

Editorial Review

From acute injury to chronic disease: pathophysiological hypothesis of an epithelial/mesenchymal crosstalk alteration in CKD

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Abstract

Observational clinical studies link acute kidney injury to chronic kidney disease (CKD) progression. The pathophysiological mechanisms that underlie this process are currently unknown but recently published papers suggest that tubular epithelial cells and interstitial mesenchymal cells emerge as a single unit, and their integrity alteration as a whole might lead to renal fibrosis and CKD. The present article reviews the biological findings supporting the hypothesis of an altered epithelial/mesenchymal crosstalk in fibrosis development and progression toward CKD.

Keywords: epithelial/mesenchymal unit; epithelial cells; fibrosis; myofibroblasts

Introduction

Acute kidney injury (AKI) is common and remains closely associated with increases in short-term mortality and health-care utilization. Observational clinical studies have linked AKI to progressive chronic kidney disease (CKD), including the development of end-stage renal disease (ESRD) [1–4]. The relevance of this AKI/CKD nexus was recently reinforced by the Chawla study, which reports that the severity of AKI is a robust predictor of progression to CKD [5]. The topic is currently a matter of debate in the renal community [6]. Recent epidemiological studies showed that AKI community-based incidence was much higher than previously thought and has increased by 60% in the last few years [7], averaging 21.7 per 1000 patient-years in the USA [8] and affecting 1.2% of all hospitalized patients according to a prospective, observational, single-center study [9] using the AKIN criteria as defined by the ADQI Working group in 2002 [10]. The annual incidence of ESRD generated from AKI survivors has also increased (2011 Annual Data Report

from the US Renal Data System) and will be precisely assessed with the prospective ASSESS-AKI Study (Assessment, Serial Evaluation, and Subsequent Sequelae of Acute Kidney Injury) [11]. Thus, the estimated incidence of ESRD attributed to patients who survived an episode of AKI could be as high as a 25% (4.9 per 100 000 person-years), similar in importance to diabetes (5.2 per 100 000 person-years) and hypertension (4.8 per 100 000 person-years), well ahead of, for example, glomerular disease (2.5 per 100 000 person-years) and cystic kidney disease (0.6 per 100 000 person-years) [12–14].

Histopathologically, AKI, and especially acute tubular necrosis, is characterized by marked epithelial damage followed by a proliferative recovery phase where highly dividing epithelial cells repopulate tubules, whereas CKD is characterized by interstitial fibrosis and inflammatory cell infiltration, tubular atrophy, glomerulosclerosis and arteriosclerosis. Clinico-pathological studies have demonstrated that the extent of tubulo-interstitial fibrosis correlates better than that of glomerular sclerosis with the degree and progression of renal impairment, regardless of the type and anatomical origin of the inciting injury [15–18]. Therefore, exploration of the molecular mechanisms of the AKI/CKD nexus, with special attention focused to the cellular players active in these two pathological processes, is a central issue in renal research.

Several older [19–21] and more recent experimental findings [22, 23], together with the common histopathological notion that regions of active interstitial fibrosis predominantly exhibit a peritubular rather than a perivascular distribution [24, 25], suggest a causal role for tubular epithelial cells (TECs) inducing the proliferation and activation of myofibroblasts in diseased kidneys. Thus, a severe AKI episode might be followed by an incomplete repair of regenerating tubules giving rise to a paracrine activity triggering fibroblast proliferation and inflammation.

The present article reviews the biological findings that support the hypothesis of an altered epithelial/mesenchymal crosstalk leading to renal fibrosis.

