How tubular epithelial cells dictate the rate of renal fibrogenesis?

Kevin Louis, Alexandre Hertig

The main threat to a kidney injury, whatever its cause and regardless of whether it is acute or chronic, is the initiation of a process of renal fibrogenesis, since fibrosis can auto-perpetuate and is of high prognostic significance in individual patients. In the clinic, a decrease in glomerular filtration rate correlates better with tubulointerstitial damage than with glomerular injury. Accumulation of the extracellular matrix should not be isolated from other significant cellular changes occurring in the kidney, such as infiltration by inflammatory cells, proliferation of myofibroblasts, obliteration of peritubular capillaries and atrophy of tubules. The aim of this review is to focus on tubular epithelial cells (TEC), which, necessarily involved in the repair process, eventually contribute to accelerating fibrogenesis. In the context of injury, TEC rapidly exhibit phenotypic and functional changes that recall their mesenchymal origin, and produce several growth factors known to activate myofibroblasts. Because they are high-demanding energy cells, TEC will subsequently suffer from the local hypoxia that progressively arises in a microenvironment where the matrix increases and capillaries become rarified. The combination of hypoxia and metabolic acidosis may induce a vicious cycle of sustained inflammation, at the center of which TEC dictate the rate of renal fibrogenesis.

Key words: Epithelium; Fibroblasts; Acute kidney injury; Chronic kidney diseases; Fibrosis

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Louis K, Hertig A. How tubular epithelial cells dictate the rate of renal fibrogenesis? World J Nephrol 2015; 4(3): 367-373 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/367.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.367

INTRODUCTION

In the clinic, decrease in glomerular filtration rate correlates better with tubulointerstitial damage than with glomerular injury. Myofibroblasts are the main source of extracellular matrix in fibrotic organs, but the view that they merely result from the proliferation of resident interstitial fibroblasts at the onset of an injury is considered simplistic. Bone marrow derived stem cells, vascular smooth muscle cells, epithelial cells, endothelial cells and, more recently, pericytes, have all been suggested as significant sources of myofibroblasts. If anything, this three-ring circus reflects a real shift in the paradigm of the cell differentiation and fate process. In contrast to the established idea that cells are terminally differentiated, a more dynamic and plastic vision of how cells behave and react to environmental constraints has emerged. With respect to epithelial cells, a switch to a mesenchymal phenotype (stricto sensu, essentially a cell program that produces the extracellular matrix) makes sense since they are mesenchymal in origin: during embryogenesis the entire nephron, apart from the collecting duct, is derived from the mesenchymal to epithelial transition of the metanephric blastema. The concept of the reverse phenomenon, epithelial to mesenchymal transition (EMT), is well known to embryologists since primary epiblasts acquire mesenchymal properties in order to disperse, and to oncologists because, at the invasive front of carcinomas, transformed epithelial cells may also acquire migratory properties and metastasize. In 1995, Strutz et al. extended the concept of EMT to the field of fibrogenesis and its occurrence in adult solid organs, and discussed the possibility that tubular epithelial cells (TEC) might also acquire migratory properties and eventually create de novo myofibroblasts. It was proposed that TEC, properly stimulated, would convert and progress from the tubular structure to the interstitium. This major new idea was corroborated by one experimental study, but contradicted by other studies. Overall, the concept of EMT has focused on the TEC phenotype as a potential contributor to fibrogenesis. Rather than suggesting epithelial cells are the main source of myofibroblasts, we use the term "epithelial phenotypic changes" (EPC) to refer to in situ EMT. Analyzing sequential surveillance biopsies performed in kidney recipients, we and others have demonstrated that EPC are detectable in TEC and are associated with accelerated fibrogenesis and poor graft outcome. Results confirmed elsewhere. How the external microenvironment influences the phenotype of TEC is an area of intense research, although it is safe to say that the members of the Smad family play a major role. The balance between pro-fibrotic Smads (Smad 2/3) and anti-fibrotic Smads (Smad 1 and Smad 7) is controlled both inside the cells, for example by micro RNAs, and outside, where growth factors such as transforming growth factor beta (TGFβ), bone morphogenetic protein 7 (BMP7), hepatocyte growth factor (HGF), their trap proteins [connective tissue growth factor (CTGF), kielin/chordin-like protein (KCP)], and their cognate membrane receptors, all regulate the transient phenotype of “bistable” TEC. Excising ALK3, the gene encoding the receptor for BMP7, specifically in TEC, is sufficient to induce a worsening of renal fibrosis in mice subjected to different models of renal injury. This demonstrates that TEC exert some control on the process of fibrogenesis. The aim of this review is to provide an update on why EMT is detrimental and contributes in situ to renal fibrogenesis. Schematically, EMT reprograms TEC in a way that allows them to produce aberrant amounts of extracellular matrix, activate myofibroblasts from a distance, and eventually impair tissue oxygenation by decreasing the secretion of vascular endothelial growth factor (VEGF) by the epithelium. Table 1 indicates the main molecules produced by TEC and involved in renal fibrogenesis.

