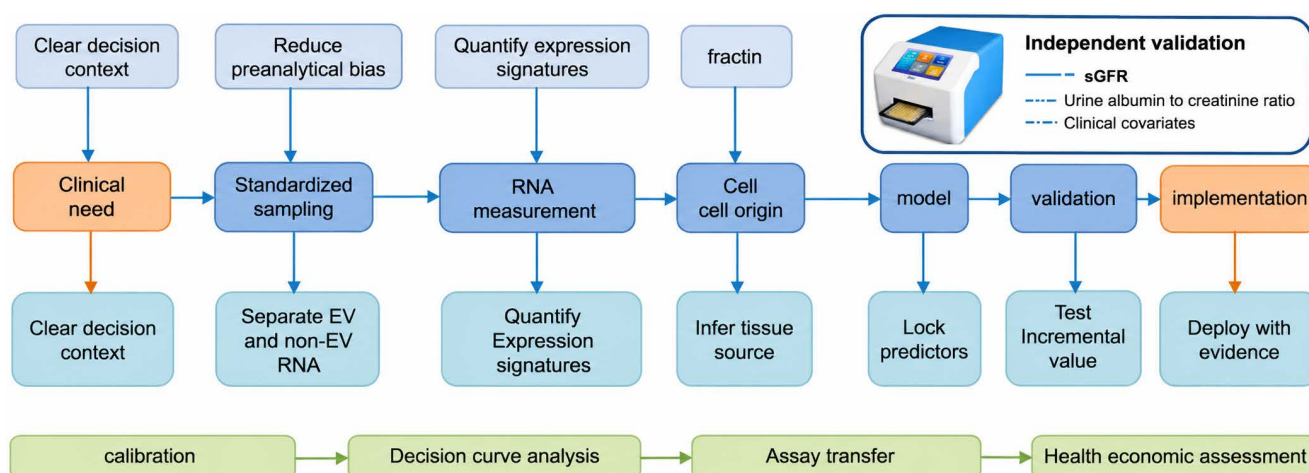


Figure 2. From exRNA discovery to a clinically useful biomarker. A clinical decision is defined and sampling standardized before discovery proceeds through EV and nonEV fractionation, RNA measurement and cell of origin analysis to development of a locked model. Incremental value over eGFR, urine albumin to creatinine ratio and clinical covariates must be assessed in independent validation. Calibration, decision curve analysis, assay transfer and health economic assessment take place before clinical implementation.

The clinical literature contains detectable signals with limited consistency across studies. In a 2026 systematic review of 24 human observational studies comprising 1171 participants, 197 miRNAs were

detected in urinary EV preparations, while only one study examined other RNA classes in depth [39]. miR-30a, miR-24-3p and miR-27b-3p were among the signals reported across albuminuria stages, with individual studies reporting area under the receiver operating characteristic curve values above 0.88 for miR-636, miR-34a or miR-15b. Methodological heterogeneity and a moderate risk of bias identified in the review limit the interpretation of these estimates. Small case-control designs, feature selection and internally optimized thresholds can inflate accuracy, with the reported AUC values remaining discovery-stage findings whose performance in real-world clinical settings has not been established.

The earliest urinary EV miRNA signatures were reported in studies of type 1 and type 2 diabetes [29,30,45–47]. Subsequent studies included biopsy-proven DKD and nonproteinuric disease and evaluated urinary EV miRNAs for differentiating DKD from focal segmental glomerulosclerosis [31,41,48,49]. The recurrent detection of miR-21, miR-30 family members and several inflammation-related miRNAs supports biological plausibility across studies whose exact signatures differ substantially. Each measurement reflects a population and mechanism defined by diabetes type, ancestry, medication, albuminuria stage, eGFR, biopsy status and technical workflow [50].

(7) Blood exRNA: systemic context and renal specificity

Systemic metabolic and vascular signals, including signals that do not enter urine, can be captured in plasma and serum. Circulating EV and miRNA profiles have been associated with albuminuria and DKD stage [51]. Preanalytical effects of platelet activation, hemolysis and clotting complicate interpretation of circulating RNA, and platelet-derived particles may dominate the measured signal. Measurements from serum and plasma are not interchangeable. Reduced kidney clearance may increase circulating RNA without an increase in production.

Systemic risk stratification and unreliable urine collection may be the most useful settings for blood assays. Urinary EVs have a stronger anatomical rationale for kidney-specific early detection. A combined model could use urine to reflect local renal injury and plasma to reflect systemic inflammation and vascular risk, together with the established prognostic information in routine clinical variables. Its evaluation needs a prespecified clinical question, with discrimination considered as part of that question rather than the sole objective.

