Kidney keratins: cytoskeletal stress responders with biomarker potential

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Keratins are cytoskeletal filamentous proteins that support the structural integrity of epithelial cells. Deficiency of the major simple epithelial keratins K8, K18, and K19 increases susceptibility to hepatobiliary injury, but keratin function in kidney injury has not been addressed. Djudjaj *et al.* examined renal keratins in health and disease, in both mice and humans. Their findings lay the foundation for pursuing keratins as markers and regulators of renal tubular epithelial injury.

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Keratins are abundant cytoskeletal proteins that belong to the large family of intermediate filaments (IFs).[1] Human keratin genes (*KRT*) encode 28 type I and 26 type II keratin proteins, which associate as obligate heterodimers to form the IF cytoskeleton of all epithelial cells.[1] A properly functioning keratin cytoskeleton ensures the structural and mechanical integrity of epithelial cells, while keratin dysfunction has been associated with over 60 individual disorders known as keratinopathies.[1] For example, mutations in epidermal keratins cause skin fragility diseases, while mutations in simple epithelial keratins predispose to acute and chronic liver diseases of multiple etiologies.[1] In addition to their fundamental roles as mechanoprotectors, keratins are known to regulate gene transcription, protein synthesis, stress signaling, cellular injury, and apoptosis. In particular, mechanistic studies involving keratin-transgenic mice have unequivocally demonstrated the importance of keratins for stress resilience in simple-type epithelia. While this has been well studied in the context of the liver and other digestive organs, the regulation and function of renal epithelial keratins have remained largely unexplored. In this issue, Djudjaj *et al.*[2] (2016) profile keratin expression, distribution, and regulation in normal and diseased mouse and human kidneys. They further evaluate the presence of urinary keratin 18 (K18) as a potential marker of renal epithelial injury (Figure 1). While the specific cell biological functions of renal keratins remain to be addressed, this study provides a comprehensive analysis of keratin fate in multiple experimental injury models and in different clinical settings of kidney disease. As such, this study should serve as a catalyst for new investigations in this area.

Simple-type epithelia express K7 and K8 (type II) and K18, K19, K20, and K23 (type I) as their predominant keratin isoforms.[1] The authors focused on K7, K8, K18, and K19 because older studies had established the presence of these 4 major isoforms in the human kidney. Healthy mouse glomerular parietal epithelial cells stained positive for K8 and K18 only, while all 4 keratins were abundant in cortical and medullary epithelial cells of collecting ducts.[2] A robust time-dependent *de novo* upregulation in the expression of the mRNA and protein for all 4 keratins in tubular epithelial cells (TECs) of the collecting ducts of distal tubules was observed upon induction of tubulointerstitial fibrosis using unilateral ureteral obstruction. This pattern persisted in other types of renal injury models, including adenine nephropathy, ischemia–reperfusion injury, folic acid nephropathy, and glomerulonephritis in the *Col4a3*$^{-/-}$ Alport mice.[2] The induction was a result of increased number of keratin-expressing TECs as well as an increase in the abundance of keratins per individual cell, based on image quantification analysis. Importantly, keratin upregulation was similarly demonstrated in patients with tubular injury due to myeloma cast nephropathy, diabetic nephropathy, and systematic lupus erythematosus.[2]

Thus, on the basis of their high abundance and further induction upon stress, keratins emerge as cytoskeletal stress proteins in the kidney. In that respect, renal keratins behave similarly to epidermal keratins in response to wound healing, stretch and inflammation, and liver keratins in response to toxicants, oxidative stress, viral infections, and other damaging insults.[3] The next critical follow-up step to these findings will be to understand what role keratins play in TECs undergoing stress. To that end, modeling TEC injury in mice engineered to lack or overexpress K8, K18, and K19 (which are available) should reveal the function of these proteins in the kidney. Variants of *KRT8* and *KRT18* are present in 5% of the human population and overrepresented in patients with acute and chronic liver diseases.[1] At this point is it not known whether *KRT* gene mutations are found in patients with kidney disease, and whether keratins serve as genetic modifiers for renal disease progression. This is another potential avenue for future investigation.

