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Extracellular vesicles and diabetic kidney disease: a systematic review

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Abstract. **Extracellular vesicles (EVs) are small lipid-encapsulated vesicles secreted from specific cells that can be taken up by other cells, thereby allowing for the efficient transport of macromolecules such as lipids, proteins, and nucleic acids between tissues and organs *in vivo*. These EVs have been found to play critical roles in normal tissue homeostasis and disease development, serving to regulate complex processes, including inflammation, immunity, and angiogenesis. At present, the leading global cause of the end-stage renal disease (ESRD) is diabetic kidney disease (DKD), with the prevalence of this latter condition being predicted to increase in the near future due to rising type 2 diabetes mellitus (T2DM) incidence. Recent work from several research groups has highlighted a potential role for EVs in the development of DKD. The present review, therefore, serves to explore the relevance of EVs to the development, detection, and treatment of DKD in an effort to better understand this complex disease state.**

Key Words:
Extracellular vesicles, Diabetic kidney disease, MicroRNAs, Stem cells.

Introduction

Diabetic kidney disease (DKD) is one of the leading causes of death among patients with type 2 diabetes mellitus (T2DM), in addition to be a primary driver of end-stage renal disease (ESRD) globally[1-3]. Patients suffering from DKD exhibit progressive renal functional deterioration that coincides with concomitant reductions in glomerular filtration rate (GFR) values, elevated albumin levels, and increased blood pressure that can contribute to serious cardiovascular complications. While DKD is generally not detected in patients with T1DM during the earlier stages of the disease, upwards of 3% of T2DM patients exhibit nephropathy upon initial diagnosis[4]. Between 25% and 45% of all ESRD patients in the USA, Japan, and Europe are believed to suffer from DKD, making it the single largest driver of this serious condition[5]. Despite their elevated risk, not all T2DM patients ultimately develop DKD, and these patients must therefore undergo regular risk screenings to detect and treat this disease during its early stages before it causes irreversible damage. Consistent with this goal, most treatments for T2DM patients are preventative and aim to lower blood glucose levels as a means of preventing the development of tissue damage and associated systemic complications. The implementation of routine screening, diagnostic, and treatment plans for DKD in recent years have been highly successful, leading to more expedient diagnoses and prolonged patient survival[6,7]. Current research efforts aim to build upon this success by facilitating even earlier DKD detection and treatment.

Extracellular vesicles (EVs) are small lipid-encapsulated vesicles that are regularly secreted by the majority of cells in both physiological and pathological contexts. Countless prior studies have shown that EVs can be isolated from both cell culture supernatants and physiological samples such as plasma, saliva, cerebrospinal fluid, and urine[8]. EVs can transport macromolecules, including proteins, nucleic acids, and metabolites between cells. Their contents can also offer insight into the intracellular state of their cells of origin. There are three primary classes of EVs that are differentiated based on their mode of generation: exosomes, microvesicles, and apoptotic bodies[9]. Exosomes are the smallest forms of EVs (30-120 nm) and are released from parental cells following multivesicular body fusion with the plasma membrane. In contrast, microvesicles are significantly larger (1000 nm) and are produced following plasma membrane blebbing. Apoptotic bodies are the largest EVs (1000-5000 nm), can contain organelles or nuclear fragments, and are generated only in the context of programmed cell death[10].

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While they can be produced via many different mechanisms, EVs invariably contain contents derived from their parental cells, including nucleic acids, proteins, lipids, and metabolites[11]. The specific contents found within EVs can vary significantly as a function of both cell type and pathological status. After they have been secreted, EVs can be internalized by recipient cells wherein they can induce significant physiological changes. Several recent studies have explored the relationship between EVs and metabolic diseases such as T2DM[12,13], while other studies have highlighted the potential value of EVs for the diagnosis and treatment of conditions such as DKD[14-16].

Diagnosis and Biomarkers of DKD

Biomarkers are specific molecular indicators that can offer insight into the status of specific biological processes, enabling clinicians to detect disease or to gauge the efficacy of a given treatment regimen[17]. When effectively leveraged, biomarkers can allow for early-stage disease detection and dynamic disease monitoring. To date, many researchers have sought to identify reliable serum or urine biomarkers of DKD in T2DM patients, as such biomarkers would enable routine screening and more efficacious early intervention efforts.

