The kidney as a target organ in pharmaceutical research

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Kidney diseases are a major source of morbidity and mortality in humans. In developed countries, mortality owing to chronic kidney disease (CKD) terminating in end-stage renal failure is comparable with that associated with cancer. A full understanding of the mechanisms implicated in the progression of CKD is needed to achieve its prevention and to delay the need for support strategies based on dialysis and transplantation. Renal fibrosis is the unifying feature of progressive renal alterations. In this review, we discuss the current status of possible mechanisms, tools and targets in CKD. Pathophysiological compound identification, biomarker discovery and accurate selection of clinical validation criteria appear to be three key elements needed to develop a successful innovative pharmaceutical approach to treating kidney diseases.

Introduction

The kidney is a complex organ involved in whole-body homeostasis in humans (and other organisms). One of its functions is the production of urine, which results from plasma filtration and modification of the urine composition via reabsorption–secretion mechanisms. Filtration and reabsorption–secretion occur in separate functional compartments: filtration occurs in special units called glomeruli, which filter low-molecular-weight plasma proteins while restricting the passage of larger macromolecules; reabsorption–secretion occurs in tubules, which extensively modify the glomerular filtrate and account for the final excretion of urine [1]. In addition to urine production, the kidney also has important roles in various physiological processes, including: erythropoiesis [via the production of erythropoietin (EPO)]; hypertension regulation (via the production of renin); and phosphocalcic metabolism (via the production of the active hormonal form of vitamin D). Avoiding or repairing tissue injuries involving lesions of the cell types involved in such processes are crucial for preserving renal function.

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The epidemiology of kidney disease

From a clinical point of view, kidney diseases can be grouped into two categories according to the onset of the renal pathology (but irrespective of the etiology of injury): (i) acute kidney injury (AKI); and (ii) chronic kidney disease (CKD). AKI, also known as acute renal failure or acute kidney failure, is a rapid loss of renal function, with variable evolution (full, partial or no recovery of a normal renal function), whereas CKD is defined as a progressive loss of function over a long period of time. Both affect patients worldwide and both are associated with a high morbidity and mortality rate. AKI community-based incidence has increased by 60% in the past few years [2,3] affecting up to 15.3% of all hospitalized patients [4]. CKD affects more than 13% of the population in the USA [5] and in Europe [6]. In addition, recent epidemiological studies have brought to light important links connecting AKI and CKD to progression toward end-stage renal disease (ESRD) [7]. Thus, a large percentage of patients who have AKI do not fully recover their renal function and go on to develop CKD. Moreover, AKI events have been demonstrated to be higher in CKD-affected patients [8], thus having an important, negative impact on the global epidemiology of ESRD [9]. This observation is

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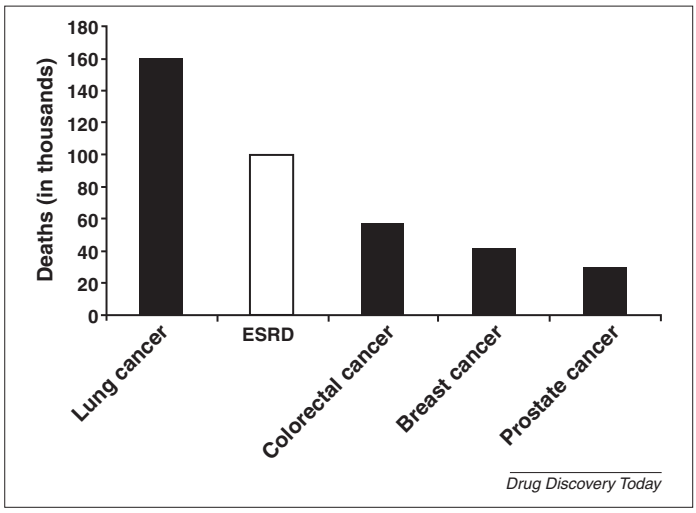

FIGURE 1

ESRD: a leading cause of patient death in developed countries. Epidemiological studies have shown that ESRD is a major cause of death, its mortality in developed countries was comparable to that of cancer in the USA in 2004 (Surveillance, Epidemiology, and End Results Program of the National Cancer Institute; http://seer.cancer.gov/).

