Am J Physiol Renal Physiol 306: F473–F485, 2014.
First published December 19, 2013; doi:10.1152/ajprenal.00503.2013.

Review

The physical basis of renal fibrosis: effects of altered hydrodynamic forces on kidney homeostasis

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Submitted 16 September 2013; accepted in final form 13 December 2013

Grabias BM, Konstantopoulos K. The physical basis of renal fibrosis: effects of altered hydrodynamic forces on kidney homeostasis. *Am J Physiol Renal Physiol* 306: F473–F485, 2014. First published December 19, 2013; doi:10.1152/ajprenal.00503.2013.—Healthy kidneys are continuously exposed to an array of physical forces as they filter the blood: shear stress along the inner lumen of the tubules, distension of the tubular walls in response to changing fluid pressures, and bending moments along both the cilia and microvilli of individual epithelial cells that comprise the tubules. Dysregulation of kidney homeostasis via underlying medical conditions such as hypertension, diabetes, or glomerulonephritis fundamentally elevates the magnitudes of each principle force in the kidney and leads to fibrotic scarring and eventual loss of organ function. The purpose of this review is to summarize the progress made characterizing the response of kidney cells to pathological levels of mechanical stimuli. In particular, we examine important, mechanically responsive signaling cascades and explore fundamental changes in renal cell homeostasis after cyclic strain or fluid shear stress exposure. Elucidating the effects of these disease-related mechanical imbalances on endogenous signaling events in kidney cells presents a unique opportunity to better understand the fibrotic process.

chronic kidney disease; EMT; fibrosis; mechnotransduction; TGF-β1

Clinical Relevance of Renal Fibrosis

RENAL FIBROSIS IS CHARACTERIZED by the development of excessive extracellular matrix plaques in the tissue interstitium that compromise kidney function and result in eventual tubular atrophy and organ failure. This destructive pathology represents the final end stage of many different kidney disorders and can be found in patients suffering from diabetes (66, 124, 146), hypertension (12, 38, 169), some autoimmune disorders (22, 82, 133), congenital defects (7, 52), and chemical toxin exposure (17, 89, 138). Although ~13% of the US population is affected with some degree of chronic kidney disease (CKD) (21), the umbrella classification that encompasses progressive loss of renal function from all fibrotic stimuli, therapeutic options are scarce and often ineffective. Moreover, although our knowledge of the complexities of fibrogenesis has improved considerably over the years, it remains far from complete. One unique factor that drives disease progression involves dysregulation of the kidney's natural filtration processes deriving from supraphysiological levels of fluid stresses and pressures. Primary treatment thus focuses on controlling these destructive physical forces via diet modification and exercise, and tight control of blood pressure with medication (98). The last recourse is eventual kidney replacement and transplantation. Since CKD is also associated with an increased risk of cardiovascular disease, most patients die before the onset of full kidney failure (124).

Epithelial-to-Mesenchymal Transition: Friend or Foe?

During the progression of renal fibrosis, the accumulation of fibroblasts and myofibroblasts, activated by many different fibrogenic cytokines, leads to excessive extracellular matrix deposition and damage to normal tissue organization. Thus understanding the physical and biological factors that contribute to the activation and generation of matrix-producing fibroblasts could provide a therapeutic approach to halting the advancement of renal diseases. Currently, many hypotheses suggest that resident epithelial cells may themselves dedifferentiate and acquire a fibroblast-like phenotype via an epithelial-mesenchymal transition (EMT). These mesenchymal-like cells lose their polarity as well as their characteristic intercellular adhesion molecules such as E-cadherin and become more motile. Overall, this process may be particularly important for tubular repair following injury (62, 70); however, it is hypothesized that dysregulation of EMT signaling pathways could actually lead to sustained renal damage (Fig. 1*A*). Interestingly, mechanical stimuli may elicit strong differentiation signals in cultured epithelia, countering this EMT response (32). A number of studies support