The normal tubulo-interstitial space

The tubulo-interstitial space is composed of tubules and the interstitium [26–28]. TECs represent the large majority of the cells of the normal tubulo-interstitial space. The interstitium consists of sparse interstitial cells (fibroblasts and inflammatory cells) and peritubular capillaries, both embedded in an extracellular matrix (ECM) network (collagens I, III, VII; fibronectin; tenascin) [28–30]. Interstitial fibroblasts are mesenchymal cells with a spindle-shaped morphology [28, 31]. The fibroblasts in the peritubular interstitium bridge the spaces between the capillaries and the epithelia [32] and form a continuous network throughout the kidney. Renal fibroblasts display similar shapes and ultrastructure as fibroblasts in the interstitium of other organs [33–35]. They synthesize many constituents of the fibrillar ECM such as type I, III, and V collagen, and fibronectin [36, 37]. They are also a major source of ECM-degrading proteases such as matrix metalloproteinases, underscoring their crucial role in maintaining ECM homeostasis via ECM turnover regulation [38, 39]. Inflammatory cells present in the normal interstitium include macrophages (or histiocytes), dendritic cells and leukocytes such as plasma cells, lymphocytes and mast cells [40]. Finally, pericytes or perivascular cells are the supportive and pro-angiogenic cells of the peritubular capillaries.

Crosstalk among tubulo-interstitial cells, and between tubulo-interstitial cells and ECM, is very probably tightly regulated in the normal renal microenvironment. However, currently available knowledge on the physiological mechanisms and feedback loops is sparse and relies upon data derived from the altered microenvironment during disease.

The fibrotic tubulo-interstitial space

In contrast to the normal renal interstitium, fibrotic tubulo-interstitium is characterized by numerous cells (fibroblasts, myofibroblasts, inflammatory cells and atrophic TECs), rarefaction of peritubular capillaries and excessive accumulation of ECM as well as proteins originating from tubular and vascular basement membranes (collagen IV, laminin) [30, 41, 42].

Key cellular mediators of fibrosis are myofibroblasts [43]. Myofibroblasts share features with smooth muscle cells including expression of α -smooth muscle actin (α -SMA) and secreting ECM [44, 45]. Despite its pivotal role in disease progression, the origin of renal myofibroblasts is currently a matter of debate. Several hypotheses have been proposed, e.g. migration of circulating fibrocytes to the lesion site [46], differentiation from local fibroblasts [47] or pericytes [48], as well as direct transformation of resident epithelial cells or endothelial cells through an ‘epithelial-to-mesenchymal transition’ (EMT) or an ‘endothelial-to-mesenchymal transition’ [49, 50]. In parallel, a growing body of evidence involves the tubulo-interstitial microenvironment as a whole, whose alterations might be a fundamental trigger leading to myofibroblast activation.

EMT and fibrosis: a fascinating hypothesis under close evaluation

Several authors have stressed the relevance of TEC contribution to the fibrotic process through a direct transformation into activated fibroblasts known as EMT [45, 51]. This hypothesis is perfectly sound as the EMT process is well known in ontogeny and cancer where it contributes, respectively, to mesodermal structure formation and metastasis diffusion. In both cases, when migration is needed, epithelial cells have to transform into a migratory phenotype corresponding, in animal cells, to a mesenchymal phenotype with a contractile apparatus and a metalloproteinase arsenal that allow movement in the dense ECM. This hypothesis has become a widely accepted mechanism by which injured renal TECs contribute to renal fibrosis development [52, 53]. However, an increasing number [22, 48] of both *in vitro* [54] and *in vivo* [55] studies is raising doubts about the role of the EMT in the fibrotic process, not only in the kidney but also in other organs.

Epithelial cells are still under the spotlight

Nevertheless, beyond EMT, TECs remain central players in fibrosis. Histopathologically, epithelial cells are, in fact, the main injured cells in AKI. Clinically, whereas the majority of AKI patients do recover, it is now increasingly recognized that patients with severe AKI can progress to CKD [1, 2, 4]. Scientific evidence supports this AKI/CKD connection and derives from some interesting experimental work by Nath *et al.* [56]. These authors showed that AKI insult, which was induced through repetitive exposure to heme proteins, was invariably accompanied by a long-term glomerular filtration rate decrease associated with chronic tubulo-interstitial damages as measured by collagen deposition and transforming growth factor (TGF)- β 1 activation. More recently, the causal association between acute epithelial injury and fibroblast activation with consequent fibrotic outcome was documented in a number of AKI experimental models, including ischemic, toxic and obstructive models [57]. The authors convincingly showed that injured TECs stopped in the G2/M phase of the cell cycle, released pro-fibrotic cytokines, and that bypassing the G2/M arrest by administration of a p53 inhibitor, reduced fibrosis. Another group [58] additionally showed that sustained p53 inhibition with prolonged pifithrin- α administration negatively affected fibrosis in a rat model of ischemic AKI. This finding could however be due to an increased viability of infiltrating macrophages, as demonstrated by immunohistochemistry and *in vitro* analyses. Indeed, other studies confirmed that p53 inhibition in macrophages sustained their survival and pro-inflammatory function [59, 60]. These results therefore underscore the importance of appropriate timing and duration of p53 inhibition and also suggest that developing pharmaceutical strategies to selectively inhibit p53 in TECs as opposed to leucocytes may be an important consideration in the AKI therapeutic approach [58].