supported by experimental evidence that shows that a reduced renal mass negatively influences repair and recovery after ischemic injury mimicking AKI [10–12]. Thus, similarly to the reduction of renal mass commonly observed in CKD patients independently of the cause, diabetic- and age-related nephropathies are expected to predispose kidneys to develop acute injuries. The global population of patients with ESRD treated with renal replacement therapy (RRT) was estimated to have reached almost 1.7 million at the end of 2003, and continues to grow at an annual rate of ~5–8%, driven by an aging population, increased incidence of diabetes mellitus, better means of detecting CKD and better access to treatment [6]. This exponential growth worldwide of patients with ESRD, whose mortality in developed countries is comparable to that owing to cancer (Fig. 1), will become a public health problem with unbearable costs of substitutive treatments.

From a pathological point of view, kidney diseases can be grouped into three categories according to the injured renal compartment, but irrespective of the etiology of injury: (i) glomerulopathies (e.g. immune complex-mediated glomerulonephritis); (ii) tubulointerstitial diseases (e.g. interstitial nephritis); and (iii) vascular diseases (e.g. hypertensive nephroangiosclerosis). However, in regard to their pathogenic interconnections, the distinction between these three conditions appears artificial, because renal fibrosis (i.e. tubulointerstitial fibrosis), is the common and final histopathological feature.

Characteristics of the glomerular and tubular compartments

Glomeruli and tubules, which are functionally and morphologically distinct, both contribute to renal fibrosis. The filtration function of the kidney relies upon the unique morphology of the glomerulus. This filtrating unit consists of a capillary tuft located inside the Bowman's capsule. The glomerular filter comprises three distinct layers: (i) a fenestrated endothelium; (ii) a glomerular basement membrane (GBM); and (iii) a slit diaphragm located between the interdigitating foot processes of the epithelial podocytes [1]. The glomeruli are affected in a variety of systemic and primary kidney diseases (e.g. autoimmune processes and inherited defects of podocytes), leading to proteinuria. Persistent proteinuria has harmful effects on the kidney and frequently leads to glomerulosclerosis, tubulointerstitial fibrosis and CKD [13]. Emphasis has been placed during the past few years on the role of podocytes and mesangial cells as causes of the proteinuria and in the progression of kidney fibrosis, respectively. Genome mapping of inherited nephrotic syndromes that do not respond to steroid treatment has led to the discovery of proteins of the podocyte slit-diaphragm [14,15]. In diabetic nephropathy, high glucose and angiotensin II (AngII) both target mesangial cells directly, modulating mesangial matrix synthesis degradation and inducing transforming growth factor (TGF)-β secretion, thus contributing to glomerular sclerosis [16].

In contrast to the filtration function of glomeruli, tubules are involved in the modification of urine composition via reabsorption and secretion processes. Morphologically, tubules are in direct contact with the interstitial compartment and are composed of extracellular matrix and cells. Injury of the tubular epithelial cells therefore also affects the interstitium. Thus, during ischemic AKI, tubular cells (especially proximal tubule cells, which are particularly sensitive to hypoxia) are severely injured, with occasional necrosis. Although patients generally recover from AKI through an efficient tubular re-epithelialization process, some of them progress to CKD. In this case, atrophic tubules contribute to the progression of CKD by eliciting transformation of interstitial cells into myofibroblasts through a variety of mechanisms, as demonstrated in the microembolic rat model of CKD [17]. Bands of fibrosis, with α-smooth muscle actin (SMA)-expressing myofibroblasts, can arise at the level of proximal tubular cells; the contribution of distal tubules to fibrosis is still not fully understood.

The fibrosis pathways

Interstitial fibrosis is a common pathological feature observed in all kinds of CKD irrespective of their etiology (Fig. 2). An important advance in the understanding of fibrosis pathogenesis was the identification of the myofibroblast, initially described in wound granulation tissue [18], as the major actor in the onset and evolution of the lesions [19,20]. The myofibroblast, characterized by the expression of α-SMA [19], contributes crucially to extracellular matrix (ECM) remodeling by the synthesis of essential matrix components, such as collagen type I and III, and cellular fibronectin. Fibroblast migration, accumulation, replication and differentiation into myofibroblasts, are observed in injured tissues under the influence of local inflammatory factors such as TGF-β1. Although myofibroblasts normally disappear as a result of apoptosis when a wound heals [21], they might persist in other situations, leading to fibrosis that, in turn, causes tissue deformation and loss of organ function.