TUBULAR EPITHELIAL CELLS AS ABERRANT PRODUCERS OF EXTRACELLULAR MATRIX

The continuous decline in renal function is closely associated with the progressive accumulation of ECM proteins such as collagens and fibronectin. Excessive matrix is scattered between tubular structures, and also around tubules in what pathologists term “tubular atrophy”. Beneath the circular ECM that surrounds it, the epithelium often appears flattened, yet Nadasdy et al. have observed a high cell proliferation rate in those atrophic tubules, i.e., higher than in normal tubules or damaged but non-atrophic tubules, which suggests that cells are actively engaged in damage repair. In non-atrophic tubules located in non-fibrotic areas, EPC may be detected by immunohistochemistry, using antibodies targeting cytoskeletal proteins typical of myofibroblasts rather than epithelial cells. For example, vimentin, alpha-smooth muscle actin, and even fibroblast-specific protein 1, may be aberrantly expressed in cortical tubules, at the expense of epithelial proteins such as cytokeratins, cadherins, or ZO-1, which are lost. Importantly, this cytoskeletal switch occurs at the same time as increased production of two proteins that help to assemble ECM components: (1) heat shock protein 47 (HSP47), a collagen-specific molecular chaperone which helps to synthesize, process and secrete procollagen from the endoplasmic reticulum, and then acts in the folding and assembly of procollagen.
Table 1 Major molecules produced by tubular epithelial cells and involved in renal fibrogenesis

| Role in renal fibrosis | Ref. |
|-----------------------|------|
| Pro-fibrotic agent via EMT, activation of myofibroblasts. | [8,15,25-27,30] |
| Trap ligand for TGFβ (promotes its action) | [21,28-31] |
| Anti-Fibrotic agent. Counteracts TGFβ | [14,15] |
| Trap ligand for BMP7 (promotes its action) | [13] |
| Promotes fibrosis through the induction of TGFβ, CTGF, PDGF, and PAI-1. | [34-36,41-42] |
| Promotes endothelial survival through the induction of VEGF. | [38-40,42-43] |
| Promotes endothelial fenestration, and survival. | [32,33] |
| Induces EMT, enhances angiotensin 2 and endothelin secretion. | [44,50,52-53] |

TGFβ: Transforming growth factor β; CTGF: Connective tissue growth factor; BMP7: Bone morphogenetic protein 7; KCP: Kielin/chordin-like protein; HIF: Hypoxia inducible factor; VEGF: Vascular endothelial growth factor; PAI-1: Type 1 plasminogen activator inhibitor.

