



A Novel Mechanism of Regulation for Exosome Secretion in the Diabetic Kidney

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Exosomes are nano-sized membrane vesicles generated by inward invagination of the endosomal membrane and released by the fusion of multivesicular bodies (MVB) with plasma membrane in various cell types (Fig. 1) (1). Once released, exosomes have diverse activities such as transmitting signals and biomolecules to neighboring or distant recipient cells and regulating the extracellular environment (2). Secreted exosomes have been shown to mediate intercellular communication among kidney cells and may play a key role in the pathogenesis of diabetic kidney disease (DKD) (3,4). Thus, it is important to understand the role and mechanisms of exosome secretion in the diabetic kidneys.

The study by Zeng et al. (5) in this issue of *Diabetes* advances our understanding of the molecular mechanism of renal exosome secretion under diabetic conditions. In this study, they provide evidence that DKD is not associated with an overall shutdown of the exosome biogenesis machinery but rather with a specific downregulation of RAB27B, a key RAB GTPase involved in exosome secretion (5,6). Overexpression of RAB27B restored the exosome secretion in renal tubular cells exposed to high glucose (5). RAB27B repression, as well as deficient exosome release, were observed in the renal cortex of both type 1 and 2 diabetic mice compared with control mice. Moreover, RAB27B mRNA expression decreased in both glomeruli and tubulointerstitium in patients with DKD when compared with those of healthy donors (5). Ostrowski et al. (6) had observed a notable reduction of exosome secretion and a characteristic asymmetrical perinuclear accumulation of CD63-positive MVB in RAB27B-silenced HeLa cells via electronic microscopic analysis, suggesting that the majority of secreted exosomes originate from a RAB27-dependent secretion event. They further showed that RAB27B/Slac2b interaction mediates the MVB

trafficking from microtubules to the actin-rich cortex at the cell periphery (6). Together, these data highlight a critical role of RAB27B in the regulation of exosome secretion.

One of the novel findings from this study was that the phosphorylation of FOXO1, a forkhead transcriptional factor that plays fundamental roles in various cellular functions (7), may prevent active FOXO1 nuclear accumulation and decreased release of exosomes via downregulation of RAB27B in proximal tubular cells with high glucose exposure. In addition, the authors confirmed that FOXO1 inactivation was associated with the reduction of RAB27B expression and exosome release from the renal cortex in diabetic mice. This observation suggests that the inactivation of the FOXO1/RAB27B pathway is a key event mediating the reduction of exosome secretion in the diabetic kidney.

The impact of the same environmental condition on exosome secretion may be different in various cell types. In contrast to the findings in the current study, prior studies investigated the effects of high glucose on exosome secretion in glomerular cells and reported that high glucose-treated mouse glomerular endothelial cells and mesangial cells secreted more exosomes than normal glucose-treated cells (8–10). According to those studies, the exosome secretion in high glucose-treated glomerular cells is increased. In addition, Lv et al. (11) observed an enhanced exosome secretion in tubular epithelial cells after albuminuria exposure, while Li et al. (12) demonstrated that tubular cells exposed to hypoxia secreted exosomes enriched with miR-23a without significant changes of exosome release. Therefore, exosome secretion by the same cell type responds differently to diverse environmental conditions.