Myofibroblasts originate mainly from local fibroblasts [18], although another local source of these cells is epithelial (or endothelial) cells that undergo epithelial-to-mesenchymal transition (EMT) [22]. EMT has been extensively investigated recently and has been demonstrated to be a crucial phenomenon in other relevant physiological and pathological situations, such as organ development

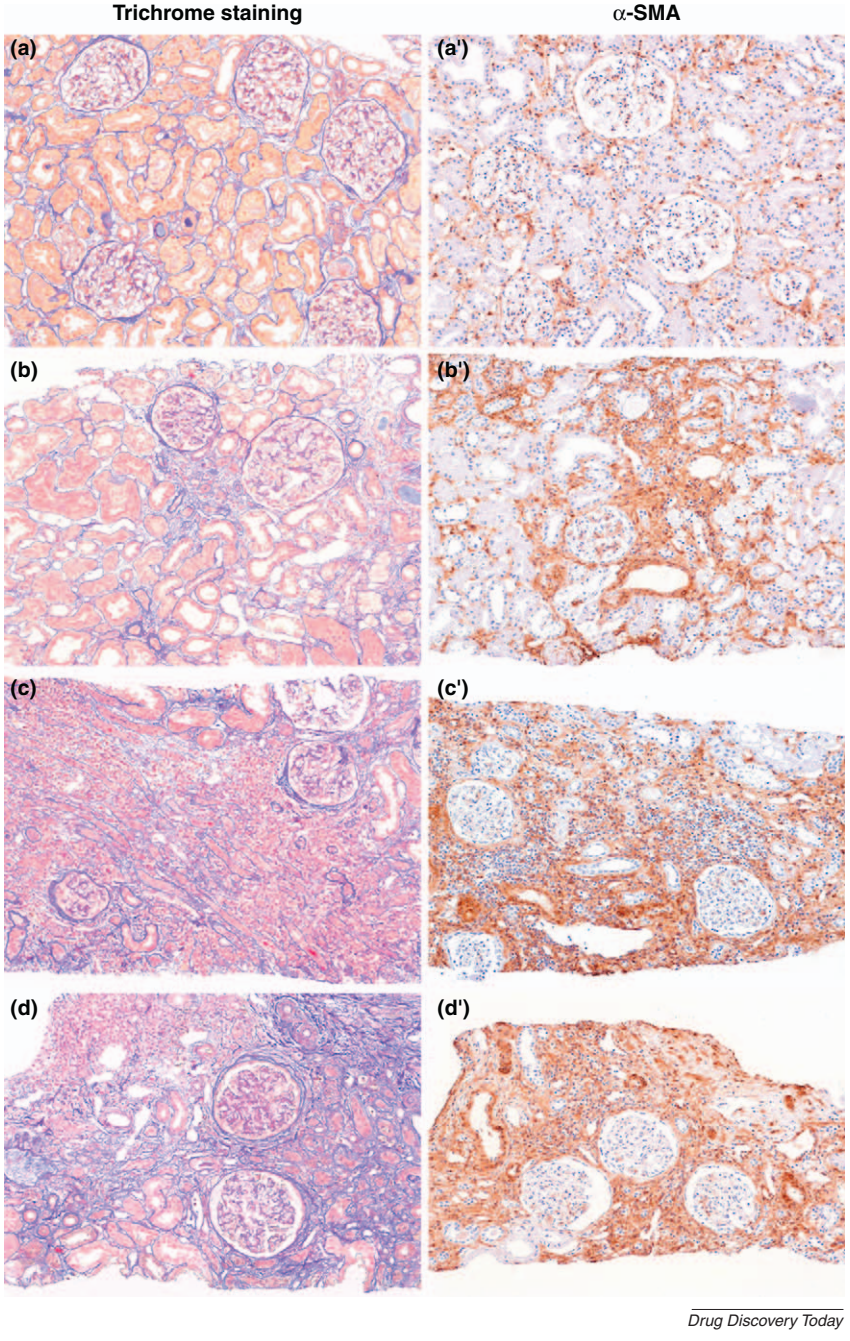

FIGURE 2

Interstitial fibrosis: a common pathological feature observed in any type of CKD irrespective of the etiology. Graded renal biopsies from minor to severe CKD patients: different methods of staining [**(a–d)** trichrome staining; **(e–h)** α-SMA immunostaining] enable researchers to understand the evolution of organ remodeling during disease progression. Biopsies from patients with low-grade fibrosis (a,e) show almost preserved epithelial tubular cell architecture and a low amount of ECM, labeled in orange and blue, respectively, in (a). In these patients, α-SMA expression is limited to vessels (e). In biopsies from patients with severe fibrosis (d,f), ECM, produced by activated myofibroblasts, accumulates in place of functional tubules [seen by comparing the orange:blue staining ratio in (d)] and α-SMA expression (f) is upregulated, revealing activation of resident myofibroblasts. Infiltrating leucocytes and macrophages, releasing profibrotic cytokines, can be observed at the lesion site [particularly evident in (c)].

during embryogenesis, stroma reaction to epithelial cancer and carcinoma progression (for review see [23,24]). On the basis of *in vitro* experiments, the main contributor to EMT in renal fibrosis appears to be tubular epithelial cells. However, the contribution of EMT to fibrosis *in vivo* was recently revised owing to conflicting experimental results [25] and the nephrology scientific community is still divided on the relative relevance and contribution of this process in patients, as summarized by the last World Congress (Nexus meeting) on fibrosis held in Geneva (Switzerland) June 2010 and recently addressed in a debate article [26]. EMT is also likely to exist *in vivo* but to contribute differentially to different kidney diseases. What appears to be increasingly relevant is that