Renal epithelial cells and mesenchymal cells interact

Several studies showed that renal TECs and mesenchymal cells modulate mutually their biological behavior, through paracrine mechanisms as well as direct intercellular contacts:

- (i) Co-cultured renal TECs (Madin–Darby canine kidney cells) were demonstrated to decrease the mesenchymal stem cell (MSC) proliferation and regeneration, and, in turn, MSCs were shown to promote TECs cell differentiation, attesting to a bi-directional epithelial/mesenchymal crosstalk [23].
- (ii) Co-cultured rat renal TECs and human stromal cells (mesenchymal multipotent stromal cells) were demonstrated to interact, with the formation of different types of intercellular contacts, including ‘tunneling nanotubes’ through which transfer and exchange of cell contents, such as cytoplasm and organelles, can occur [61].
- (iii) Co-cultured renal TECs (proximal) were shown to increase cortical fibroblast proliferation and collagen synthesis through paracrine mechanisms, including the production and release of TGF- β 1 and platelet-derived growth factor (PDGF)-AB [19, 20].
- (iv) Co-cultured renal TECs (porcine LLC-PK1 and rat IRPTC cell lines) and rat MSCs were shown to interact in a paracrine manner [62]. Paracrine factors secreted by MSCs in response to injured TECs which were submitted to stressful conditions mimicking AKI, i.e. ATP depletion and/or serum free starvation were demonstrated to promote renal epithelial cell proliferation and to protect them against cell death. In particular, only conditioned medium recovered from the ‘homeostatic microenvironment’ (previous TECs/MSCs co-culture), but not from MSCs alone (without previous interaction with TECs), which were capable of inducing both the TECs proliferative response and cell death arrest. These results, which indicate that MSCs require a previous stimulus to secrete their bioactive molecules in order to have beneficial effects on injured TECs, clearly demonstrate a mutual crosstalk through a paracrine mechanism.
- (v) In a tetracycline-controlled transgenic mouse model, conditional overexpression of TGF- β 1 confined to renal TECs was demonstrated to induce widespread peritubular proliferation of resident fibroblasts, differentiation into myofibroblasts and subsequent proliferation, and progressive deposition of ECM [63].
- (vi) Injury of TECs and the consequent activation of the Notch signaling pathway, whose major biological role is to control the cell fate determination, differentiation and patterning of highly organized tissues [64], was demonstrated to play a key role in tubulo-*interstitial* fibrosis development. Thus, a study using *in vitro* and *in vivo* genetic and pharmacologic experiments [65] demonstrated that TGF- β 1-treated TEC induced Notch1 pathway activation. TEC expression of Notch1 correlated with both epithelial and interstitial cell proliferation, and TEC specific expression of Notch1 was necessary and sufficient for tubulo-*interstitial* fibrosis development.
- (vii) TEC injury and consequent deregulation of the BMP-7 signaling pathway was demonstrated to play a role in renal fibrosis in *in vitro* and *in vivo* studies. BMP-7 was shown to be expressed in tubules and might maintain a healthy differentiated epithelial cell phenotype [66, 67]. BMP-7 expression was found to be decreased in both acute [67] and chronic [68] renal disease models, and recombinant BMP-7 administration reduces the severity of acute injury [69] and prevents renal fibrogenesis in chronic renal disease [70–72]. In contrast, the BMP receptor activin-like kinase 3 (Alk3), whose tubular deletion was shown to enhance both epithelial damage with TGF- β 1 signaling and fibrosis, was found to be up-regulated early in diseased kidneys after injury [73]. Administration of a synthetic small peptide agonist of BMP signaling that functions through the Alk3 receptor (THR-123) was shown to reverse established fibrosis in different mouse models of acute and chronic renal injury [73]. (If, for one moment, we make an abstraction of EMT as an explanation for BMP-7-mediated effects, epithelial cell protection would be advocated as the causal role of reduced fibrosis.) Thus, a possible hypothesis would be that the degree of BMP-7-mediated effects on EMT determines the reduction in renal fibrosis.
- (viii) TEC injury and the consequent up-regulation of the chemokine CTGF, which is mainly expressed in tubular cells [74], could also play a role in renal fibrosis. In an *in vitro* co-culture model of TECs and tubule-*interstitial* fibroblasts (TFBs) that mimic the renal subepithelial mesenchyme, tubular TGF- β 1-induced CTGF was demonstrated to increase pro-fibrotic mRNA molecular levels in TFBs [75, 76]. In subtotaly nephrectomized TGF- β 1 transgenic mice, where an enhanced CTGF expression correlated with an accelerating renal fibrogenesis [75, 76], CTGF antisense oligodeoxynucleotides treatment significantly blocked CTGF mRNA expression in the proximal TECs, despite the presence of sustained levels of TGF- β 1 mRNA [75, 76]. This tubular CTGF mRNA level reduction paralleled an mRNA levels reduction in matrix molecules, suppressing renal *interstitial* fibrosis [75, 76]. Similar results were obtained with hepatocyte growth factor administration with respect to its effect on CTGF [75, 76].