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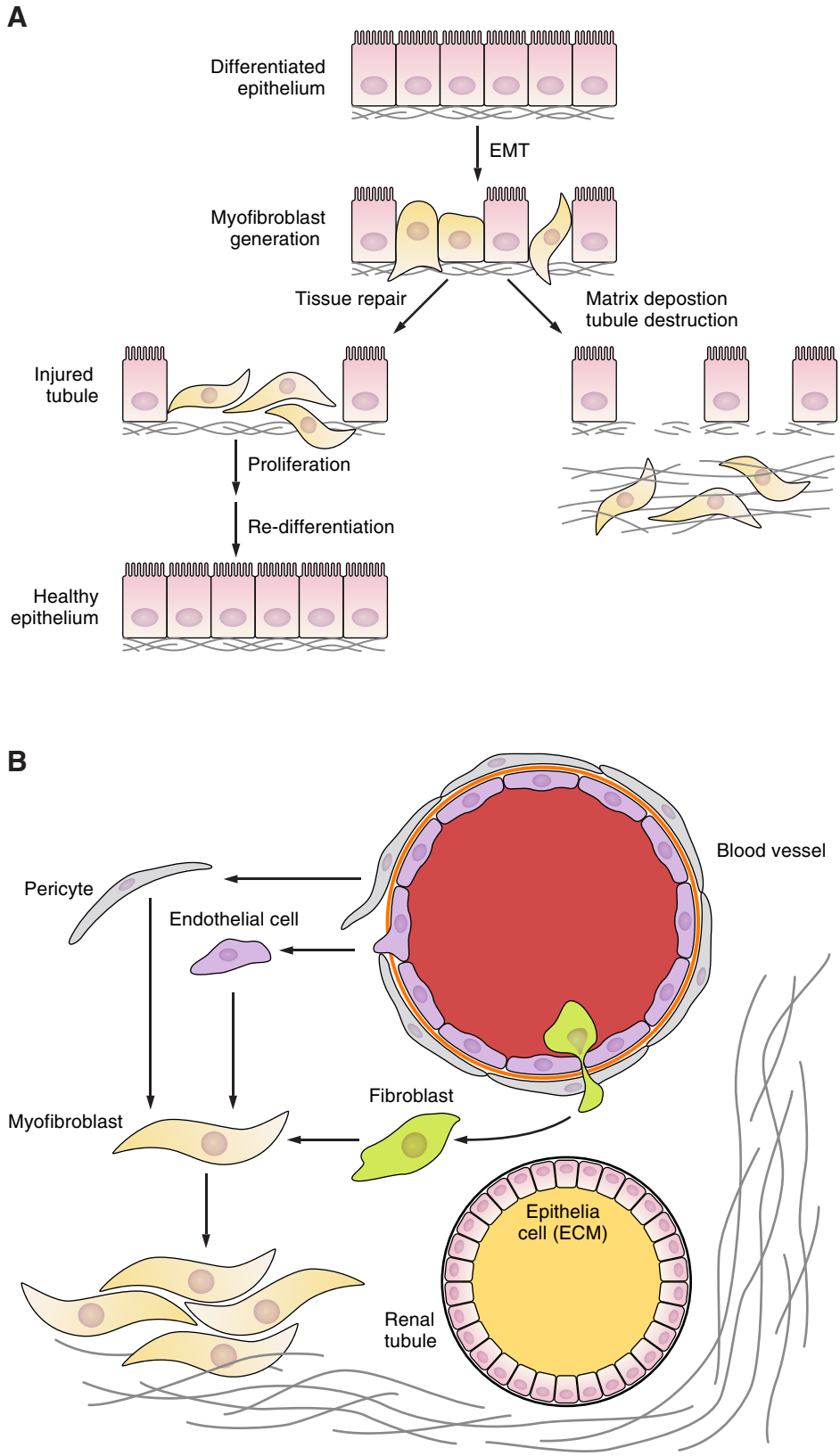


Fig. 1. Identifying the source of matrix-producing cells within the renal interstitium. *A*: epithelial-mesenchymal transitions (EMT), or the dedifferentiation of epithelial cells into a more fibroblast-like phenotype, are crucial elements of tubular repair. Following injury or denudation of one portion of the renal tubule, nearby epithelial cells could undergo EMT, migrate to the afflicted area, and proliferate. Given the appropriate biochemical cues, the mesenchymal cells could then regain their proper epithelial phenotype and restore tubule integrity. Dedifferentiated epithelia arising from dysregulation of this process have been hypothesized to be the primary sources of extracellular matrix accumulation in renal fibrosis, although the evidence regarding the role of EMT in disease progression has been contradictory. *B*: a number of fate-tracing studies have identified numerous different sources of matrix-producing cells: pericytes surrounding the renal microvasculature; circulating bone-marrow derived fibrocytes; and dedifferentiated endothelium from renal blood vessels.

the importance of EMT in renal matrix accumulation by tracing the lineage of plaque-producing fibroblasts from their epithelial origins (26, 72, 179). Furthermore, successful blockade of EMT seems to prevent kidney fibrosis, and vice versa, under some conditions (75, 76, 114, 176, 178). Still, this widely accepted premise is being hotly contested by other evidence identifying pericytes (63, 92), perivascular fibroblasts (93, 151), bone marrow-derived circulating fibrocytes (81), and even the adjacent

endothelium itself (88, 177) as the source of matrix synthesis (Fig. 1*B*). Additionally, multiple studies have now observed fibrosis occurring in the apparent absence of an EMT (33, 86, 118, 122). It is not immediately apparent how to reconcile these conflicting sets of data. One possible explanation is that the role of EMT in fibrogenesis is tissue and context specific. Also, since most studies identify EMT via somewhat random, ad hoc expression of an assortment of characteristic marker proteins, perhaps the importance of each individual marker varies across multiple models of disease progression. Finally, assessment of EMT as a phenotypic switch within a single time point, as occurs in most studies, may be inadequate if EMT is itself transient and part of a complex temporal program that cumulatively results in the progression of fibrosis.

Transforming Growth Factor-β1: A Potent EMT Initiator and Hypothesized Mediator of the Fibrotic Response

Transforming growth factor (TGF)-β1 is a potent pleiotropic growth factor with many diverse functions. In the kidney, TGF-β1 has been hypothesized as the primary mediator of renal fibrosis and eventual organ failure (143). In particular, TGF-β1 is a potent initiator of EMT (24, 48, 56, 90). The TGF-β1 signaling cascade is itself somewhat complex, with multiple points of regulation. Newly synthesized protein is secreted, possessing a latency peptide which effectively blocks activity until properly acted upon by a potential host of molecules, such as integrins, matrix metalloproteinases (MMPs), or other proteases (54, 107, 145, 164). Cleaved, active TGF-β1 then binds a type II receptor, which subsequently recruits one of many type I receptors responsible for activating downstream ligands. Canonical signaling involves phosphorylation and activation of the receptor-regulated SMAD transcription factors SMAD2 and/or SMAD3 (65, 120), but MAP kinases can also be stimulated (19, 64). Both of these SMAD proteins are also subject to regulation via inhibitory SMAD proteins such as SMAD7, or even ubiquitination by a number of different proteins (68, 148, 157, 163, 174).

