Tell-Tale Signs of Perturbed Podocytes

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It is well recognized that differentiated cells in solid organs are in constant communication with each other through a variety of signals. Similar to communication with landlines, emails, social media, and texts, cells in the kidney can communicate with each other via hormones, autocrines, growth factors, cytokines, metabolites, and even portions of themselves. The latter form of communication may take the form of exosomes, microvesicles, damage-associated membrane proteins, and apoptotic bodies.

The physical segmentation of the cell may be an important mechanism by which an organ can communicate with distal neighbors within the organ or via organ to organ communication. At a basic level, the budding off of cell membranes into vesicles is a mysterious and confusing state of affairs. Apart from the budding-off process acting as a waste disposal system, the functional consequences of this process remain unclear. Indeed, the budding-off process is reminiscent of the budding off of yeast hyphae and may be a vestigial process.

Exosomes and microparticles (or microvesicles) are sometimes lumped together, but they are distinct entities separated by physical characteristics, methods of isolation, and biogenesis.1 By virtue of size, exosomes are 40–100 nm and microvesicles are 0.1–1.0 μm. Exosomes are derived from the endosomal network. The endosomal compartment sorts the various intraluminal vesicles and directs them to their correct destinations. Early endosomes fuse with endocytic vesicles and incorporate their content destined for recycling, degradation, or exocytosis. Apart from recycling endosomes, the rest of the early endosomes undergo transformations to become late endosomes. Late endosomes that fuse with the plasma membrane result in secretion of 30- to 100-nm vesicles and are termed exosomes. Microparticle formation is distinct from exosome formation. The leaflets of the plasma membrane can exchange their phospholipids via aminophospholipid translocases, called flippases and floppases. Under normal conditions, phosphatidyserine is exclusively in the inner monolayer of the plasma membrane.2 Upon cell activation, membrane budding or microparticle formation is induced by translocation of phosphatidylserine to the outer membrane leaflet via actin-myosin interactions.

There is a growing body of literature indicating that urine exosomes may provide important insights into the development and progression of diabetic kidney disease. Exosomes from patients with established diabetic kidney disease (eGFR<60 ml/min per 1.73 m2 and albuminuria) have reduced levels of mitochondrial DNA compared with healthy controls.3 Furthermore, the reduction in urine exosomal mitochondrial DNA was supported by the reduction of mitochondrial protein in human diabetic kidney tissues. Urine exosomes were found to be produced in epithelial cells and contain TGF-β1 mRNA, among many other transcripts, microRNAs and proteins. Exosomal-derived TGF-β1 mRNA from tubular epithelial cells was shown to contribute to fibroblast activation and production of matrix molecules, and may be an intrarenal path for progression of fibrosis linking tubular epithelial cells to fibroblasts.4 Hypoxic stress may be an stimulus for exosome release from tubular epithelial cells. Endothelial-derived exosomes were also shown to deliver mRNA to recipient endothelial cells and to regulate angiogenesis.5 The production of endothelial exosomes may be partly under the control of microRNAs (specifically miR-214). However, the regulation of exosomes from renal cells in vivo remains poorly understood and significant challenges remain to isolate and standardize urine exosomes and their intraexosomal constituents.

Microparticles have been described primarily in plasma and there is a large body of literature that demonstrates that microparticles are produced after various cell stresses. Microparticles have been established to be circulating in the bloodstream and approximately 80% of circulating microparticles retain markers consistent with platelet origin.2 However, endothelial cells, red blood cells, and leukocytes are also sources of circulating microvesicles. A recent study showed that microparticles derived from endothelial cells...
and monocytes may regulate podocyte function.⁶ Although microparticles are well described, there are few studies to examine the origin and role of microparticles in urine and originating from renal cells.

In this issue of JASN, Burger et al. evaluate the role of microparticles to discern the intraorgan state of podocytes using several models of diabetic mice.⁷ The authors addressed the question of whether podocyte microparticles are produced in excess from known stresses relevant to diabetic kidney disease and whether urine exosomes may provide added value with respect to the development of albuminuria. Isolation of exosomes from podocyte media and urine was performed with serial centrifugation steps and microparticles were quantitated using FACS analysis using Annexin V antibody to detect all microparticles. Podocyte-derived microparticles were quantitated using antibody to podocalyxin and/or podoplanin. Electron microscopy was performed to visualize the microparticles. Burger et al. used an additional novel method to quantitate the size of the microparticles via a nanoparticle detection system using a Nanosight LM10 instrument.⁷ This is a light-scattering technique that uses video analysis for sizing and enumeration of extracellular vesicles. The combination of methods and quantitation of microparticles provided added confidence that these methods could be utilized for future comparative studies.

Burger et al. convincingly showed that the number of microparticles increases dramatically from podocytes upon exposure to high glucose and stretch.⁷ High glucose, but not mannitol an osmotic control, stimulates a 5-fold increase in microparticle production from podocytes. Surprisingly, both angiotensin II and TGF-β failed to stimulate an increase in podocyte microparticles. The authors also showed that the high-glucose stimulation of microparticle release was blocked by the Rho kinase inhibitor, fasudil, implicating cytoskeletal reorganization as a contributor to microparticle release. Because angiotensin II and TGF-β have also been shown to stimulate the Rho kinase and affect cytoskeletal organization, there are likely other distinct mechanisms by which high glucose stimulates microparticle release from podocytes. Nevertheless, the authors clearly demonstrated that podocytes respond to high glucose with a robust increase in microparticle release.