molecules\(^{19}\); and (2) prolyl 4-hydroxylase (P4H), which stabilizes collagen triple helix molecules\(^{20}\). We have reported on the de novo expression of HSP47 in proximal TEC from human renal allografts, which strongly suggests collagen synthesis\(^{21}\). Alpha and beta chains of P4H were similarly found in the tubular cells of most biopsy samples (but not in normal kidneys\(^{17}\). ECM proteins, in particular collagens and laminins, were indeed shown to be synthesized by TEC: Rastaldi et al\(^{17}\), using in situ hybridization, were the first to demonstrate that, in a number of human diseases affecting the native kidneys, TEC produce detectable amounts of collagens even before they lose cytokeratins\(^{17}\). Of note, the fact that TEC are able to produce ECM is not surprising, since TEC must build their own basement membrane. Nevertheless, manufacturing significant amounts of ECM and modifying the cytoskeleton in the same way as mesenchymal cells, attests to a cell reprogramming which precisely mirrors mesenchymal function (and as such would help to “contain” the injured area). One last point should be highlighted: cell matrix interactions also regulate the epithelial phenotype, hence qualitative changes in the matrix also matter. For instance, the deposition of fibrillar collagen types I and II (but not type IV) might further divert TEC from a normal (epithelial) differentiation, thus creating a vicious circle\(^{22,23}\).

Importantly, the intensity of EPC was found to be predictive of a more rapid progression of interstitial fibrosis and tubular atrophy in renal grafts undergoing sequential biopsies taken for immunological surveillance, and of a poorer allograft function in the long run\(^{10,21}\). To what extent TEC contribute to net fibrogenesis by the direct production of ECM is, however, unknown. EPCs may still serve as biomarkers to identify patients who have a high propensity for renal fibrosis, although the anti-fibrotic intervention required for these patients has yet to be developed. We have used two robust markers of EPC which resemble EMT, namely, the de novo expression of vimentin, and the translocation of beta-catenin into the cytoplasm, in the decision tree of the Certitem study, a prospective, multicenter trial performed in France. In this study, patients were stratified depending on the presence of EPC on a graft biopsy sample taken at three months’ post-transplant, and then randomized either to a conventional immunosuppressive regimen to prevent graft rejection, or to discontinue cyclosporine A and replace it with a mammalian target of rapamycin (mTOR) inhibitor\(^{24}\). This strategy was chosen because at the time the trial was designed, calcineurin inhibitors were regarded as the main cause of graft fibrogenesis. The main results of the Certitem trial are that the conversion from cyclosporine to everolimus at 3 mo (a timepoint at which interstitial fibrosis was not present or was very mild) failed to protect EPC\(^+\) grafts from fibrogenesis, since conversion to everolimus increased both clinical and infra-clinical graft rejection episodes. Any benefit that could have been expected from cyclosporine withdrawal was thus masked by inflammatory lesions. However, the predictive value of EPC was good, especially for patients who had a pristine kidney at three months’ post-transplant, and this study may serve as a proof of concept that the epithelial phenotype can be used in everyday practice. Should an anti-fibrotic agent enter our materia medica in the future, these markers would undoubtedly be helpful.

**TUBULAR EPITHELIAL CELLS SECRETE PRO-INFLAMMATORY AND PRO-FIBROTIC AGENTS**

TEC placed under cellular stress may produce various cytokines and chemokines promoting the recruitment of leukocytes. Interstitial inflammation is frequently present in fibrotic areas, such that pathologists often
disregard this kind of inflammation. For obvious reasons, it is difficult to measure the respective contribution of each cell type in this production (a complex crosstalk probably exists between epithelial and inflammatory cells, and potentially between endothelial cells as well). Among factors that sustain the growth and the activation of fibroblasts, TGFβ1 is a powerful cytokine. TGFβ1 signals through its cognate receptor, ALK5, and induces Smad 2/3 phosphorylation. By doing so, TGFβ1 contributes to multiple tubular phenotypic changes in epithelial cells, including EMT and death by apoptosis, but conversely promotes activation and proliferation in fibroblasts[25]. Bechtel et al[26] elegantly demonstrated that durably exposed to TGFβ1, fibroblasts will undergo epigenetic changes that will auto-perpetuate their proliferation. TEC were repeatedly found to be a source of TGFβ1 themselves, and thereby contribute to fibrosis progression[27]. CTGF is also an important molecule, since it can act as a positive trap for TGFβ1 (i.e., facilitating its binding to ALK5) and as a negative trap for BMP7 (preventing its binding to ALK3)[28]. Of note, TGFβ1 increases the transcription of CTGF, and this positive feedback loop amplifies the process. In renal allografts, we have detected that TEC also produce CTGF, and that, unlike Banff acute or chronic scores, the intensity of CTGF staining in TEC correlates well with graft dysfunction and proteinuria at the time of allograft biopsy[29]. In observations made by others, tubular cells were also found to produce CTGF in diabetes mellitus nephropathy[30], IgA nephropathy[31] and renal allografts[32]. Type 1 plasminogen activator inhibitor (PAI-1) is another important target gene of TGFβ1: by controlling the production of plasmin, PAI-1 regulates the activation of matrix proteases and of TGFβ1 itself, and is involved in inflammatory pathways[32]. Many studies have demonstrated that it may be secreted by renal epithelial cells during pathol[33].