epithelial cell health might be pivotal in preserving normal kidney architecture. Injured tubular epithelial cells have recently been demonstrated to behave as a major source of pro-inflammatory cytokines in experimental induced kidney fibrosis [27]. In addition, Udo *et al.* [28] were able to show that this is also true in physiological conditions *in vitro*, where differentiated MDCK distal tubular epithelial cells, but not 3T3 fibroblasts, inhibited regeneration of mesenchymal stem cells from co-cultured adipose tissue fragments, therefore confirming that renal tubular cells contribute to the general maintenance of the static state of mesenchymal cells. Results that suggest the presence of a pro-fibrotic microenvironment perpetuating the activation of myofibroblasts are similar to the scenario already established in cancer biology [29,30].

Mimicking of tubular injury by confined overexpression of TGF-β1 in renal epithelial cells [31] confirmed the absence of EMT *in vivo* in mice and reconfirmed the importance in keeping epithelial cells healthy. In this model, TGF-β1 drove autophagy in tubular epithelial cells in the absence of EMT, leading to tubular atrophy, with concomitant interstitial fibrosis that resulted from myofibroblasts derived from local fibroblasts. Neither tracing of injured tubules in electron micrographs nor genetic tagging of tubular epithelial cells revealed cells transgressing the tubular basement membrane, a typical feature observed in EMT.

Another possible myofibroblast precursor is bone marrow-derived cells (fibrocytes), which have been identified in several fibrotic organs, such as skin, liver and lung [32]. However, the contribution of local versus circulating cells to fibrosis process remains to be established. Different mechanisms, among them proteinuria, hypoxia and inflammation, might drive myofibroblast activation within the kidney.

As early as 1914, Volhard and Fahr [33] found that renal damage was related to copious protein excretion in the urine and they suggested that proteinuria could be the result of impaired plasma protein reabsorption. Epidemiological studies have since confirmed the detrimental effect of proteinuria on renal function, showing a clear correlation between renal failure progression and proteinuria, which appears as an independent risk factor [34]. Moreover, several recent *in vitro* studies using proximal tubular epithelial cells led to renewed interest in proteinuria as a causative agent of tubular damage, myofibroblast activation and consequent CKD progression [35,36].