In addition to the observed quantitative changes, keratins were shown to undergo significant morphologic changes in TECs during kidney injury.[2] In healthy mouse and human TECs keratins exhibited membrane-proximal distribution, which was primarily basolateral in the medullary compartment

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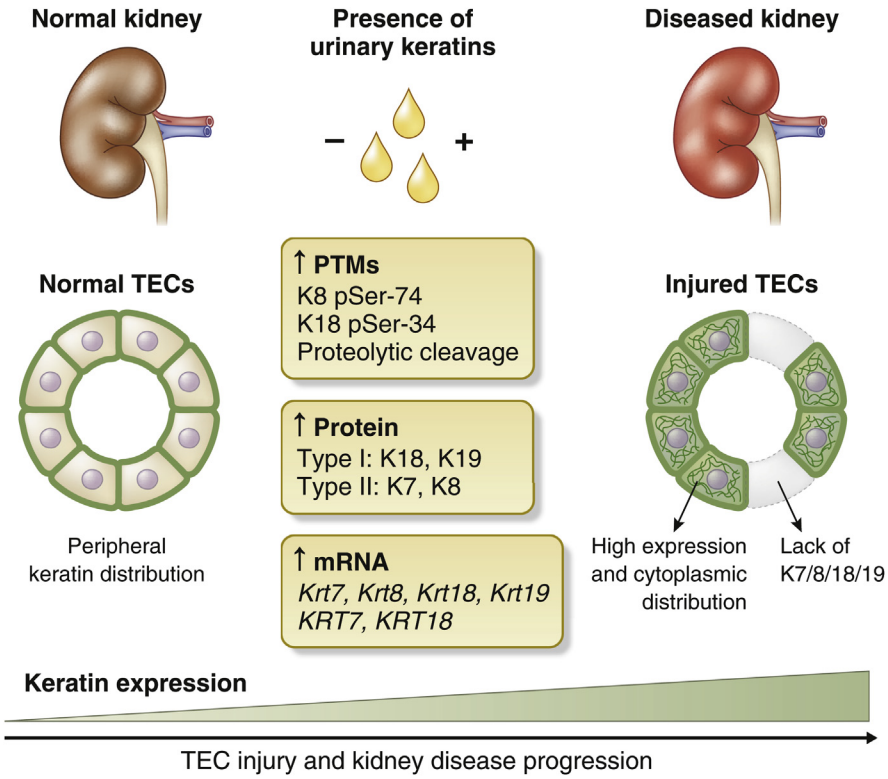

Figure 1 | Keratins are stress-inducible markers of renal epithelial injury. The simple epithelial keratins K7, K8, K18, and K19 are significantly upregulated at the mRNA level, the protein level, or both levels in mouse and human renal tubular epithelial cells (TECs) in response to injury. Subcellular keratin distribution patterns change from membrane-proximal to cytoplasmic in injured TECs, while some cells completely lack keratin expression. An increase in stress-dependent phosphorylation of K8 and K18, and release of full-length and cleaved K18 in urine of mice and humans with kidney injury, are also observed. Note that K8 Ser-74 and K18 Ser-34 correspond to amino acid residues in the human proteins. PTM, post-translational modification.

and apicolateral in cortical collecting ducts.[2] Upon injury keratins underwent circumferential redistribution, partly occupying the entire cytoplasmic space (Figure 1). The significance of this is not clear at present, although similar keratin changes have been observed in the pancreas during injury. One possibility for this redistribution is a stress-induced shift in the dynamics of the keratin filament cycle. The keratin filament cycle is a biosynthesis-independent turnover mechanism that facilitates rapid remodeling of the keratin network via dynamic filament formation and disassembly.[4] Phosphorylation is known to be critically involved in the assembly and disassembly dynamics of keratins and other IFs.[5] Djudjaj *et al.* detected significant phosphorylation of mouse K18 at Ser-33 in diseased but not in healthy kidneys.[2] This site was previously shown to be important for the association of K18 with the adapter protein 14-3-3 and to be critical for keratin organization and distribution.[6] Therefore, modulation of this K18 phosphorylation site by stress-activated kinases may account, at least in part, for the observed dramatic reorganization of the keratin filament network. Additionally, K8 phosphorylation at Ser-73 was also detected in a subset of TECs upon injury, and this site is known to be critically important for the cytoprotective roles of K8 in the liver.[5] Future studies employing unbiased proteomic analysis should reveal a more extensive repertoire of IFs, their associated post-translational modifications, and IF protein binding partners that may mediate these stress-dependent changes in the kidneys.