The capacity for activated TEC to produce pro-fibrotic and pro-inflammatory agents directly can be enhanced by various circumstances, including renal hypoxia.

TUBULOINTERSTITIAL INJURY, INTRARENAL HYPOXIA AND FIBROSIS

Although renal blood flow represents 20% of the cardiac output, the kidney is physiologically at risk of hypoxia because of the presence of a complex arteriovenous oxygen shunt[34]. Hypoxia is instantly sensed by and in cells by the oxygen-dependent hypoxia inducible factor (HIF) pathway. HIF proteins (HIF-1 in epithelial cells, and HIF-2, also known as EPAS-1, in endothelial cells and fibroblasts) are heterodimeric transcription factors, composed of an α subunit and a common β subunit[35,36]. These two units only assemble under hypoxic conditions, because otherwise oxygen causes the ubiquitination of HIF-α through a complex system involving prolyl hydroxylases (PHDs) and Von-Hippel-Lindau (VHL) proteins. In the absence of oxygen, HIF-α heterodimerizes with HIF-1β, and the complex enters the nucleus to promote the expression of target genes. Of note, many growth factor stimulating fibroblasts, such as TGFβ1, CTGF and PDGF, are also induced by HIF[37,38]. In addition, glycolytic enzymes which facilitate anaerobic production of ATP, and angiogenic factors including VEGF, are among HIF-target genes. In turn, VEGF promotes endothelial functions and survival. VEGF is constitutively and selectively expressed in podocytes and TEC in normal kidneys, whereas expression of the VEGF receptor (KDR/VEGFR2) is largely restricted to adjacent peritubular capillaries[39]. Transcription and translation of VEGF-A in TEC is up-regulated by hypoxia, and VEGF expression correlates with expansion or regression of peritubular capillaries[40]. To what extent is the epithelial secretion of VEGF important in the context of a renal injury? It has been found that the conditional knockout of VHL in tubular cells (artificially increasing HIF-α even in the absence of hypoxia) resulted in the enhancement of VEGF and PDGF-B expression, an increase in endothelial cell proliferation and an attenuation of the tubulointerstitial damage following ischemia/reperfusion injury[41]. Accordingly, the specific ablation of VEGF-A in tubules leads to a specific dropout of peritubular capillaries, and reflects the importance of an intimate tubulo-vascular crosstalk to maintain peritubular microvascularization. Conversely, inhibitors of PHD (and thus upregulation of HIF and hence of VEGF) were recently shown to exert a protective role in a model of diabetic nephropathy where carbonyl and oxidative stress are particularly high.

A loss of VEGF expression by TEC has been documented in progressive renal diseases[42,43]. This data is counterintuitive since interstitial fibrosis could theoretically alter oxygen supply. By increasing the distance between capillaries and TEC, accumulation of ECM probably impairs oxygen diffusion. However, tissue oxygenation is decreased early in chronic renal failure and this precedes the accumulation of ECM, suggesting causality the other way around, i.e., a primary endothelial defect is probably there in the first place[35,37]. It could be speculated that the cell reprogramming that induces EPC also includes the decrease in secretion of VEGF, an important epithelial function. This would in turn promote capillary loss and, eventually, hypoxia[43]. Under hypoxia, TEC may either undergo apoptosis or survive with a mesenchymal phenotype[35].