Although multiple studies establish the fibrogenic potential of TGF-β1 (55, 67, 100, 130, 135), we and others are starting to challenge the simplicity of this finding. For example, a number of reports suggest overexpression of latent TGF-β1 exerts renoprotective effects, although the authors link these consequences to overall decreases in active TGF-β1 signaling via feedback inhibition (60, 166). Furthermore, while a number of diabetic mouse models demonstrate a therapeutic effect of TGF-β1 neutralization on renal function (142, 181), a phase I/II trial of anti-TGF-β1 therapy in systemic sclerosis failed to note any efficacy in halting the development of fibrotic lesions (27). Similarly, recent work employing homozygous deletion of TGF-β1 alleles in mouse macrophages succeeded in significantly reducing renal TGF-β1 expression as well as downstream SMAD activation in both ischemic and obstruction injury models, yet failed to completely attenuate fibrotic marker expression or halt interstitial matrix accumulation (61).

One potential explanation for the lack of a pathological role of TGF-β1 during the progression of renal diseases may involve the ability of TGF-β1 to regulate inflammation and immune cell activity. Although TGF-β1 may induce some inflammatory signaling events (129, 162), it primarily exerts protective anti-inflammatory effects that directly interfere with tumor necrosis factor (TNF)-α and interleukin (IL)-1 signaling, two potential mediators of renal injury (83, 101, 103). Further inhibition of tissue inflammation occurs via deactivation of infiltrating macrophages and direct inhibition of their attachment within the blood vessel (110, 155). Finally, exciting in vitro and in vivo work by Fragiadaki and colleagues (41) has recently discovered that low doses of TGF-β1 may stimulate expression of collagen I, while high doses of TGF-β1 trigger inhibition of collagen synthesis via the negative transcriptional regulator CUX1. Thus the phenotypic consequences of TGF-β1 signal transduction, or even the predominant signaling mechanism, could be entirely context dependent. There is extensive evidence of these specific, contradictory roles TGF-β1 plays in cellular physiology in the literature (1, 20, 23, 99). This possibility suggests that limited in vitro studies employing exogenous TGF-β1 treatment or forced ectopic expression would be inferior to other approaches that examine endogenous TGF-β1 signaling under patho-/physiologically relevant mechanical stimuli. Overall, these data also indicate that the sole focus on TGF-β1 alone as a therapeutic target in renal fibrosis may be inadequate.

The Endothelium and Vascular Components of Injury

All blood vessels, including the multiple capillary beds surrounding the nephron, are composed of a monolayer of endothelial cells that serves as a physical barrier, preventing the movement of large molecules into the surrounding tissues. Additionally, the endothelium is capable of regulating vascular tone, contributes to the local balance of pro- and anti-inflammatory mediators, and modulates coagulation cascades (49). Endothelial cell dysfunction is then often accompanied by inflammatory or thrombotic imbalances as well as improper vasodilatory phenotypes (95). Ongoing damage to the renal capillaries is thought to play a role in progressive loss of renal function (6), and a plethora of evidence at least supports the concurrence of endothelial damage and renal damage (10, 73, 85), but it remains to be seen whether these symptoms are a fundamental cause or indirect effect of renal pathologies. Increasing loss of renal function may result in less clearance of toxins or inflammatory byproducts that contribute to blood vessel rarefaction (116, 149). On the other hand, chronic hypertension or some other dysregulation of blood flow anterior to the kidneys would result in altered fluid dynamics and potentially disrupt the integrity of the glomerular tuft, not only affecting filtration capacity but allowing leakage of hazardous molecules that would otherwise remain in the bloodstream.

Regardless of the precipitating event, the effects of mechanical stresses on endothelial cells have been more extensively characterized than the corresponding response of epithelial cells. For example, fluid shear stress results in endothelial production of nitric oxide (NO) (5, 11, 69, 170), a potent vasodilator, as well as modulation of the clotting pathway and fibrinolytic activity via both thrombomodulin and tissue plasminogen activator (tPA), respectively (8, 79, 97, 156). In contrast, circumferential stretch enhances vascular tone (9, 25, 115, 134) and initiates rearrangements of the cytoskeleton (53, 58, 71). Additionally, mechanical stimulation results in the activation of a number of growth factors and paracrine signaling molecules such as inflammatory cytokines (3, 16, 96), TGF-β1 (35, 125, 144), and vascular endothelial growth factor

(VEGF) (44, 102, 167). Of particular note, pioneering work by Lin and coworkers (91) demonstrated that targeted inhibition of endothelial VEGF and platelet-derived growth factor (PDGF)-β signaling cascades not only prevented vascular deterioration but also attenuated the development of interstitial renal fibrosis. These results suggest that both the vascular endothelium and the renal epithelium likely cooperate in the progression of renal disease via a complex web of interactions and feedback loops. Other work by Miya and colleagues (104) further reinforces this possibility, establishing that human renal epithelial cells exhibit enhanced tubulogenesis, a sign of tubular repair mechanisms, when cocultured with endothelial cells. Unfortunately, despite extensive characterization of endothelial stress responses, our knowledge of the effects of such mechanical strains on epithelial cells of the kidney is woefully inadequate.