In summary, these results suggest that epithelial/mesenchymal cells in the context of the physiological renal microenvironment constitute an ‘epithelial/mesenchymal unit’ (Figure 1). Imbalance of the normal homeostatic

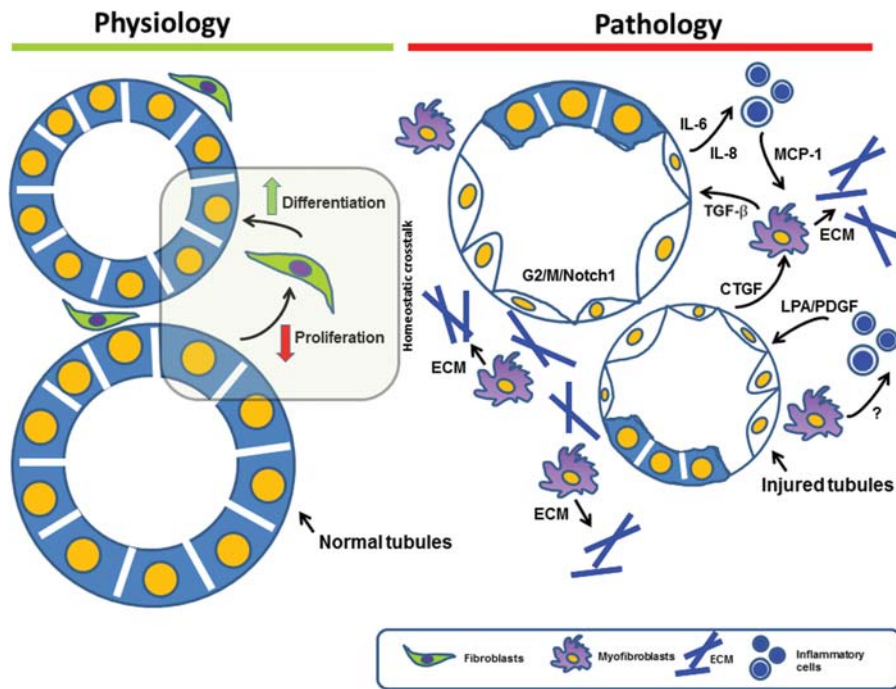


Fig. 1. Epithelial, mesenchymal and inflammatory cells interactions in physiologic and pathologic conditions.

