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How tubular epithelial cells dictate the rate of renal fibrogenesis?

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Abstract

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

Core tip: In this review, we explain why and how tubular epithelial cells should be regarded not only as victims in the context of chronic kidney disease, but also as actors playing an ambiguous role. In particular, we report on studies which demonstrated that they can actively contribute to fibrogenesis itself, either directly, because their function has been reprogrammed in a way reminiscent of their mesenchymal origin, or from a distance, by influencing endothelial and myofibroblast functions. Last, they are seen as potential targets for new drugs aiming at controlling fibrosis.

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INTRODUCTION

In the clinic, decrease in glomerular filtration rate correlates better with tubulointerstitial damage than with glomerular injury[1]. 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[2-4]. 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[5]. 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[6]. The concept of the reverse phenomenon, epithelial to mesenchymal transition (EMT), is well known to embryologists since primary epiblasts acquire mesenchymal proteins 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*[7] 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[7]. 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[3], but contradicted by other studies[2,8,9]. 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[10,11]. Analyzing sequential surveillance biopsies performed in kidney recipients, we and others have demonstrated that EPC are detectable in TEC[12] and are associated with accelerated fibrogenesis and poor graft outcome[10], 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 β (TGFβ), bone morphogenetic protein 7 (BMP7), hepatocyte growth factor (HGF), their trap proteins [connective tissue growth factor (CTGF), kielin/chordin-like protein (KCP)[13]], 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[14,15]. 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*[16] have observed a high cell proliferation rate in those atrophic tubules, *i.e.,* higher than in normal tubules or damaged but non-atrophic tubules[17,18], 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.
Transforming growth factor beta pathway		
TGFβ	Pro-fibrotic agent *via* EMT, activation of myofibroblasts.	[8,15,25-27,30]
CTGF	Trap ligand for TGFβ (promotes its action)	[21,28-31]
BMP7	Anti-Fibrotic agent. Counteracts TGFβ	[14,15]
KCP	Trap ligand for BMP7 (promotes its action)	[13]
Hypoxia pathway		
HIF	Promotes fibrosis through the induction of TGFβ, CTGF, PDGF, and PAI-1. Promotes endothelial survival through the induction of VEGF.	[34-36,41-42]
VEGF	Promotes endothelial fenestration, and survival.	[38-40,42,43]
PAI-1	Pro-fibrotic agent. Inhibitis plasmin formation.	[32,33]
Ph		
Acidotic pH	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 Ⅲ (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 leucocytes. 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, TGFβ is a powerful cytokine. TGFβ signals through its cognate receptor, ALK5, and induces Smad 2/3 phosphorylation. By doing so, TGFβ 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 TGFβ, fibroblasts will undergo epigenetic changes that will auto-perpetuate their proliferation. TEC were repeatedly found to be a source of TGFβ themselves, and thereby contribute to fibrosis progression[27]. CTGF is also an important molecule, since it can act as a positive trap for TGFβ (*i.e.,* facilitating its binding to ALK5) and as a negative trap for BMP7 (preventing its binding to ALK3)[28]. Of note, TGFβ 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[21]. In observations made by others, tubular cells were also found to produce CTGF in diabetes mellitus nephropathy[29], IgA nephropathy[30] and renal allografts[31]. Type 1 plasminogen activator inhibitor (PAI-1) is another important target gene of TGFβ: by controlling the production of plasmin, PAI-1 regulates the activation of matrix proteases and of TGFβ itself, and is involved in inflammatory pathways[32]. Many studies have demonstrated that it may be secreted by renal epithelial cells during pathology[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 arterio-venous 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 TGFβ, 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[40,42]. 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[44]. 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 trans-differentiation[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[52,53].

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.

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