Mechanotransduction in Kidney Cells

Cells lining the nephron are continuously exposed to fluid shear as the blood is filtered. The physiological magnitudes of shear stress in the proximal tubule are an order of magnitude less than those experienced in the vasculature or <1 dyn/cm^2. Typical reported values range from 0.06 to 0.3 dyn/cm^2 (13, 37, 42). Following surgical nephrectomy, flow in the remnant nephrons has been shown to increase approximately threefold (57, 78) (Fig. 2*A*). Similarly, intratubular pressure increases dramatically from basal levels of ~0–10 cmH_2O following urological obstruction (126) (Fig. 2*B*). Renal epithelial cells have three main mechanisms of sensing these changes in externally applied forces: bending moments along the primary cilium, similar transmission of torque via the numerous apical microvilli, and the activation of mechanosensitive ion channels that trigger specific signaling events (Fig. 2, *C* and *D*). In models of human and murine polycystic kidney disease, mutations in the cilia-associated proteins polycystin 1 and 2 abrogate shear-induced spikes of intracellular calcium concentration ($[Ca^{2+}]_i$) (111, 131, 147, 173) (Fig. 2*C*). Recent work also suggests that cilia may be responsible for inhibiting fluid resorption in response to fluid shear stress (14). Such results indicate that cilia may be important mechanosensors on epithelial cells. In particular, increases in $[Ca^{2+}]_i$ may contribute to the progression of renal fibrosis by stimulating pathological signaling cascades. For example, Chiluiza and colleagues (18) demonstrated that ion channel-mediated calcium influx activated ERK kinases, which contribute to EMT and fibrosis, in both podocytes and HEK-293 cells. Additionally, inhibition of L-type calcium channels successfully reduced perivascular renal fibrosis in a rat model (141). Indeed, multiple reports are starting to suggest that calcium is a critical factor driving multiple stages of disease progression (84).

Fluid shear stress across the luminal surface of some kidney cells results in a $[Ca^{2+}]_i$ response even in the absence of a functioning cilium (32, 94, 171). Furthermore, the data supporting primary cilium as the main mechanosensor directly contradict the existence of glomerulotubular balance, whereby shear forces increase tubular fluid resorption (30, 31, 139). Accumulating evidence demonstrates that bending of the microvilli transmitted to the underlying cytoskeleton may directly increase Na^+ and HCO_3^- ion transport (Fig. 2*C*), thus stimulating fluid flow and governing this important physiological response (31, 50, 165). These two divergent effects of fluid shear occurring through distinct sensory mechanisms may provide context-dependent regulation of kidney filtration processes. Readers interested in a more thorough discussion of mechanotransduction mechanisms in kidney epithelial cells are directed to an excellent review by Weinbaum and colleagues (168).

Ureteric obstruction generally causes a transient but dramatic increase in intratubular pressure due to urinary pooling (126) (Fig. 2*B*). However, this initial stress declines over time due to dilation of the renal pelvis, as well as reduced renal blood flow and glomerular filtration (87, 160). Still, due to disruption of sodium transport in obstructive models of nephropathy, pooling urine may exert hypotonic swelling-induced mechanical strains on the tubules even after this relaxation occurs (51, 117). Although obstruction could occur naturally, this phenomenon is particularly relevant in a widely accepted animal model of kidney disease in which the ureter of one kidney is surgically ligated [unilateral ureteral obstruction (UUO)]. Cells are capable of sensing these circumferential strains primarily through stretch-activated ion channels (59, 113). As with fluid flow stimulation, mechanical strains initiate transient calcium-dependent signaling events in renal epithelial cells (39, 94).

Effects of Fluid Shear Stress on Kidney Tubular Epithelial Cells

The importance of fluid shear as a physiologically relevant pathological stimulus has already been discussed; however, very few studies have examined the effects of applied fluid flows on the progression of fibrotic diseases in kidney cells. Since tubulointerstitial damage correlates well with overall decline in renal function (109, 137) and involvement of tubular epithelial cells may be critical even in the early stages of renal dysfunction (45, 159), the response of these cells to shear stress warrants attention. In particular, direct application of supraphysiological levels of fluid shear via flow chamber allows precise examination and temporal resolution of key signaling events, including the possible role of an EMT in these diseases. The work of Essig et al. (37) was one of the first studies to demonstrate that shear stress (0.17 dyn/cm^2) altered cellular fibrinolytic activity via reduced expression of tissue plasminogen activator and urokinase. Similar results were subsequently obtained in a rat proximal tubule cell line under stresses of 5–10 dyn/cm^2 (123). Interestingly, Essig et al. (37) also observed the disappearance of cytosolic stress fibers on the basal surface and a reinforcement of the lateral actin network. Analogously, more recent data also demonstrate that moderate (1 dyn/cm^2) laminar fluid stress results in the increased formation of tight junctions and adherens junctions and an accumulation of focal adhesion proteins in the basement membrane (32) (Fig. 3, modified from Ref. 32 with permission). Together, these phenotypic changes, collectively dubbed "junction-buttressing," result in an overall more cohesive cell layer and are fundamentally inconsistent with the characteristic changes that occur during the transformation to a mesenchymal phenotype during an EMT. More importantly, these results are corroborated by recent evidence of increased E-cadherin expression, a well-established epithelial marker crucial for intercellular cohesion, in a mouse UUO model (29). Intriguingly, this increase