(8) Beyond single miRNAs

Single marker strategies are unlikely to capture the biological heterogeneity of DKD. Whole-transcriptome studies have identified differentially expressed urinary EV mRNAs, lncRNAs, circRNAs and small RNAs, enabling pathway-level and competing endogenous RNA network analyses [44,52]. Coherent biological patterns can emerge from these approaches, although ceRNA networks are particularly prone to overinterpretation. Sequence complementarity and correlated expression do not establish stoichiometrically meaningful competition *in vivo*.

Multianalyte panels can improve prediction if they capture nonredundant processes, such as podocyte stress, tubular injury, inflammation and fibrosis. Machine learning is useful for feature integration, not for compensating for weak study design. External validation data must not be used for candidate selection, model training or threshold choice. Assessment of panel performance should include explicit tests for batch, center and treatment effects alongside calibration and decision curve analyses. The panel must provide value beyond the information already available from age, diabetes duration, blood pressure, eGFR, urine albumin to creatinine ratio and contemporary treatment.

(9) From association to clinical utility

Cohort selection, sampling and outcomes should match the intended clinical use, which for early detection requires prospective follow-up of normoalbuminuric people with diabetes and preserved eGFR using reproducible kidney endpoints. Prognostic studies require samples obtained before the subsequent eGFR slope, sustained percentage decline, kidney failure or validated surrogate outcome is assessed. Treatment monitoring depends as much on within-person biological variation and response kinetics as on differences between groups, and differential diagnosis requires biopsy or an adjudicated clinicopathological reference standard.

Validation requires reproducible results from the same specimen across runs, operators and laboratories to establish analytical validity. Clinical validity concerns association with the intended state or outcome in the target population; evidence that using the result improves decisions or outcomes is required for clinical utility. Work on DKD exRNA has largely focused on analytical and clinical validity, often in single-center discovery studies (Table 2). exRNA signatures are promising candidates, and translational claims need to remain prospective and restrained because the signatures are not yet ready to replace albuminuria or eGFR.

Table 2. Minimum translational framework for DKD exRNA studies.

Domain	Minimum requirement	Preferred advance
Clinical question	Prespecified intended use and target population	Decision threshold linked to a care pathway
Cohort	Clear diabetes type, DKD definition, eGFR, albuminuria and treatment exposure	Multicenter, prospective, serial sampling with adjudicated outcomes
Preanalytics	Collection, processing, storage and freeze-thaw fully reported	Harmonized protocol and inter-laboratory ring testing
EV characterization	Operational isolation method, recovery and contamination controls	Cell-origin-resolved or single-particle characterization
RNA assay	Extraction controls, limit of detection and normalization plan	Absolute quantification and reference materials
Statistics	Locked features, internal validation and confidence intervals	External validation, calibration and decision-curve analysis
Mechanism	Carrier association, uptake and target engagement	Cell-specific cargo perturbation at physiological dose with rescue
Therapy	Biodistribution, dose-response and short-term safety	Chronic toxicology, combination with standard care and scalable manufacture

2.4. Therapeutic strategies

(1) Direct modulation of pathogenic or protective RNAs

Antisense oligonucleotides, locked nucleic acids, antagomirs and miRNA mimics can be used to inhibit pathogenic RNAs or restore protective ones. In diabetic mice, miR-21 inhibition has reduced fibrosis and renal injury; restoration of miR-30 or miR-26 activity has shown protective effects in experimental renal systems [24,26,27,34]. Many organs and hundreds of targets can be affected by systemic miRNA manipulation despite the experimental tractability established by these studies. The therapeutic window may differ across DKD stages, and a miRNA can be harmful in one cell state yet adaptive in another (Figure 3).

Kidney delivery remains a major constraint. Oligonucleotide chemistry, size, charge, protein binding and filtration determine exposure to glomerular and tubular cells. Target engagement should be measured

in the relevant cell population, not inferred from whole kidney RNA. Long term studies must evaluate immune activation, hepatic and hematological toxicity, effects on host defense and oncogenic risk. Because DKD is chronic, a treatment that works after short term dosing in a rodent model must also be manufacturable and tolerable over repeated administration.

(2) EVs as endogenous therapeutics and delivery vehicles

EVs from mesenchymal stromal cells, urine derived stem cells and other progenitor populations have reduced renal injury in diabetic animals and other experimental kidney injury models. Reported cargoes include miR-26a-5p, miR-29b, miR-16-5p and other molecules that modulate inflammation, autophagy, apoptosis and fibrosis [40,53–56]. For example, adipose stromal cell EVs carrying miR-26a-5p protected podocytes through TLR4-NFκB related signaling, urine derived stem cell EVs protected podocytes and vascular cells, and liver stem cell EVs delivered miR-29b in fibrotic models. These data support therapeutic potential at the EV product level, but they do not yet establish which cargo or combination is necessary and sufficient.

