Proteinuria: it is time to look beyond the proximal tubule

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Tubulointerstitial fibrosis is a prominent feature of end-stage kidney disease. However, the mechanism of tubulointerstitial injury preceding chronic kidney disease remains to be determined. The degree of proteinuria has been utilized as a surrogate marker for prognosis and response to medical therapy in glomerular diseases. Proteinuria was proposed to induce progression by facilitating tubulointerstitial inflammation and fibrosis. Controversial hypotheses were put forward in regard to the mechanism of proteinuria-induced progression in glomerular diseases (1). It was suggested that increased protein trafficking as a result of albumin overload in the proximal tubule epithelial cells facilitates tubulointerstitial inflammation and fibrosis that is observed in proteinuric states independent of the glomerular pathology. Another hypothesis proposes that proteinuria-induced tubulointerstitial injury originates from tubular alterations as a result of encroachment of the glomerulotubular junction caused by glomerular crescents. However, there is a little doubt that in vivo and in vitro exposure to high concentrations of albumin result in production of proinflammatory and profibrogenic molecules in the proximal tubule cells (3, 4, 10).

The role of proximal tubule in albumin endocytosis renders it the focus of attention in proteinuric states. Proximal tubule epithelial cells handle 3–5 g of albumin in the glomerular filtrate via receptor-mediated endocytosis (9). In in vivo and in vitro albumin overload models, it was demonstrated that albumin endocytosis in the proximal tubule through megalin and cubilin receptor complex trigger production of proinflammatory and profibrogenic mediators such as transforming growth factor-β1 (TGF-β1), regulated on activation normal T-expressed and presumably secreted (RANTES), monocyte chemoattractant protein-1 (MCP-1), endothelin, and NF-κB that results in tubulointerstitial inflammation, fibrosis, and tubular apoptosis (10). Despite the convincing in vitro data, glomerular proteinuria models in mice with mosaic knockout of megalin have revealed conflicting results depending on the type of glomerular insult. Podocyte injury with mild glomerular disease and proteinuria has caused apoptosis and expression of monocye chemoattractant factor (MCP) in megalin-expressing proximal tubule cells whereas in the crescentic GN mice model lack of megalin in the proximal tubules was not protective of tubulointerstitial injury, suggesting that albumin endocytosis in the proximal tubule cells may not be the sole mechanism for tubulointerstitial fibrosis and inflammation in glomerular diseases (6, 8).

In a recent issue of American Journal of Physiology-Renal Physiology, Dizin et al. (2) have presented convincing evidence that proximal tubule may not be the only culprit in proteinuria induced tubulointerstitial injury. Their data suggested that proteinuria-induced damage may be multifaceted and a more thorough examination of distal nephron may be required to elucidate the cause of proteinuria induced tubulointerstitial damage in proteinuric states. The authors have demonstrated that in fact albumin overload/proteinuria may promote production of proinflammatory and profibrogenic molecules in the collecting duct cells contributing to tubulointerstitial fibrosis and inflammation. In glomerular diseases, high concentrations of albumin in the glomerular filtrate that was not captured by the overwhelmed endocytic machinery in the proximal tubule results in an increase in albumin delivery to the distal parts of the nephron. Previous studies have supported that cortical collecting duct (CCD) cells were not immune to the affects of proteinuria. It was proposed that cleavage of epithelial sodium channel (ENaC) γ-subunit leading to full activation of the channel and downregulation of renal outer medullary K+ channel (ROMK) in collecting duct cells could mediate sodium retention and decreased potassium excretion in proteinuric states (7). Apoptosis in proximal and distal/collecting tubules in correlation with the degree of proteinuria was reported on patient kidney biopsies with focal segmental glomerulosclerosis (FSGS; Ref. 5).

Authors have demonstrated that CCDs have the potential to contribute to proteinuria induced tubulointerstitial damage by triggering inflammation and fibrosis by combination of multiple experimental tools including in vivo, in vitro experiments and microdissection of CCDs. Albumin uptake in CCDs and connecting tubules was demonstrated by immunofluorescence staining of kidney sections and microdissected CCDs in puromycin-aminonucleoside (PAN) rats in addition to cell culture model. Furthermore, CCDs were able to respond to albumin overload via an autocrine mechanism by upregulation of profibrogenic, TGF-β1, SNAIL, vimentin, and α-SMA and activation of NF-κB and NF-κB target genes such as TNF-α, IκBα, and RANTES activation in association with tubulointerstitial fibrosis surrounding CCDs. Interstitial fibrosis was evident in the vicinity of normal looking glomeruli, which indicates that albumin caused tubulointerstitial damage was independent of the glomerular injury. Furthermore they have identified a novel receptor neutrophil gelatinase-associated lipocalin (NGAL)/lipocalin-2/24p3 receptor (F24p3R) responsible for albumin endocytosis in the CCDs. Expression of proinflammatory and profibrotic molecules was downregulated with inhibition of 24p3R, suggesting that this molecular network is linked to albumin endocytosis. TGF-β1 treatment has resulted in a decrease in 24p3R expression reminiscent of downregulation of megalin and cubilin in the proximal tubule under similar conditions.

This study explores an interesting and unknown area of research regarding the origins of tubulointerstitial injury observed in proteinuric states. The data presented in this study clearly show that CCDs respond to albumin overload similar to proximal tubule epithelial cells by promoting the production of proinflammatory and profibrogenic molecules. Any strategy directed only to block albumin endocytosis in the proximal tubule cells may not be protective against tubulointerstitial injury; on the contrary, this may increase albumin delivery to distal and collecting tubules initiating the aforementioned mo-

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lecular network. Therefore, the profibrogenic and proinflammatory affect of albumin overload in CCDs may explain the lack of protective effect of isolated megalin deletion in proximal tubules.

This study not only enhances our understanding of mechanism of proteinuria-induced tubulointerstitial damage but also paves the way for future studies to dissect the trafficking of albumin in CCD. The future questions include the role of clathrin and adaptor molecules in albumin handling in CCD. Despite >80% of inhibition in 24p3R expression with small interfering RNA, the decrease in albumin uptake was ∼30%, which brings the possibility of another receptor contributing to albumin endocytosis in CCD. The possible cross talk between 24p3R and sodium channels in CCDs may reveal an overlap between albumin endocytosis and trafficking of sodium channels. If so manipulating the overlapping pathways may attenuate tubulointerstitial damage and hypertension in proteinuric states.

Future research will shed light to the mechanism proinflammatory/profibrogenic response and downstream molecules involved in albumin endocytosis in distal nephron and whether blocking this pathway may lead to promising therapies to halt progression of the glomerular diseases.

DISCLOSURES
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