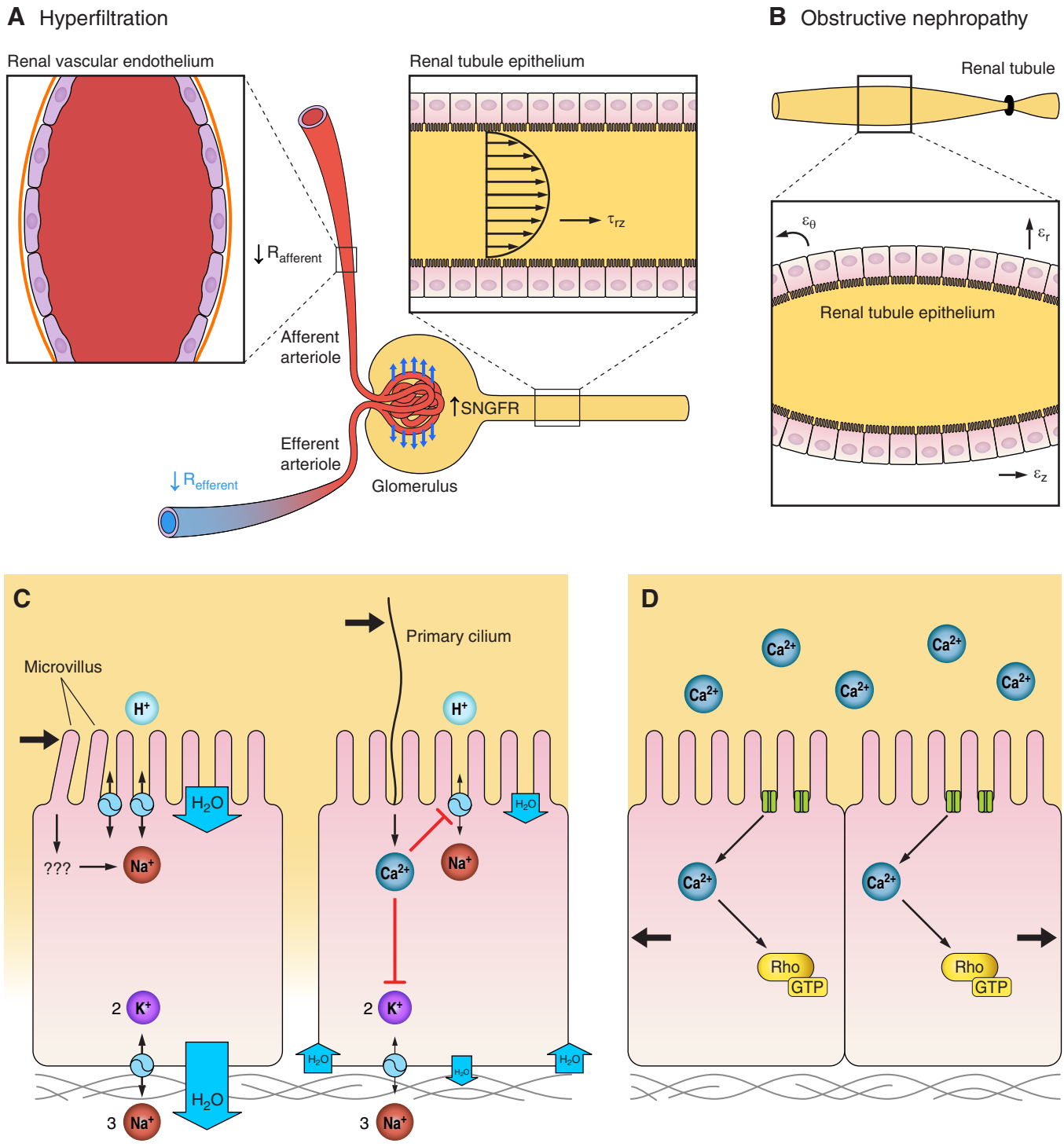
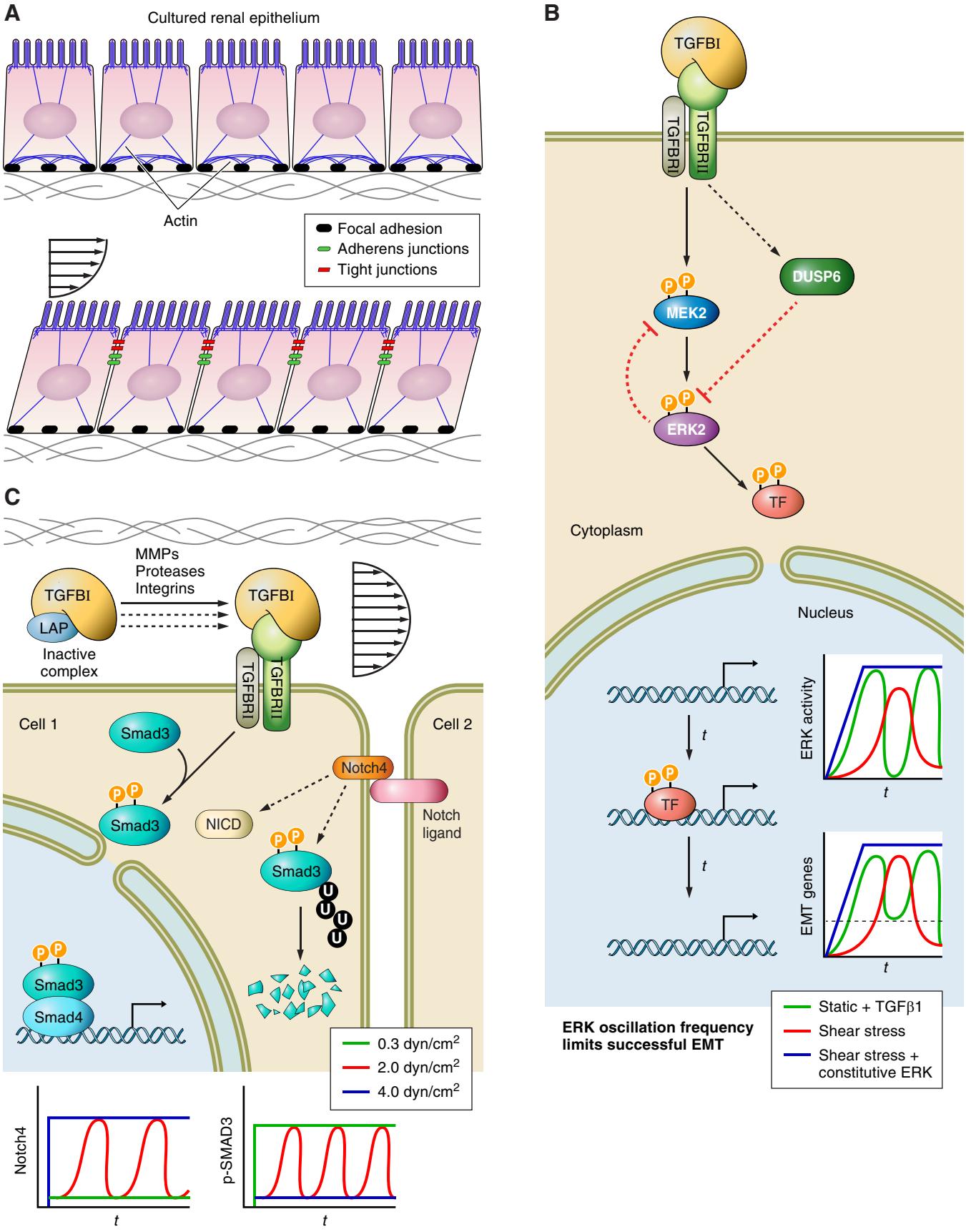