The most common clinical biomarkers currently used to monitor for DKD progression include serum creatinine levels, estimated glomerular filtration rates (eGFR), and levels of protein, albumin, and urea in the blood. Of these biomarkers, eGFR is the most reliable and direct means of measuring renal functionality, but its utility is limited during the earlier stages of disease prior to the development of significant renal structural alterations[18]. This eGFR value is primarily calculated based upon measurements of circulating creatinine levels. While some studies have attempted to measure alternative markers such as cystatin C in an effort to improve the reliability of eGFR estimates, these approaches are highly susceptible to individual variability[19-23]. The earliest known clinical biomarker of DKD at present is microalbuminuria, but even this is generally only detectable when renal functionality has been significantly impaired in affected individuals. In some cases, T2DM patients develop DKD without exhibiting any changes in urinary albumin levels, highlighting the limitations of this biomarker as a screening tool[24-26]. Elevated albumin excretion can also manifest as a result of dietary changes, exercise, infection, obesity, or other factors[27-31]. As such, the reliability of albumin and other current biomarkers of DKD is somewhat limited and often only allows for disease detection at a point when the disease has become irreversible.

The Diagnostic Utility of Urinary EVs

Most renal cells are capable of producing small exosomes (40-100 nm) that are excreted into the urine[32]. As these small EVs offer potential insight into the intracellular state of renal cells and are readily collected, they represent an ideal tool for the detection of DKD-associated biomarkers in a noninvasive manner in T2DM patients as a component of diagnostic or disease-monitoringtests[33]. To that end, many studies to date have explored potential urinary EV biomarkers of DKD.

In one study, Alvarez et al[34] tested a range of different urinary EV isolation protocols, leading them to determine that urinary exosomes and the nucleic acids therein (mRNAs and microRNAs [miRs]) could be readily isolated using a modified exosome precipitation protocol. As shown in Table I, Zang et al[35] determined that urinary exosomes isolated from T2DM patients with DKD contained elevated miR-21-5p levels and reduced miR-30b-5p levels. A separate study by Eissa et al[36] similarly observed elevated urinary EV levels of miR-34a, miR-636, and miR-15b in DKD patients, while Jia et al[37] detected increases in miR-192, miR-194, and miR-215 levels in EVs from a comparable patient population. In line with these findings, Xie et al[38] assessed urinary EVs from T2DM patients with DKD and detected elevated levels of miR-362-3p, miR-877-3p, and miR-150-5p as well as reduced miR-15a-5p levels in these samples, whereas Delić et al[39] were able to identify significantly elevated miR-320c and miR-6068 levels and markedly reduced miR-30d-5p and miR-30e-5p levels in the urinary exosomes of T2DM DKD patients. Prabu et al[40] separately found the expression of let-7i-3p, miR-24-3p, and miR-27b-3p to be increased in urinary EVs from T2DM patients with DKD, while miR-15b-5p levels in these same samples were decreased. In contrast, Abe et al[41] measured a significant increase in the expression of Wilm's Tumor-1 in comparable samples. In another analysis, Gudehithlu et al[42] found that urinary exosomes from T2DM patients with DKD exhibited significant reductions in gelatinase expression and significantly increased ceruloplasmin levels.

Table I. Studies of EVs derived from urine and serum samples.

First author in research	Molecule	Tendency	Species	Type of extracellular vesicles	Type of diabetic kidney disease
Zang et al[35]	miR-21-5p miR-30b-5p	Increased Decreased	Human	Exosomes	T2DKD
Eissa et al[36]	miR-34a miR-636 miR-15b	Increased Increased Increased	Human	Exosomes	T2DKD
Jia et al[37]	miR-192 miR-194 miR-215	Increased Increased Increased	Human	Exosomes	T2DKD
Xie et al[38]	miR-362-3p miR-877-3p miR-150-5p miR-15a-5p	Increased Increased Increased Decreased	Human	Exosomes	T2DKD
Ghai et al[39]	miR-941-1-3p miR-9-1-3p let-7c-5p	Decreased Increased Increased	Human	Extracellular vesicles	T1DKD
Barutta et al[40]	miR-130a miR-145 miR-155 miR-424	Increased Increased Decreased Decreased	Human	Exosomes	T1DKD
Denis Delić et al[41]	miR-320c miR-6068 miR-30d-5p miR-30e-5p	Increased Increased decreased decreased	Human	Exosomes	T2DKD
Prabu et al[42]	let-7i-3p miR-24-3p miR-27b-3p miR-15b-5p	Increased Increased Increased Decreased	Human	Extracellular vesicles	T2DKD
Abe et al[43]	Wilm's Tumor-1	Increased	Human	Exosomes	T2DKD
Kalani et al[44]	Wilm's Tumor-1	Increased	Human	Exosomes	T1DKD
Gudehithlu et al[45]	Gelatinase Ceruloplasmin	Decreased Increased	Human	Exosomes	T2DKD
Musante et al[46]	Myeloblastin and its natural inhibitor elafin	Increased	Human	Extracellular vesicles	T1DKD
Zubiri et al[47]	Regucalcin	Decreased	Rats	Exosomes	Rat model of STZ-induced diabetes
Raimondo et al[48]	Xaa-Pro dipeptidase Major Urinary Protein 1	Increased Decreased	Rats	exosomes	Zucker Diabetic Fatty rats
De et al[49]	Full-length megalin	Increased	Human	Extracellular Vesicles	T2DKD
Zubiri et al[50]	MLL3 AMBP VDAC1	Increased Increased Decreased	Human	Exosomes	Types of diabetes not recorded
Yamamoto et al[51]	Uromodulin	Increased	Human	Extracellular Vesicles	T2DKD
Sun et al[52]	Dipeptidyl peptidase-IV		Increased	Human	Microvesicles T2DKD
Lou et al[53]	Uromodulin	Increased	Human	Microvesicles	T2DKD
Kim et al[54]	miR-122-5p miR-432-5p miR-193b-5p miR-3656 miR-6087 miR-8485 miR-6739-5p miR-1273a miR-7641 miR-4461 miR-6751-3p miR-4484	Increased Increased Increased Increased Increased Decreased Decreased Decreased Decreased Decreased Decreased Decreased	Human	Exosomes	Types of diabetes not recorded