Table 1. Inflammatory cells and fibrotic microenvironment interactions: data from the literature

Finding	Reference
Macrophages depletion ameliores fibrogenesis in mice	101
Macrophages induce apoptosis of proximal TECs	83
CD11c(+) F4/80(+) dendritic subset depletion is associated with persistent kidney damage after ischemia–reperfusion injury	102
Rag-2-null mice lacking both mature B and T lymphocytes are protected from renal interstitial fibrosis	103
FoxP3 ⁺ regulatory T cells (Tregs) were required for physiologic kidney regeneration	104
Renal collecting duct epithelial cells regulate inflammation in tubulointerstitial damage in mice	78
TECs regulate NK cell-mediated kidney ischemia–reperfusion injury through osteopontin expression	79

microenvironment might be, *per se*, a cause of fibroblast proliferation and myofibroblast differentiation leading to fibrosis. Hence, therapeutic targets which preserve the structural integrity of the epithelial/mesenchymal unit might represent an alternative to blockading the myofibroblast activation

Epithelial/mesenchymal unit interact with inflammatory cells

Interstitial inflammation is a well-known condition accompanying fibrotic lesions [77]. The epithelial/mesenchymal unit interacts with inflammatory cells, but details about these interactions are unclear, in part due to the complexity and heterogeneity of the inflammatory

infiltrate [77]. Following renal injury, both TECs and interstitial fibroblasts express receptors or produce soluble mediators, such as chemokines, cytokines, growth factors and lipid mediators [77–79], which induce interstitial mononuclear inflammation [77, 80–82]. Experimental attenuation of inflammation has been shown to influence the fibrosis process (Table 1). On their own, macrophages, with still unidentified soluble factors [83], have been shown to induce apoptosis of TECs, hence creating a vicious cycle.

In contrast, in the embryo, tissue damage can be repaired without any inflammation, scarring or fibrosis. Differences in both the inflammatory response (lower number of less differentiated inflammatory cells) and the growth factor profile (low levels of TGF-β1 and TGF-β2, low levels of PDGF and high levels of TGF-β3) were observed in scar-free healing in embryonic wounds in comparison to scar-forming healing in adult wounds [84].

Epithelial/mesenchymal unit alteration hypothesis in the CKD progression

Alterations of the epithelial/mesenchymal crosstalk might contribute to the progression of AKI to CKD. Progression might be due to processes unrelated to the original pathology that initiated AKI, occurring without any external novel or repeated obvious insult but through an internal, continuing and self-reinforcing pathological process resembling a vicious circle.

It could be hypothesized that pathological processes developing in regenerating tubules after a severe AKI episode, characterized by differentiation failure (stop in

the G2/M phase) and persistently high signaling activity (TGF- β paracrine secretion), might be the proximate cause that drives downstream interstitial events (fibroblast activation and proliferation) leading to tubulo-interstitial fibrosis and loss of functional renal parenchyma. Concomitantly, the same pathological processes could develop in the remnant healthy tissue, leading to CKD and finally ESRD. Indeed, in analogy to the experimental remnant kidney model [85], simulating human disease-associated loss of renal parenchyma, an alteration of the epithelial/mesenchymal unit through internal and continuing ischemic and/or toxic injuries might be the main factor responsible of the tubulo-interstitial fibrosis onset [86]. In the experimental remnant kidney model, compensatory adaptations, characterized by increased blood flow and glomerular hyperfiltration, maintain function at increased levels per nephron, leading to hypertrophy of the glomeruli and tubules [85]. As a consequence, renal structure and function deteriorate steadily, reaching an end-stage within several months. Therefore, it can be speculated that tubular ischemia due to tubular hypertrophy with increased epithelial transport, higher rates of O₂ consumption, with concomitant consecutive reduction in oxygen tension and hypoxic signaling together with tubular toxic-type injury due to glomerular hyperfiltration with protein overload and increased tubular reabsorption [87] might be the main process inducing alterations of the epithelial/mesenchymal crosstalk. It is interesting to note that this tubular-driven hypothesis is supported by Megyesi *et al.*'s study [88] reporting that partial kidney ablation in p21-deficient mice did not lead to tubulo-interstitial fibrosis and chronic renal failure. The P21 gene is induced to very high levels by oxidative stress and DNA damage, and the p21-derived protein is implicated in the control of the G1 to S phase transition in mammals, effectively stopping cell-cycle progression [89]. Thus, in Megyesi *et al.*'s study [88], the lack of a functional p21 gene induced an increased hyperplastic tubular reaction, as demonstrated by an increased proliferating cell nuclear antigen expression, which is a marker of cell-cycle progression, and inhibited the development of tubulo-interstitial fibrosis.