The in vivo significance of microparticle release from podocytes was examined in different models of diabetic kidney disease. The authors studied three mouse models: streptozotocin (STZ)-induced, Akita, and OVE26 diabetic mice. A benefit of these various models was that hyperglycemia was induced via different pathways and the onset of albuminuria, as a clinically relevant outcome, varied in timing and degree in these various models. Using highly quantitative techniques in mouse urine, the authors found that there was a global increase in Annexin V–positive microparticles in the urine of the diabetic mice. Interestingly, the Akita mice exhibited an increase in urine microparticles weeks before demonstrable increases in albuminuria were detected. However, there was a large SEM in the values among mice in each group. More dramatically, the increase in podocyte-derived microparticles was more consistent with respect to the magnitude of release. In the nondiabetic mice, across the strains examined, the urine podocyte microparticles ranged from means of 5 to 339 microparticles per milligram of creatinine. In STZ-induced and OVE26 diabetic mice, the urine podocyte microparticles ranged from 4000 to 12,000 microparticles per milligram of creatinine. Again, the Akita mice released fewer microparticles; however, there was a gradual increase from 49 microparticles at 4 weeks to 96 at 8 weeks, 509 at 12 weeks, and 845 at 16 weeks. The increases in podocyte microparticles were detected before the onset of albuminuria in the Akita mice. Furthermore, there was a significant correlation between the number of microparticles and the degree of albuminuria across all three models, with coefficients of determination of 0.76 in STZ mice and 0.26 in Akita mice. This set of data across several models of diabetes provides added confidence that the biology is true across states and strains of mice.

Further studies to understand the biogenesis of exosomes and microparticles from renal cells will provide an improved biological perspective to decide how to use the information derived from these vesicles. Studies such as that of Burger et al.⁷ are especially valuable because the regulation of microparticles from podocytes was assessed, and quantitative measures were used in the urine studies. If similar studies are performed in urine samples from patients with diabetes who are well phenotyped, important insights could be obtained to identify and follow patients who are at risk for progressive kidney disease.

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DISCLOSURES

None.

REFERENCES

Tick Tock: Time to Recognize the Kidney Clock

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A variety of physiologic functions related to the kidney, including BGP, GFR, renal blood flow, and urinary sodium excretion, exhibit a circadian pattern of variation (reviewed by Stow and Gumz1). Whereas these clinical observations are well established, the underlying molecular mechanisms are not completely characterized. On the molecular level, the circadian clock consists of a complex series of transcriptional, translational, and post-translational feedback loops. More simply, the four core circadian proteins—Bmal1, CLOCK, Period (Per1, Per2, Per3) and Cryptochrome (Cry1, Cry2)—regulate expression of thousands of target genes via a transcriptional mechanism to perpetuate rhythms in physiologic function.3

There has long been a debate in the circadian field regarding the relationship between the central clock, located in the suprachiasmatic nucleus (SCN) of the brain, and the peripheral clocks, located in nearly every cell type and tissue of the body. Zeitgebers, or “time-givers,” act as inputs to the circadian clock and these cues include light and food (reviewed by Richards and Gumz4). The prevailing model at the present time is that the central clock in the SCN, entrained by light, acts as a “conductor” to coordinate the physiologic functions of the “orchestra” made up of peripheral clocks located throughout the body (reviewed by Richards and Gumz4). Neuronal and humoral signaling is involved in the function of the conductor to synchronize the peripheral clocks in the orchestra.

Generation of cell type–specific knockouts (KOs) has recently begun to shed light on the extent to which the peripheral clocks may independently contribute to physiologic function. For example, studies by Young and colleagues in the cardiomyocyte–specific clock mutant mouse have demonstrated a clear role for the CLOCK protein in the metabolic function of the heart (reviewed by Richards and Gumz4).

Specific deletion of Bmal1 in pancreatic β cells demonstrated a role for the circadian clock in glucose-stimulated insulin secretion and oxidative stress–induced β-cell failure.5 Until now, studies like these have been lacking for the kidney. In a groundbreaking report presented in this issue of JASN, Tokonami et al. demonstrate a role for a kidney–specific peripheral clock in the regulation of renal function and BP.6

The first report of a circadian clock–controlled gene in the kidney came from Saifur Rohman et al., with the demonstration that the Na/H exchanger NHE3 was directly regulated by Bmal1 and CLOCK via a transcriptional mechanism.7 Our subsequent reports showed that Per1 regulates the expression of the α subunit of the epithelial sodium channel and the activity of epithelial sodium channel.8,9 Consistent with these mechanistic molecular findings in renal models, studies in circadian KO mice have consistently demonstrated a role for each of the core clock proteins in BP control.10–13 An important role for the kidney in these BP phenotypes has often been proposed, but the lack of renal cell type–specific KO models of circadian genes has prevented the use of a genetic model to directly test this hypothesis.

In the report by Tokonami et al., floxed Bmal1 mice were crossed with Ren1Cre mice to generate mice lacking Bmal1 expression in renin–producing cells of the kidney. Specifically, Bmal1 expression was significantly reduced in the renin–secreting granular cells of the juxtaglomerular apparatus and in principal cells of the cortical collecting duct and the outer medullary collecting duct. Less dramatic decreases in Bmal1 expression were also observed in the medullary thick ascending limb. Reduction of Bmal1 expression in these specific