Engineering can increase RNA loading, kidney targeting or endosomal escape. Surface ligands, donor cell modification and post isolation loading have all been proposed. Changes in product identity accompany each intervention and can affect biodistribution, immunogenicity and potency. Batch variation in the proteins, lipids, DNA and metabolites carried by native EVs can also change activity. A clinically credible product needs its source cell, culture and purification process to be defined, together with identity and purity assays, a potency assay linked to mechanism, stability criteria and quantitative biodistribution.

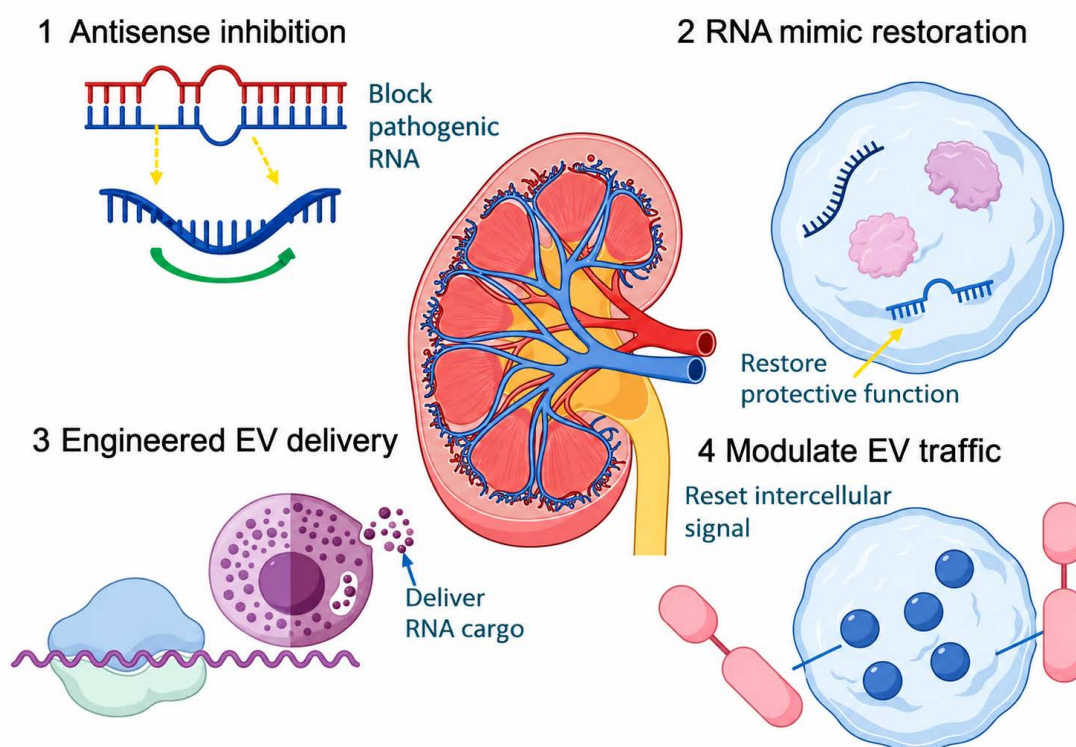


Figure 3. Therapeutic entry points in exRNA biology. Pathogenic RNAs can be inhibited by antisense agents and protective RNAs restored with mimics. Engineered EVs can deliver RNA to selected renal cells, with modulation of pathological EV release or uptake as another intervention point. Translation requires delivery and target engagement, assessment of offtarget exposure and immune risk, feasible manufacturing and safety with chronic dosing.

(3) Modulating pathological EV release or uptake

EV release, cargo loading and recipient cell uptake are potential points for interrupting endogenous pathological communication. In experimental DKD, injury has been attenuated by inhibition of vesicle release machinery or by drug induced changes in tubular EV release [20]. The approach may be less cargo specific and could suppress a coordinated pathogenic program. The role of EV secretion in normal tissue homeostasis and waste disposal creates broader risks for this approach that would require cell selective targeting and temporal restriction.

(4) Standardize the specimen before optimizing the model

Standardization begins with a common preanalytical record covering participant fasting and activity, collection time, urine volume and concentration, and the plasma anticoagulant. This record also needs time to processing, centrifugation, storage, freeze thaw cycles and checks for hemolysis or contamination. EV separation reports need to specify operational recovery and specificity, which cannot be inferred from a brand name alone. MISEV2023 and uEV recommendations call for orthogonal particle characterization and non EV contamination markers [5,6]. EV TRACK and related frameworks can improve the transparency of this reporting [57].