De et al[43] determined that urinary EVs from T2DM patients with DKD contained higher levels of full-length megalin. In an analysis of urinary EVs from T2DM DKD patients, Yamamoto et al[44] determined that the levels of uromodulin in these samples were significantly increased, while Sun et al[45] observed increased Dipeptidyl peptidase-IV levels in urinary microvesicles from T2DM DKD patients, and Lou et al[46] detected a similar increase in uromodulin levels in comparable samples. In a separate study, Musante et al[47] showed higher levels of myeloblastin and its inhibitor elafin in T1DM DKD patient urinary EVs, while Ghai et al[48] reported DKD-associated increases in miR-9-1-3p and let-7c-5p expression and reductions in miR-941-1-3p expression in samples from T1DM patients with DKD. Barutta et al[49] found that urinary exosomal levels of miR-130a and miR-145 were increased, and levels of miR-155 and miR-424 were reduced in T1DM DKD patients. In line with this, Kalani et al[50] detected significantly higher levels of Wilm's Tumor-1 in urinary EVs from T1DM patients with DKD, highlighting the potential relevance of this biomarker, while Zubiri et al[51] observed significant reductions in the levels of MLL3, AMBP, and VDAC1 in the urinary EVs of DKD patients. In a rat model of STZ-induced diabetes, Zubiri et al[52] observed increased regucalcin levels in isolated exosomes, while in a separate Zucker Diabetic Fatty rat model system, Raimondo et al[53] demonstrated significantly elevated Xaa-Pro dipeptidase and markedly reduced Major Urinary Protein 1 in isolated exosomes.

Serum EVs as Tools for DKD Diagnosis

While urinary EVs have been extensively analyzed as a potential source of DKD biomarkers, there have been fewer studies to date aimed at identifying similar biomarkers in blood, serum, or plasma samples. In one analysis, Kim et al[54] attempted to classify such biomarkers by isolating samples from healthy volunteers (HVs), diabetic patients without DKD (DMs), and DKD patients. By comparing EV miRNA contents between these three patient cohorts, they were able to determine that DKD patient EVs had significantly elevated miR-122-5p, miR-432-5p, miR-193b-5p, miR-3656, and miR-6087 levels relative to those from HVs, whereas DKD patient EVs contained lower levels of miR-8485, miR-6739-5p, miR-1273a, miR-7641, miR-4461, miR-6751-3p, and miR-4484 than did those from HVs. They further determined that DKD patient EVs contained increased levels of miR-6751-3p, miR-4449, and miR-4644 and reduced levels of miR-6739-5p, miR-7641, miR-3613-5p, miR-4485-5p, and miR-590-3p relative to EVs isolated from DM patients. Through further analyses, they established that these differentially expressed miRNAs were associated with TNF signaling, MAPK signaling, and TGFβ signaling. While preliminary, this study highlights the potential relevance and utility of serum EVs as a source for DKD diagnostic biomarkers and as a potential tool that can be used to understand the development and progression of this disease.