TUBULAR CELL METABOLISM, RENAL TISSUE ACIDOSIS AND FIBROSIS

Proximal tubular cells, the predominant cell type in the interstitium, are notable in that they have a high
level of energy consumption because of multiple functions such as fluid and electrolyte homeostasis, active solute secretion and hormonal production[44]. They depend solely on aerobic oxidative metabolism[45] and, like cardiomyocytes, they use fatty acid oxidation (FAO) to produce energy. An abnormal accumulation of lipids was recently identified in epithelial cells in both mouse and human kidneys presenting fibrotic lesions, suggesting that β-oxidation is altered because of hypoxia. This accumulation might also alter epithelial functions and phenotype, and even lead to apoptosis[46].

In homeostasis, 80% of renal oxygen consumption is used for the tubular sodium reabsorption driven by Na-K-ATPase, which creates a negative membrane potential and a Na+ gradient. Na+-dependent co-transporters and counter-transporters use the energy of this gradient to promote the uptake of HCO3- and the secretion of H+ which both ensure the systemic acid-base balance[44,45]. Proximal TEC respond to acidosis by an increased bicarbonate reabsorption and transport into the blood and an increased extraction and catabolism of plasma glutamine, which allows for increased ammoniagenesis[46]. But the significant plasticity of intercalated cells eventually prevents acidosis in the collecting duct. They may alternatively secrete protons or bicarbonates, a phenotypic switch which is not due to EMT, but to a process of transdifferentiation[47]. However, how acidosis is sensed by cells from the collecting ducts remains unelucidated. Despite the fact that “systemic” metabolic acidosis usually appears at a late stage of chronic kidney disease[48], acid retention occurs earlier in the renal tissue. Thus, mice subjected to a 2/3 nephrectomy have H+ retention, but without alteration of the renal function. Intrarenal acidosis, or even dietary H+, can activate the renin angiotensin system, and increase intrarenal angiotensin 2 activity[49]. An oral alkali diet preserves GFR better than angiotensin 2 receptors or endothelin antagonists in experimental models of moderate chronic kidney disease in mice. In these models, H+ renal retention is present but not sufficient to induce a metabolic acidosis in plasma[50,51]. Thus, a dysfunction of TEC metabolism, in particular of acid base regulation, probably contributes to renal fibrogenesis and reduction of GFR. Clinical studies are ongoing to determine whether an alkali diet or an increased fruit consumption (i.e., a basic as opposed to acid dietary regimen) will affect the deterioration of GFR in patients with chronic kidney disease[44,52].

CONCLUSION
Preventing the progression of chronic kidney disease is still a major goal of modern medicine. It requires interventions that target and ideally reverse renal fibrogenesis. Of all the renal cell populations, whether resident and injured, or infiltrating and exacerbating injury, TEC are under closest scrutiny since they play a pivotal role in the process. They contribute directly to fibrogenesis by secreting aberrant amounts of extracellular matrix, and indirectly through the production of pro-fibrotic factors, which will act in a paracrine way and stimulate myofibroblasts and inflammatory cells. Progressively isolated by the surrounding matrix, and placed in a microenvironment where hypoxia and oxidative stress increase, they can no longer perform a protective function, including the promotion of endothelial cell survival and sufficient secretion of acid, in the absence of which fibrosis and inflammation increases. This circle is vicious on many levels, but also offers points of therapeutic intervention for the future.

ACKNOWLEDGMENTS
Alexandre Hertig is the recipient of a Contrat d’Interface Hospitalier with INSERM (2014-2017).