Fig. 2. Pathophysiological relevance of mechanical stimuli and mechanosensing in kidney epithelial cells. *A*: glomerular hyperfiltration is an adaptive response to renal injury. Preferential dilatation and reduction in the resistance of the afferent arteriole results in increased fluid pressures in the glomerulus and the afferent vessel. Endothelial cells lining the vasculature anterior to the glomerulus experience tensile strains associated with distension of the vessel walls due to this decreased vascular resistance. Stress responses of these endothelial cells may initiate and/or contribute to progressive renal deterioration. Higher glomerular fluid pressures also result in greater filtration (blue arrows) and exposure of epithelial cells lining the kidney tubules to pathological levels of fluid stress. Eventually, hyperfiltration leads to fibrotic scarring and tubular atrophy. *B*: in contrast, urological obstruction, a widely employed animal model of kidney disease, involves direct surgical ligation of the ureter. Subsequent pooling of urinary fluid results in circumferential strains in the tubules and stretching of the epithelial monolayer. *C*: both primary cilia and brush border microvilli have been proposed as the primary mechanosensor of fluid flow in kidney epithelial cells. In response to bending moments of individual villi, sodium transport via apical and basal ion pumps increases, leading to significantly higher water resorption. Bending of the cilium, on the other hand, results in a transient Ca^{2+} response and reduction of fluid transport either via decreased pump activity or increased paracellular permeability. *D*: finally, kidney epithelial cells detect tensile strains primarily via stretch-activated ion channels, which allow calcium influx and cytoskeletal rearrangement via Rho-GTPase activation.



was insensitive to TGF-β1 treatment, reflecting active inhibition of TGF-β1-mediated EMT during the disease response. Collectively, these results call into question the necessity of dedifferentiated epithelium in the progression of tubulointerstitial fibrosis.

Recent work in our laboratory employing a shear-induced model of fibrosis offers further insight into the role of TGF-β1 and EMT in fibrogenesis (Fig. 3, *B* and *C*). Utilizing an immortalized human proximal tubule cell line, HK-2, our data reveal that PTECs stimulated by supraphysiological levels of fluid shear exhibit a decrease in migratory potential as measured via wound migration and Transwell assays. These shear-activated PTECs failed to display any characteristic EMT changes but exhibited increased type I collagen deposition. These observations are altogether inconsistent with an acquired mesenchymal phenotype and exclude the occurrence of EMT in concomitant type I collagen expression (46). Furthermore, we identify inherent oscillatory, TGF-β1-mediated activation of ERK2, a key signaling molecule responsible for the progression of EMT in many different cell lines (28, 119, 150, 172). In static cells subjected to high concentrations of exogenous TGF-β1, oscillatory ERK2 activation is observed but nevertheless results in successful EMT and relatively persistent expression of downstream mesenchymal genes (Snail1, vimentin, and N-cadherin). In contrast, fluid shear stress elicits more sporadic bursts of ERK2 activity and effective transmission of the oscillatory response to downstream gene expression (Fig. 3*B*). These unique, divergent responses of the TGF-β1 cascade could potentially be due to kinetic and mechanistic constraints imposed by other pathways activated under shear conditions (46). Importantly, the more sporadic ERK2 oscillations exhibited by shear-stimulated PTECs are insufficient to sustain a successful EMT response. Only transfection of PTECs with a constitutive ERK2 mutant before shear exposure results in sustained expression of EMT marker proteins as well as compensation of shear-induced reductions in cell motility; however, such persistent ERK2 activity does not result in excessive matrix deposition. Cumulatively, our data suggest that temporal patterns of ERK activity may define unique phenotypic outcomes. One potential limitation of this hypothesis is the use of an in vitro cell culture system, which may not accurately represent the physiological environment; however, in conjunction with the aforementioned evidence, these results provide further corroboration that EMT and fibrosis may be mutually exclusive events.