Therefore, it could be speculated that tubular injury might induce epithelial/mesenchymal crosstalk alterations with consecutive myofibroblasts activation and fibrosis, accounting for the AKI-CKD connection and the therefore CKD progression. Thus, microtubular internal events due to an altered parenchymal microenvironment, occurring without any external AKI factor and without any detected (or currently detectable) clinical expression, might lead to microfoci of interstitial fibrosis which progressively expand, to become diffuse and clinically expressed as CKD. It should however be emphasized that there are currently no human data to provide supporting evidence for epithelial/mesenchymal crosstalk alteration hypothesis in injured renal microenvironment. Therefore, analyses of human kidney diseases, such as diabetic glomerulosclerosis, IgA glomerulonephritis or primary FSGS, as well as on chronic allograft nephropathy are needed to demonstrate the relevance of an epithelial/mesenchymal crosstalk alteration in human nephropathies leading to CKD.

Conclusion: more research needed to pave the way to new anti-fibrotic medicines

Altered epithelial/mesenchymal crosstalk emerges as an interesting pathophysiological hypothesis to explain the clinically observed AKI/CKD interaction. Beyond the supposed or real role of EMT, epithelial cell phenotype retains a clinical and research interest. Thus, the experimental data in this review provide supportive evidence for a role of epithelial cells in fibrosis progression beyond the mere activation of fibroblasts toward an activated myofibroblast. Epithelial and mesenchymal cells emerge as a single unit whose integrity ensure normal renal structure and function. Correspondingly, fibrosis emerges as an alteration of that unit. The idea of a renal tubulo-interstitial unit would match perfectly with other pathological processes, e.g. stromal reaction to a carcinoma, where fibrotic cells have been already shown to play a pivotal role in disease onset and progression [90]. Striking similarities to alteration of epithelial/mesenchymal unit as a whole in kidney disease have been shown in idiopathic pulmonary fibrosis (IPF) where fibrosis has been shown to be the result of an altered epithelial/mesenchymal interaction [91]. Various human studies performed on IPF tissues demonstrated that activated alveolar epithelial cells were the main site of synthesis of TGF- β , PDGF and *tumor necrosis factor- α* [92–95]. *In vitro* studies suggested that during repair, regenerating epithelial cells lose their ability to inhibit fibroblast proliferation [96].

Beyond mere scientific interest, the hypothesis of curing fibrosis by preserving the structural integrity of the epithelial/mesenchymal unit is an attractive concept and might represent an alternative to the blockade of myofibroblast activation. Many pharmacological approaches targeting fibroblast activation-driven mechanisms, such as TGF- β 1, have, in fact, shown shortcomings due to the multiple functions of these mediators. In addition, rather different results have been obtained in animal models [97–99] that have not been confirmed in humans [100]. Strategies aimed at preserving the epithelial/mesenchymal homeostatic microenvironment will also have the advantage of allowing earlier intervention, for instance in AKI survivors at risk for CKD progression [2]. Earlier intervention might result in longer preservation of organ function, delaying the need for replacement therapy and improved cardiovascular protection. Therefore, basic and clinical studies are required in order to confirm the role of the epithelial/mesenchymal unit in fibrosis and to analyze which targets would protect epithelial cells without any risk of cancer induction.

Conflict of interest statement. M.P., D.C.B., M.M., G.H., I.F. and S.P. are employees and shareholders of Hoffmann-La-Roche Ltd, Basel, Switzerland.

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Received for publication: 16.1.12; Accepted in revised form: 22.5.12