The normalization strategy needs to reflect the study purpose and the source of variation it is intended to address. Exogenous spike-ins track extraction and amplification without measuring biological sampling, and creatinine-based correction for urine concentration remains sensitive to muscle mass and kidney function. EV counts may be imprecise and do not capture shifts in cellular origin; endogenous reference RNAs need to be validated for stability in the study population. Sensitivity analyses comparing more than one normalization approach can test whether a signature is robust or method dependent.

(5) Resolve cell of origin and functional dose

Interpreting a biofluid exRNA signal in terms of mechanism and disease specificity depends on identifying the cells that released it. Surface proteins, single-EV assays, spatial transcriptomics and cell-specific genetic labeling offer ways to relate biofluid particles to renal cell states. Single-cell RNA sequencing can identify candidate donor and recipient populations and expected target programs [58], without measuring extracellular transfer; spatial methods and imaging can test particle movement within tissue.

Many EV experiments use particle-to-cell ratios above plausible *in vivo* exposures, making quantification of functional dose a priority. Dose assessment needs cargo copies per particle, the fraction of cargo-positive particles, uptake per recipient cell and target repression at the measured concentrations. These measurements are needed to distinguish endocrine or paracrine signaling from rare particle associations that are analytically detectable but have negligible biological effects.

(6) Design clinically anchored cohorts

Discovery cohorts should represent the intended clinical population, including, where appropriate, people receiving renin angiotensin system blockade, sodium glucose cotransporter 2 inhibitors, non-steroidal mineralocorticoid receptor antagonists or glucagon like peptide-1 receptor agonists. These treatments can alter kidney physiology and exRNA profiles, making their exclusion or omission from analysis a source of limited generalizability. Prospective multicenter studies need prespecified outcomes, serial samples and

relevant comparators, including non-diabetic chronic kidney disease and kidney disease of a nondiabetic cause in people with diabetes.

Within well-phenotyped cohorts, nested case-control sampling can improve discovery efficiency; validation across the full target spectrum is still needed to obtain unbiased estimates of performance. Event rates and incremental model performance should determine sample size; the number of candidate RNAs is not an adequate basis. Selective publication can be reduced by reporting assay failures and negative findings together with locked analysis plans.

(7) Match the product to a decision

The clinical usefulness of an exRNA test depends on whether its result changes care. Possible decisions include intensifying monitoring in normoalbuminuric patients, selecting individuals for biopsy, enriching prevention trials for rapid progressors or identifying a pathway responsive subgroup. Acceptable false-positive rates, sampling intervals and comparator models depend on the application. Robust external validation is needed before decision-curve analysis or cost-effectiveness assessment.

Therapeutic development should address renal cell type exposure and target engagement alongside pharmacokinetics and dose response. Chronic toxicology and compatibility with standard DKD therapy also need assessment. EV products additionally require scalable production, removal of adventitious agents and non-vesicular contaminants, release specifications and batch to batch potency. Rapid EV isolation technologies may facilitate diagnostic workflows when recovery and specificity are reproducible. Processing speed alone cannot replace these requirements.

3. Conclusions

Work on urinary EV-associated miRNAs is furthest advanced in exRNA research, and repeated associations with DKD stage and renal function justify prospective validation. Studying exRNA links the transmission of diabetic stress between renal cells to the prospects for measuring these processes noninvasively and manipulating them therapeutically. Evidence from mechanistic studies implicates miR-21 and the miR-29, miR-30 and miR-146a families in selected pathways, often without fully resolving extracellular localization, carrier identity or the dose needed for a causal effect. lncRNAs, circRNAs and multiRNA networks extend the candidates available for study and increase analytical and interpretive complexity.

Analytically standardized measurements, paired urine, blood and tissue studies, and cell-of-origin resolution should support fewer, better-anchored claims that undergo independent and prospective validation. Therapeutic work needs demonstrated kidney delivery and cargo specific target engagement. Under these conditions, exRNA could add a dynamic molecular layer to DKD management. Failure to meet these requirements risks accumulating biologically interesting signatures that lack sufficient reproducibility or clinical actionability.

Declaration of generative AI and AI-assisted technologies

The authors used ChatGPT during revision to assist in reviewing similarity and AI-detection reports. The authors take full responsibility for the content of the manuscript.

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Authors' contribution

Y.L.: writing—original draft, visualization, formal analysis, data curation; M.Z.: writing—original draft, investigation, funding acquisition, data curation, conceptualization. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

We declare that we have no competing interests (financial or nonfinancial).

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