We also identified dynamic Notch4-dependent regulation of the TGF-β1 signaling axis in part via targeted degradation of downstream SMAD3 protein (47) (Fig. 3*C*). PTECs subjected to low, more physiological shear stress levels (0.3 dyn/cm^2) exhibited negligible levels of the Notch4 intracellular domain, the cleavage product of successful Notch4 ligand-receptor binding responsible for generating Notch-dependent transcriptional responses. This absence of the Notch4 signaling domain occurred concomitantly with high levels of circulating active TGF-β1 protein and persistent SMAD3 phosphorylation. Higher shear stress exposures (2 or 4 dyn/cm^2), in contrast, dramatically reduced the levels of active TGF-β1 protein in the surrounding media (46). While similar variations in TGF-β1 gene expression were also observed over this range of shear stresses, it is not clear to what extent these differences in the levels of active TGF-β1 protein reflect posttranslational control of the TGF-β1 latency complex via MMPs, integrins, or some other catalytic protease. More importantly, shear-stimulated PTECs exhibit synchronous temporal oscillations in the levels of active, phosphorylated SMAD3 under a moderate shear stress regime (2 dyn/cm^2). These oscillations disappear as the shear stress level is increased to 4 dyn/cm^2, where the levels of Notch4 intracellular domain reach their zenith and phosphorylated SMAD3 protein declines to negligible levels. The importance of these unique temporal variations is further reinforced by our observations that the levels of active TGF-β1 the cells experience during shear exposure oscillates over all of the magnitudes of stress examined. These data, in concert with the additional discovery that overexpression of TGF-β1 abrogates shear-induced collagen deposition altogether (46), suggest that TGF-β1 itself may exert context-dependent effects with the overall phenotypic consequence determined by the precise temporal characteristics of the stimulus and downstream signal response. For example, work by a number of different groups has also demonstrated this phenomenon whereby differences in pulse frequency of exogenously supplied TNF-α resulted in observable differences in transcriptional activation of different classes of downstream genes (4, 154, 158). It is critically important to reinforce that one limitation of any analysis based primarily on in vitro studies is whether these results accurately reflect real in vivo phenomena. While these preliminary data may provide a novel perspective on the complex and controversial effects of TGF-β1 on the progression of fibrotic diseases, further validation and corroboration in both clinical and in vivo settings is required. At the very least, the complex time- and dose-dependent behavior exhibited by the TGF-β1 cascade suggests that only a system-level analysis of TGF-β1 signaling events provides an ade-

Fig. 3. Fluid shear stress maintains epithelial phenotype via oscillatory modulation of the ERK cascade and inhibition of SMAD-dependent transforming growth factor (TGF)-β1 signaling. *A*: recent reports examining the response of kidney epithelial cells to fluid shear have led to the development of the "junction-buttressing" model. Epithelial cells grown in static culture exhibit extensive stress fibers on their basolateral surface that maintain firm adhesion of cells to their underlying substrate. Furthermore, this tension is transmitted to the lateral membrane and causes individual cells to separate and pull away from their neighbors. With the application of an external fluid flow, basolateral stress fibers disappear and the lateral actin network is reinforced. Both tight and adherens junctions form as mechanically deformed cells contact one another. This enhancement of cellular cohesion is directly antithetical to the mesenchymal characteristics acquired after an EMT. *B*: we discovered that shear stress induces oscillatory activation of the key EMT signaling component ERK2. Feedback inhibition from the DUSP6 phosphatase and ERK-mediated suppression of MEK2 activation together contribute to transient expression of Snail1 and downstream mesenchymal markers. Our observations suggest oscillating expression of these molecules fails to overcome a possible threshold required for initiation of phenotypic transition in shear-activated proixmal tubule epithelia; cells (PTECs). In contrast, statically cultured PTECs treated with ectopic TGF-β1 exhibit higher frequency ERK oscillations that allow for more sustained expression of downstream EMT marker proteins and successfully result in detectable EMT-associated phenotypic changes. *C*: we also identify divergent temporal patterns of TGF-β1-mediated SMAD signaling across multiple shear stress regimes due to Notch4-dependent degradation of SMAD3. Thus Notch4 activity represents a critical molecular cue regulating the accumulation of extracellular matrix in shear-stimulated PTECs and may be a potential therapeutic target.

quate framework for the interpretation of data and comprehension of relevant disease phenomena.

Effects of Tensile Strain and Pressure on Tubular Epithelial Cells

Mechanical stretch and deformation is an important physical stimulus during urological obstruction, a widely accepted animal model for studying the progression of renal fibrosis. As previously discussed, in vivo obstruction models result in dramatic increases in fluid pressure that deform and strain the renal tubules. During these experiments, renal epithelial cells are exposed to increased hydrostatic pressure and deformation; however, tubule distension in these models is also accompanied by tissue hypoxia, which might obscure the effects of strain alone (87, 160). The bulk in vitro studies cited herein examine cellular strain responses utilizing vacuum-mediated deformation of cell-coated silicone membranes without consideration of any potential effects of increased hydrostatic pressure. It remains to be seen which model is more physiologically relevant. In response to directly applied biaxial cyclic strain, kidney cells rearrange their cytoskeleton and form radial stress fibers with an actin-rich center. This cellular-strengthening response is similar to that observed in kidney cells exposed to fluid flow and occurs via calcium-dependent Rho activation (36, 180). In general, stress fiber formation upon mechanical deformation appears universal and has been observed in many disparate cell types (132, 175). More importantly, continuous stretch has been shown to induce both TGF-β1 expression and successful EMT in mouse and rat renal epithelial cells (15, 74, 105, 135). New evidence even suggests that blocking activation of Rho or ROCK, its downstream effector, can directly inhibit this response to therapeutic effect. Mice supplied with a ROCK inhibitor in a UUO model of injury exhibit decreased interstitial expansion and reduced expression of both the mesenchymal marker smooth muscle actin, as well as fibrotic mediators such as TGF-β1 (106, 108).