REFERENCES
1 MacKenzie-Haen S, Bader R, Grund KE, Bohle A. Correlations between renal cortical interstitial fibrosis, atrophy of the proximal tubules and impairment of the glomerular filtration rate. Clin Nephrol 1981; 15: 167-171 [PMID: 7237863]
2 Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AF, DuffIELD JS. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol 2010; 176: 85-97 [PMID: 20008127 DOI: 10.2353/ajpath.2010.090517]
3 Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neison EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest 2002; 110: 341-350 [PMID: 12163453 DOI: 10.1172/JCI215518]
4 LeBlue VS, Taduri G, O’Connell J, Teng Y, Cooke VG, Woda C, Sugimoto H, Kalluri R. Origin and function of myofibroblasts in kidney fibrosis. Nat Med 2013; 19: 1047-1053 [PMID: 23817022 DOI: 10.1038/nm.3218]
5 Neison EG. Plasticity, nuclear diapause, and a requiem for the terminal differentiation of epithelia. J Am Soc Nephrol 2007; 18: 1995-1998 [PMID: 17568015 DOI: 10.1681/ASN.2007040457]
6 Galichon P, Hertig A. Epithelial to mesenchymal transition as a biomarker in renal fibrosis: are we ready for the bedside? Fibrogenesis Tissue Repair 2011; 4: 11 [PMID: 21470408 DOI: 10.1186/1755-1536-4-11]
7 Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, Neison EG. Identification and characterization of a fibroblast marker: FSP1. J Cell Biol 1995; 130: 393-405 [PMID: 7615639 DOI: 10.1083/jcb.130.2.393]
8 Koesters R, Kaissling B, Lehr M, Picard N, Theilig F, Gebhardt R, Glick AB, Hähnel B, Hosser H, Gröne HJ, Kriz W. Tubular overexpression of transforming growth factor-beta1 induces autophagy and fibrosis but not mesenchymal transition of renal epithelial cells. Am J Pathol 2010; 177: 632-643 [PMID: 20616344 DOI: 10.2353/ajpath.2010.091012]
9 Kriz W, Kaissling B, Le Hir M. Epithelial-mesenchymal transition (EMT) in kidney fibrosis: fact or fantasy? J Clin Invest 2011; 121: 468-474 [PMID: 21370523 DOI: 10.1172/JCI44595]
10 Hertig A, Anglicheau D, Verine J, Pallet N, Touzot M, Ancel PY, Mesnard L, Brousse N, Baugey E, Glotz D, Legendre C, Rondeau E, Xu-Dubois YC. Early epithelial phenotypic changes predict graft fibrosis. J Am Soc Nephrol 2008; 19: 1584-1591 [PMID: 18434568 DOI: 10.1681/ASN.2007101160]
11 Galichon P, Vitoz N, Xu-Dubois YC, Coramine E, Vandermeersch S, Mesnard L, Hertig A, Rondeau E. Epithelial phenotypic...
changes detect cyclosporine in vivo nephrotoxicity at a reversible stage. *Transplantation* 2011; 92: 993-998 [PMID: 21900956 DOI: 10.1097/TP0b013e31822fa495]

12 **Hertig A**, Verine J, Moguena B, Jouanneau C, Ouali N, Sepe P, Glotz D, Ancel PY, Rondeau E, Xu-Dubois YC. Risk factors for early epithelial to mesenchymal transition in renal grafts. *Am J Transplant* 2006; 6: 2937-2946 [PMID: 17061992 DOI: 10.1111/j.1600-6143.2005.01559.x]

13 **Lin J**, Patel SR, Cheng X, Cho EA, Levitan I, Ulenbruch M, Phan SH, Park JM, Dressler GR. Ki67/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. *Nat Med* 2005; 11: 387-393 [PMID: 15793581 DOI: 10.1038/nm1217]

14 **Sugimoto H**, LeBlue VS, Dosukonda D, Keck P, Tadari G, Bechtel W, Okada H, Carlson W, Bey P, Rusckowski M, Tanpe B, Tanpe D, Kanasaki K, Zeisberg M, Kalluri R. Activin-like kinase 3 is important for kidney regeneration and reversal of fibrosis. *Nat Med* 2012; 18: 396-404 [PMID: 22306733 DOI: 10.1038/nm.2629]

15 **Zeisberg M**, Hanai J, Sugimoto H, Manmoto T, Charytan D, Strutz F, Kalluri R. BMP-7 counteracts TGF-beta-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 2005; 9: 964-968 [PMID: 12808448]