The authors also highlighted the presence of a small number of TECs in collecting ducts in both healthy and injured kidneys that completely lacked expression of all 4 keratins. Furthermore, at least as shown using immunohistochemistry, the proximal tubular epithelial cells had no detectable expression of K7, K8, K18, or K19, neither in healthy nor in injured murine kidneys. The functional distinction between the K7-, K8-, K18-, or K19-positive and K7-, K8-, K18-, or K19-negative TECs is not known at this point. It is also unclear whether these cells express or have upregulated another keratin or a different IF type, such as vimentin or nestin, both of which are also known to be expressed in the kidney. However, previous work showed that vimentin-null mice have normal tubular regeneration after ischemic injury,[7] suggesting that transient tubular expression of vimentin may not be important for mediating TEC injury responses. Further studies using single-cell analysis will be needed to ascertain the phenotypic and functional properties of the K7-, K8-, K18-, or K19-negative TECs in kidney injury.

Keratins are often used as disease markers in pathology, embryology, and experimental research.[1] The study of Djudjaj *et al.*[2] suggests that simple renal keratins might be a broad and helpful marker of tubular cell stress, in particular in murine kidneys. In addition, keratins might also serve as markers of parietal epithelial cells in healthy and diseased murine and human kidneys. This is of particular interest given the recent findings that these cells are crucially involved in the pathogenesis of crescentic glomerulonephritis and focal segmental glomerulosclerosis.[8]

Because of their abundance, circulating levels of keratins and their proteolytic fragments, released by dying cells, have been used as tumor markers for monitoring disease progression in a number of cancer types.[1] However, clinical utility of keratins as serum markers has been hampered by the lack of tissue specificity. On the other hand, significantly increased urinary levels of total and caspase-cleaved K18 in chronic kidney disease were shown to have predictive value for disease staging.[9] With that in mind, Djudjaj and colleagues investigated whether urinary levels of K18 are increased in mouse and human kidney injury. Immunoblot

analysis of the urine from normal, adenine nephropathy, and Alport mice, in addition to human urine from normal and acute kidney injury patients, revealed significant presence of full-length K18 and a 28-kDa K18 fragment in kidney disease.[2] The K18 fragment that was detected could represent the well-known caspase-generated product that is released by apoptotic epithelial cells.[3] Analysis for caspase activation and quantitative detection of apoptotic K18 fragments using specific antibodies were not undertaken. Nevertheless, the study provides strong evidence that urinary keratins may have utility as biomarkers for various forms of kidney epithelial cell injury. The predictive and prognostic value of specific keratins with particular post-translational modification signatures remains to be addressed in future clinical studies.

In summary, the comprehensive work by Djudjaj *et al.*[2] demonstrated significant and consistent induction of simple epithelial keratins K7, K8, K18, and K19 in different types of mouse and human kidney injury, which was accompanied by changes in their subcellular distribution and phosphorylation status (Figure 1). The authors also show that TEC injury is associated with urinary excretion of full-length and fragmented K18. Major strengths of the work include the use of numerous injury models and disease specimens, a complete evaluation of mRNA expression, protein expression, and protein localization, and quantitative analysis to compare normal and disease states. As such, the study sets the stage for future biochemical, cell biological, and physiological studies into the function, regulation, and disease significance of renal keratins. Rapid advances in our fundamental understanding of the IF cytoskeleton may fuel translational progress on keratins as novel biomarkers or therapeutic targets for human kidney diseases.

DISCLOSURE
The author declared no competing interests.

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Targeting the fatty acid transport protein CD36, a class B scavenger receptor, in the treatment of renal disease

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Augmentation of the CD36 pathway, which involves the uptake of several endogenous ligands including free fatty acids and oxidized low-density lipoprotein, contributes to the damage of proximal tubules and podocytes, whereas ablation of CD36 attenuates renal injury. Souza *et al.* demonstrate that 5A peptide can inhibit CD36 signaling, attenuate chronic kidney disease progression, and ameliorate inflammation and tubulointerstitial fibrosis by reducing the expression of inflammatory cytokines and chemokines, suggesting the therapeutic potential of 5A peptide against CD36-mediated renal injury.

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Dyslipidemia has attracted considerable attention as a factor that accelerates cardiovascular diseases, including renal injury. The condition can manifest as an increase in levels of free fatty acids and oxidized low-density lipoprotein (oxLDL). The deposition of visceral fat elicits metabolic syndrome and causes elevated cardiovascular risks. Visceral fat is an important marker of excess free fatty acids. Alteration in fatty acid metabolism, increase of very low-density lipoprotein, chylomicrons, free fatty acids, oxLDL, and reductions of high-density lipoprotein can be documented in patients with chronic kidney diseases. Excess lipid content has been shown to be a pathologic factor in renal disease, including obesity-related glomerulopathy and diabetic nephropathy. Increased levels of free fatty acids and oxLDL contribute to lipotoxicity in the renal tubular cells and glomerular cells, including podocytes. Fatty acids must

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