Stretching of tubular epithelial cells has also been shown to activate mitogen-activated protein kinase (MAPK) cascades such as ERK, JNK, and p38 (2, 112). Specifically, Nguyen and colleagues (43) demonstrate JNK and p38 cooperation drives stretch-induced apoptosis in tubular epithelial cells while more recent reports identify the same phenomenon dependent upon ERK and p38 instead. Similarly, additional reports observe stretch-induced caspase-dependent apoptosis occurring via the generation of reactive oxygen species (ROS) and simultaneous suppression of an intrinsic antioxidant response (121, 127) (Fig. 4). Such a conclusive, overwhelming apoptotic stimulus would amplify tubule deterioration and loss of renal filtration capacity in the progression of nephropathies. Of particular note, Alexander and colleagues (2) observed that stretch applied to primary rabbit proximal tubule cells results in both time-dependent and dosage-dependent ERK signaling similar to that discovered in sheared cells (46). Cyclic stretch has also been established as a key initiator of inflammation via increased expression of molecules such as osteopontin, a monocyte chemotactic glycoprotein (36, 140), and ICAM-1, a cell surface protein crucial for leukocyte attachment (128). Despite all of these important observations, few studies have fully examined the independent effect of applied strain on the fibrogenic process. Ureteral obstruction recapitulates fibrosis in animal models (40, 153, 161) but in truth represents a complex set of stimuli and sets such an aggressive pace of disease progression that some have questioned its relevance as a model of CKD (34).

Considerably fewer studies have examined the effects of increased fluid pressure on renal cells. In contrast to some

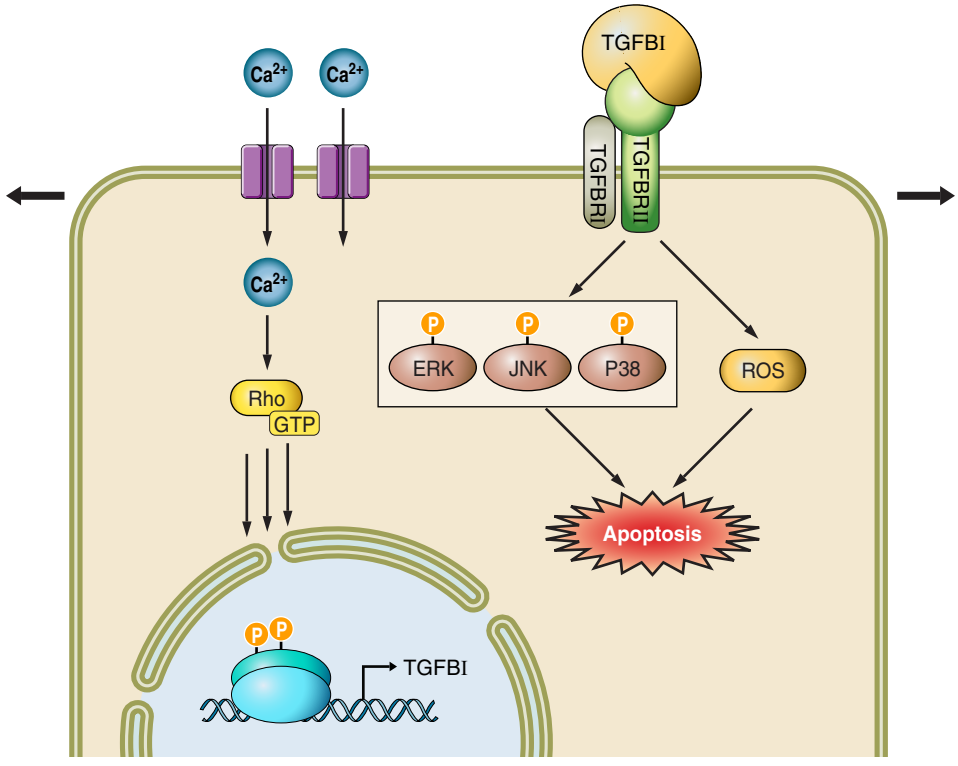


Fig. 4. Mechanical strain induces apoptosis in PTECs via calcium-dependent ROCK-mediated TGF-β1 expression. The Rho-GTPase has long been identified as a key modulator of cytoskeletal organization, but recent data also suggest it serves as a therapeutic target capable of halting the progression of renal fibrosis. Pharmacological inhibition of the Rho effector ROCK1 has been shown to abrogate TGF-β1 expression and attenuate renal matrix accumulation. Additionally, overwhelming evidence demonstrate strain induces TGF-β1-mediated apoptosis in kidney epithelial cells via stimulation of the ERK, JNK, and/or p38 mitogen-activated protein kinases. Generation of reactive oxygen species (ROS) as well as inhibition of intrinsic antioxidant pathways within the cell may also play a role in transmitting this proapoptotic signal.

tensile strain experiments, mesangial cells subjected to increased hydrostatic pressure exhibit increased MAPK activity and enhanced proliferation (80), potentially due to increased PDGF production (77). However, more recent work has shown that the levels of ERK kinase activity increase upon exposure to pressure loading and drive increased expression of the proinflammatory macrophage attractant MCP-1 (152). These results are more congruous with what is observed in specimens undergoing tensile strain and further reinforce the importance of mechanical stimuli as critical mediators of disease.

Conclusions

This review has addressed the important role physical forces play in the progression of kidney disorders. In particular, we have briefly examined effects of mechanical stress on endothelial cells which line the renal vasculature, where extensive work has already elucidated deleterious signaling events. Additionally, we have discussed how kidney epithelial cells sense and respond to pathological levels of fluid shear and circumferential strain. Although our knowledge of mechanosensitive signaling events in epithelial cells is still in its infancy, a body of impressive work is growing rapidly. In summary, stretch, pressure, and fluid shear represent important stimuli regulating kidney cell death, inflammation, and fibrogenesis. Mechanistic studies examining stretch and shear exposure are already providing new insights into the potentially negligible role of EMT in fibrotic progression, and full understanding of the effects of these stimuli on TGF-β1, inflammatory cascades, and other potential mediators of injury will undoubtedly help identify novel therapeutic targets that can halt disease progression.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: B.M.G. prepared figures; B.M.G. and K.K. drafted manuscript; B.M.G. and K.K. edited and revised manuscript; B.M.G. and K.K. approved final version of manuscript.

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