16 **Nadasdy T**, Laszik Z, Blicx KE, Johnson DL, Silva FG. Tubular atrophy in the end-stage kidney: a lectin and immunohistochemical study. *Hum Pathol* 1994; 25: 22-28 [PMID: 7906246 DOI: 10.1016/0148-8179(94)90166-X]

17 **Rastaldi MP**, Ferrario F, Giardino L, Dell'Antonio G, Grillo C, Grillo P, Strutz F, Müller GA, Colasanti G, D'Amico G. Epithelial-mesenchymal transition of tubular epithelial cells in human renal biopsies. *Kidney Int* 2002; 62: 137-146 [PMID: 12081572 DOI: 10.1046/j.1523-1755.2002.00403.x]

18 **Risdon RA**, Sloper JC, De Werdere HE. Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 1968; 2: 363-366 [PMID: 4173786 DOI: 10.1016/S0146-7269(68)90589-8]

19 **Nagata K**. Hsp47: a collagen-specific molecular chaperone. *Trends Biochem Sci* 1996; 21: 22-26 [PMID: 8848834 DOI: 10.1016/S0968-0004(96)80023-X]

20 **Kivirikko KL**, Myllärä P, Rihijärvi M. Protein hydrationlock: prolyl 4-hydroxylase, an enzyme with four co-substrates and a multifunctional subunit. *FASEB J* 1989; 3: 1609-1617 [PMID: 2537773]

21 **Xu-Dubois YC**, Baugey E, Peliter J, Colombat M, Ouali N, Jouanneau C, Rondeau E, Hertig A. Epithelial phenotypic changes are associated with a tubular active fibrogenic process in human renal biopsies. *Hum Pathol* 2013; 44: 1251-1261 [PMID: 23332931 DOI: 10.1016/j.humpath.2012.10.010]

22 **Zeisberg M**, Bonner G, Maershima Y, Colorado P, Müller GA, Strutz F, Kalluri R. Renal fibrosis: collagen composition and assembly regulates epithelial-mesenchymal transdifferentiation. *Am J Pathol* 2001; 159: 1313-1321 [PMID: 11583959 DOI: 10.1016/S0002-9440(10)62518-7]

23 **Becker GJ**, Hewitson TD. The role of tubulointerstitial injury in chronic renal failure. *Curr Opin Nephrol Hypertens* 2000; 9: 133-138 [PMID: 10757217 DOI: 10.1097/00015452-200003000-00006]

24 **Rostaing L**, Hertig A, Albano L, Anglicheau D, Durrbach A, Vuiblet V, Moulain B, Merveille P, Hazzan M, Lang P, Touchard G, Hazzan M, Rostaing L. Risk factors for kidney allograft fibrosis. *Kidney Int* 2011; 80: 944-950 [PMID: 21418855]

25 **Loeffler I**, Wolf G. Transforming growth factor-beta and the progression of renal disease. *Nephrol Dial Transplant* 2014; 29 Suppl 1: 137-145 [PMID: 24030832 DOI: 10.1093/ndt/gfu267]

26 **Abreu JG**, Keptra NI, Reversade B, De Robertis EM. Connective tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-beta. *Nat Cell Biol* 2002; 4: 599-604 [PMID: 12134160 DOI: 10.1038/nclab262]

27 **Burns WC**, Twigg SM, Forbes JM, Pete I, Tikellis C,Thallas-Bonke V, Thomas MC, Cooper ME, Kantharidis P. Connective tissue growth factor plays an important role in advanced glycation end product-induced tubulointerstitial-to-mesenchymal transition: implications for diabetic renal disease. *J Am Soc Nephrol* 2006; 17: 2484-2494 [PMID: 16914537]

28 **Nonaka Takahashi S**, Fujita T, Takahashi T, Wada Y, Fujie Y, Satomura A, Matsumoto K. TGF-beta1 and CTGF mRNAs are correlated with urinary protein level in IgA nephropathy. *J Nephrol* 2000; 13: 53-63 [PMID: 18264937]