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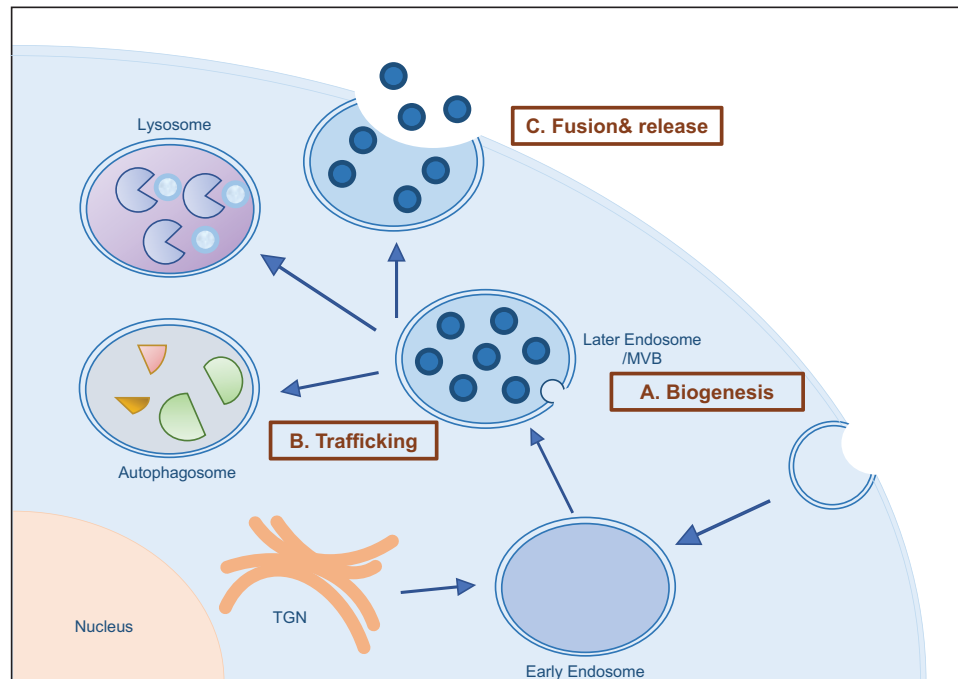


Figure 1—Schematic representation of exosome biogenesis, transport, and secretion. Briefly, the exosome generation consists of three steps: biogenesis, intracellular transport, and release. **A:** Biogenesis. An inward invagination of the plasma membrane or membrane fractions from the *trans*-Golgi network (TGN) form early endosomes, which later mature into late endosomes/MVBs via inward budding of the endosomal membrane as intraluminal vesicles (conventional mediators include endosome sorting complex required for transport [ESCRT], ceramide/neutral sphingomyelinase 2 [nSMase2], tetraspanins, ALG-2 interacting protein X [ALIX], etc.). **B:** MVB trafficking. The fate of MVBs may vary and depend on molecules and effectors mediated (e.g., RAB27A/B fuses with the plasma membrane, RAB7/11 fuses to lysosome/autophagosome, etc.). **C:** Exosome release. The membrane of MVB fuses with the plasma membrane, which results in the release of exosomes (e.g., soluble NSF attachment protein receptors [SNAREs], etc.).

Prior studies have suggested that secreted exosomes could have both protective and harmful roles in kidney cells depending on their contents and cell-specific effects. For example, many cells alleviate cellular stress by clearance of harmful materials such as heavy metals in exosomes (13). In addition, when bladder epithelial cells are infected with uropathogenic *Escherichia coli*, they secrete the invading bacteria in exosomes (14). Prior studies from the authors of the current report demonstrated that reduced secretion of exosomes from high glucose-exposed tubular cells results in proliferation and activation of fibroblasts that contribute to renal fibrosis in DKD (15). However, Wu et al. (8) showed that exosomes from high glucose-treated glomerular endothelial cells trigger the epithelial-mesenchymal transition and dysfunction of podocytes. Liu et al. (9) also demonstrated that tubule-derived exosomes induce fibroblast activation and kidney fibrosis. Therefore, the effects of secreted exosomes on kidney cell injury and disease progression depend on the cell types and contents of exosomes.

There are a few limitations of the study of Zeng et al (5). The authors only examined the effects of high glucose in exosome secretion by renal tubular cells in vitro. Further studies are required to verify the impact of the factors other than glucose per se, such as advanced glycation

end products (AGE) and TNF- α . Moreover, proximal tubule-specific genetic studies of FOXO1 and RAB27B on exosome secretion and DKD progression are needed to validate the authors' hypothesis in vivo. As numerous biomolecules are enriched in the exosomes, this makes them excellent candidates for the development of new biomarkers. It would also be interesting to investigate whether specific bioactive molecules or drugs could be loaded in tubule-specific exosomes as a drug delivery system for the treatment of DKD.

In summary, exosomes have divergent effects depending on their contents, cellular origin, and regulation of secretion. As illustrated by Zeng et al. (5), the study of exosomes will provide novel insights into the mechanisms of pathogenesis of DKD. Unfortunately, limited technologies are currently available to segregate exosomes based on their cell origins. The development of such technologies will significantly enhance our understanding of the cell-specific role of exosomes in the pathogenesis of DKD.

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