DKD Treatment Options

For many years, the best treatment options for DKD patients have included the control of hyperglycemia and hypertension through traditional means together with the inhibition of RAS. More recent research suggests that agonists of the glucagon-like peptide 1 (GLP-1) receptor and inhibitors of sodium-glucose cotransporter 2 (SGLT2) may be able to more effectively reduce hyperglycemia while simultaneously providing some degree of renal protection. DKD is a complex disease that is driven by numerous factors, including abnormal cellular signaling, reactive oxygen production, epigenetic factors, and the accumulation of advanced glycation end-products (AGE). As such, there are many potential targets for future clinical intervention. Currently, blood pressure control together with the use of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin 2 receptor blockers (ARB) remains the standard of prophylactic care in patients with DKD. This therapeutic regimen was developed based on the results of major studies, including the Collaborative study (captopril), RENAAL (losartan), IRMA (irbesartan), and IDNT (irbesartan) studies[55-58].

The Relevance of EVs to the Treatment of DKD

In a recent study, Zhu et al[59] isolated exosomes that had been produced by macrophages treated with high levels of glucose, and they then monitored the uptake of these particles by mesangial cells via confocal microscopy (Table II). They found that exosome uptake led to the activation of these mesangial cells, leading to their increased production of inflammatory factors and extracellular matrix components. They further determined that the TGF-β1/Smad3 signaling pathway is a key mechanistic regulator of this exosome-induced activation. Ling et al[60] found that glomerular endothelial cells (GECs) treated with high concentrations

Table II. Studies of EV-related in vitrotreatment efforts.

First author	EV origin	Type of extracellular vesicles	Research subject	Results
Zhu et al[59]	High glucose-treated macrophages	Exosomes	Mesangial cells	Exosomes from high glucose–treated macrophages activated glomerular mesangial cells via the TGF-b1/Smad3 pathway
Ling et al[60]	High glucose-treated glomerular endothelial cells	Exosomes	Mesangial cells	CircRNAs in exosomes from high glucose-treated glomerular endothelial cells activated mesangial cells
Wang et al[61]	High glucose-induced mesangial cells	Exosomes	Podocytes	Podocyte injury was inducedby exosomes derived from high glucose-induced mesangial cells through the TGFβ1-PI3K/AKT pathway
Wu et al[63]	High glucose-treated glomerular endothelial cells	Exosomes	Podocytes	Exosomes from high glucose- treated glomerular endothelial cells triggered epithelial-mesenchymal transition and dysfunction of podocytes
Li et al[64]	Podocytes	Microparticles	Podocytes	High glucose conditions provoked microvesicle production from glomerular podocytes via the NOX4/ROS pathway
Ravindran et al[65]	Renal proximal tubular cells	Microparticles	Proximal tubule cells	MPs activated key signaling pathways in RPTCs under high glucose conditions.
Munkonda et al[66]	Podocytes	Microparticles	Proximal tubule cells	Podocyte-derived micro particles promoted proximal tubule fibrotic signaling via p38 MAPK and CD36

of glucose secreted greater numbers of exosomes and that these exosomes contained higher levels of circRNAs than did exosomes released by GECs cultured under normoglycemic conditions. They further found that siRNAs targeting circRNF169 and circSTRN3 were able to drive the upregulation of collagen IV in these GMC supernatants, demonstrating that this coincided with an increase in α-SMA expression. This suggests that circRNAs may be transported in exosomes between different renal cell types, potentially shaping the course of DKD disease. Wang et al[61] determined that high glucose-induced mesangial cell-derived exosomes were capable of injuring podocytes via a mechanism dependent upon TGF-β1/PI3K-AKT signaling. Specifically, they observed that these exosomes led to increased podocyte TGFβ1R expression that could be reversed using berberine (BBR), which is an isoquinoline alkaloid derivative of *Coptidisrhizoma*. BBR has been shown to be capable of reducing inflammation, hyperglycemia, and oxidative stress, making it potentially ideal for the treatment of DKD[62]. Wu et al[63] similarly revealed that high glucose-treated GEC-derived exosomes were capable of triggering podocyte dysfunction and epithelial-mesenchymal transition, with the inhibition of TGF-β-containing exosome secretion being a potentially viable means of preventing fibrotic damage associated with DKD. Li et al[64] demonstrated that high-glucose conditions were capable of promoting EV production from podocytes via a mechanism dependent upon the ROS/NOX4 pathway.