29 **Cheng O**, Thuillier R, Sampson E, Schultz G, Ruipé Z, Zhang X, Yuan PS, Mannon RB. Connective tissue growth factor is a biomarker and mediator of kidney allograft fibrosis. *Am J Transplant* 2006; 6: 2292-2306 [PMID: 16889607 DOI: 10.1111/j.1600-6143.2006.01493.x]

30 **Eddy AA**, Fogo AB. Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action. *J Am Soc Nephrol* 2006; 17: 2999-3012 [PMID: 17035608 DOI: 10.1681/ASN.2006050503]

31 **Strutz F**, Kalluri R. Renal fibrosis: collagen composition and assembly regulates epithelial-mesenchymal transdifferentiation. *Am J Pathol* 2001; 159: 1319-1323 [PMID: 11583959 DOI: 10.1016/S0002-9440(10)62518-7]

32 **Becker GJ**, Hewitson TD. The role of tubulointerstitial injury in chronic renal failure. *Curr Opin Nephrol Hypertens* 2000; 9: 133-138 [PMID: 10757217 DOI: 10.1097/00015452-200003000-00006]
44 Curthoys NP, Moe OW. Proximal tubule function and response to acidosis. *Clin J Am Soc Nephrol* 2014; 9: 1627-1638 [PMID: 23908456 DOI: 10.2215/CJN.10391012]

45 Epstein FH. Oxygen and renal metabolism. *Kidney Int* 1997; 51: 381-385 [PMID: 9027710 DOI: 10.1038/ki.1997.50]

46 Kang HM, Ahn SH, Choi P, Ko YA, Han SH, Chinga F, Park AS, Tao J, Sharma K, Pullman J, Bottinger EP, Goldberg IJ, Susztak K. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat Med* 2015; 21: 37-46 [PMID: 25419705 DOI: 10.1038/nm.3762]

47 Hagège J, Gabe M, Richet G. Scanning of the apical pole of distal tubular cells under differing acid-base conditions. *Kidney Int* 1974; 5: 137-146 [PMID: 4205572 DOI: 10.1038/ki.1974.18]

48 Hsu CY, Chertow GM. Elevations of serum phosphorus and potassium in mild to moderate chronic renal insufficiency. *Nephrol Dial Transplant* 2002; 17: 1419-1425 [PMID: 12147789 DOI: 10.1093/ndt/17.8.1419]

49 Wesson DE, Jo CH, Simoni J. Angiotensin II receptors mediate increased distal nephron acidification caused by acid retention. *Kidney Int* 2012; 82: 1184-1194 [PMID: 22832514 DOI: 10.1038/ki.2012.267]

50 Wesson DE, Simoni J. Acid retention during kidney failure induces endothelin and aldosterone production which lead to progressive GFR decline, a situation ameliorated by alkali diet. *Kidney Int* 2010; 78: 1128-1135 [PMID: 20861823 DOI: 10.1038/ki.2010.348]

51 Manotham K, Tanaka T, Matsumoto M, Ohse T, Miyata T, Inagi R, Kurokawa K, Fujita T, Nangaku M. Evidence of tubular hypoxia in the early phase in the remnant kidney model. *J Am Soc Nephrol* 2004; 15: 1277-1288 [PMID: 15100368 DOI: 10.1097/01.ASN.0000125614.35046.10]

52 Goraya N, Simoni J, Jo CH, Wesson DE. Treatment of metabolic acidosis in patients with stage 3 chronic kidney disease with fruits and vegetables or oral bicarbonate reduces urine angiotensinogen and preserves glomerular filtration rate. *Kidney Int* 2014; 86: 1031-1038 [PMID: 24694986 DOI: 10.1038/ki.2014.83]

53 Goraya N, Simoni J, Jo CH, Wesson DE. A comparison of treating metabolic acidosis in CKD stage 4 hypertensive kidney disease with fruits and vegetables or sodium bicarbonate. *Clin J Am Soc Nephrol* 2013; 8: 371-381 [PMID: 23393104 DOI: 10.2215/CJN.02430312]