Ravindran et al[65] isolated microparticles (MPs) from renal proximal tubular cells (RPTCs) treated with high levels of glucose. They subsequently determined that these MPs were capable of facilitating DKD progression by increasing phosphorylated 4E-binding protein 1, phosphorylated ERK1/2, phosphorylated-eIF2 α, alpha-smooth muscle actin, and phosphorylated-SMAD2 levels and SMAD4 nuclear translocation within cells. Such work suggests that efforts aimed at preventing MP production may be able to prevent DKD progression. Munkonda et al[66] also determined that podocyte MPs contained elevated levels of p-p38, p-Smad3, collagen, and fibronectin such that these MPs were able to promote fibrosis upon interacting with proximal tubule cells.

Stem Cell-Derived EVs and the Treatment of DKD

In recent years, there have been many promising studies of stem cell-derived EVs as therapeutic candidates for halting the progression of DKD. As shown in Table III, Jin et al[67] found that adipose stem cell-derived EVs were capable of inhibiting podocyte apoptosis and inducing autophagy in a murine

Table III. Studies of stem cell-derived EVs.

First author	EV origin	Type of extracellular vesicles	Disease Model	Results
Jin et al [67]	Adipose-derived stem cells	Exosomes	db/dbmouse model of spontaneous diabetes	Exosomes secreted from adipose-derived stem cells attenuated diabetic nephropathy by promoting autophagic flux and inhibiting apoptosis in podocytes
Grange et al[68]	Human bone marrow mesenchymal stem cells (MSCs) and human liver stem-like cells(HLSCs)	Extracellular vesicles	STZ-induced murine model of diabetes	The administration of stem cell-derived EVs reduced the progression of the pro-fibrotic processes
Zhong et al[69]	Human umbilical Mesenchymal stem cells	Microvesicles	STZ-induced murine model of diabetes	Mesenchymal stem cell–microvesicle-derived miR-451a ameliorated early diabetic kidney injury by negatively regulating P15 and P19
Jiang et al[70]	Human urine-derived stem cells	Exosomes	STZ-induced murine model of diabetes	USCs-Exo inhibited podocyte apoptosis and promoted vascular regeneration and cell survival
Ebrahim et al[71]	Rat bone marrow-derived MSCs	Exosomes	STZ-induced murine model of diabetes	MSC-derived exosomes were able to upregulate autophagy via suppressing themTOR pathway, and exhibited anti fibrotic effects
Nagaishi et al[72]	Rat Bone marrow-derived mesenchymal stem cells	Exosomes	STZ-induced murine model of diabetes	Mesenchymal stem cell therapy ameliorated diabetic nephropathy via paracrine renal effects
Duan et al[73]	Human urine-derived stem cells	Exosomes	HG-treated human podocyte (HPDCs) cultures	Trophic factors including exosomes ameliorated diabetic nephropathy via protecting podocytes

model of spontaneous diabetes (db/db), thereby protecting against DKD. In a similar vein, Grange et al[68] found that human bone marrow mesenchymal stem cell (hMSC)-derived EVs and human liver stem-like cells (HLSCs) were able to revert fibrotic progression in a murine model of STZ-induced diabetes. Zhong et al[69] indicated that hMSC-derived EVs containing miR-451a were capable of negatively regulating P15 and P19 in a murine model of STZ-induced diabetes, thereby protecting against renal damage. Jiang et al[70] detected that exosomes from human urine-derived stem cells (USCs) were similarly able to prevent podocyte apoptotic death and to promote angiogenesis as a means of preventing renal damage in an STZ-induced diabetic model system. Ebrahim et al[71] determined that rat bone marrow-derived MSC-derived exosomes were capable of modulating the mTOR pathway to induce autophagy and protect against DKD in an STZ-induced diabetes model, while Nagaishi et al[72] similarly established that rat bone marrow MSC-derived exosomes could protect against DKD through the paracrine transport of renal trophic factors. Lastly, Duan et al[73] showed that human USC-derived exosomes containing miR-16-5p were able to protect against DKD in a high sugar-treated human podocyte (HPDC) culture model system.

Conclusions

This review highlights the high volume of recent research aimed at characterizing potential EV-encapsulated protein and miRNA biomarkers of DKD. As urinary exosomes can be readily isolated, they represent an ideal sample source for disease detection and monitoring efforts. As discussed above, more recent work has also sought to explore the potential relevance of stem cell-derived EVs in DKD. While further work is required to validate an optimal subset of DKD biomarkers suitable for clinical utilization and to determine the most reliable means of extracting EVs from complex patient biosamples, our review underscores the clear therapeutic relevance of these EVs and the macromolecules contained therein. With further optimization, these samples and analytical approaches have the potential to enable more reliable and efficient DKD diagnosis, treatment, and prognostic assessment in the coming years.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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