However, it is still unclear how proteinuria produces fibrosis and worsens renal function. Proteins in the urine are normally absorbed by endocytosis in the proximal tubules. During periods of heavy proteinuria, the filtered proteins accumulate in lysosomes in the proximal tubular cells, causing cell disruption and injury [37,38]. Proteins might also incite a toxic response through the stimulation of the expression of proinflammatory cytokines [39]. Tubular reabsorption of albumin has been shown to alter the biology of proximal tubular cells [40] and to upregulate synthesis of various inflammatory mediators [41], thus contributing either to phenotypical modulation of proximal epithelial cells or to leukocyte interstitial infiltration with consequent release of pro-fibrotic cytokines. Thus, cultured proximal tubule cells that are exposed to albumin, transferrin, or immunoglobulin G, produce increased amounts of monocyte chemotactic protein-1 (MCP-1), which is preferentially excreted into the basolateral compartment, suggesting a mechanism for interstitial inflammatory infiltrates *in vivo* [42]. Excessive urinary protein excretion and reabsorption has also been observed to induce rough endoplasmic reticulum (RER) stress in proximal epithelial tubular cells. Whereas RER-stress resolves rapidly upon accumulation of misfolded proteins, it can lead to apoptosis under persistent proteinuria [43], as indicated by numerous reports in experimental and human renal pathology [44–47].

Interestingly, conditions perturbing the homeostasis of the RER might induce EMT. This was demonstrated not only in *in vitro* studies on epithelial cells [48–50], but also in human studies on 'uromodulin (UMOD)-related diseases'. These familial forms of tubulointerstitial fibrosis, reported previously under different names, including medullary cystic kidney disease type 2 (MCKD2), familial juvenile hyperuricemic nephropathy (FJHN) and glomerulocystic kidney disease (GCKD), have a common defect in the gene encoding uromodulin. It was recently demonstrated that intracellular trapping of uromodulin within the RER was associated with the development of typical tubulointerstitial fibrosis [51].

Complement is also implicated in the pathogenesis of proteinuria-associated tubulointerstitial injury [52,53]. During glomerular proteinuria, complement proteins are likely to be present in the glomerular filtrate, and proximal tubular cells from both rat and human kidneys activate complement [54,55]. There is also evidence that tubular cells are an important local source of complement [52]. It was demonstrated that massive proteinuria induced tubulointerstitial injury associated with a marked deposition of C5b-9 on the apical membrane of proximal tubular cells [56], and that membrane attack complex (MAC) formation inhibition ameliorated tubulointerstitial injury in proteinuric [57], but not in nonproteinuric [58] animal models. In addition to C5b-9, complement C5, and in particular C5a, might also become a target in tubulointerstitial injury [59]. In the mouse unilateral ureteral obstruction (UUO) model, genetic C5 deficiency or pharmacological inhibition of the C5a receptor (C5aR) potently reduced tubulointerstitial fibrosis [60]. C5aR antagonists and anti-C5 antibodies (eculizumab) have been, or are currently being, evaluated in clinical trials. Complement regulatory proteins, such as Crry (a structural and functional rodent analog of human CR1, which inhibits C3 activation) or CD59 (which inhibits C5b-9 formation) might also be of interest [61,62].

Endothelin is also an important mediator of proteinuria-associated renal fibrosis [63]. Cultured proximal tubule cells that are exposed to albumin, transferrin, or immunoglobulin G produce increased amount of endothelin-1 (ET-1) [64]. Studies in a variety of experimental models demonstrated that gene encoding endothelin and/or endothelin expression is increased during nephropathy and colocalizes with fibrotic lesions [63]. Inversely, pharmacological antagonism of endothelin receptors delayed the evolution and/or prevented renal failure. Although bosentan, a dual inhibitor of ET_A and ET_B, is in clinical use, its benefits versus side effects (mainly hepatotoxicity and fluid retention) in CKD patients require additional evaluation [53].

Hypoxia is the second presumed contributor to the progression of kidney fibrosis. Chronic ischemia in the tubulointerstitium space can occur via several mechanisms, including intrarenal vasoconstriction [secondary to renin angiotensin aldosterone system (RAAS) local activation or loss of vasodilatory

nitric oxide (NO)] or structural lesions that impair blood flow delivery, such as glomerulosclerosis, with impairment of the glomerular capillary bed and consecutive impairment of the peritubular perfusion and of the tubular oxygen supply. Tubulointerstitial ischemic damage also results in the loss of peritubular capillaries, as demonstrated in histological studies of human kidneys [65]. Furthermore, interstitial fibrosis impairs tubular oxygen supply owing to the increased diffusion distance between peritubular capillaries and tubular cells. Mechanisms of hypoxia-induced tubulointerstitial damage are multifactorial. Hypoxia can activate fibroblasts, change the extracellular matrix metabolism of resident renal cells, and lead to eventual fibrogenesis [66,67]. Furthermore, mild hypoxia was demonstrated to induce tubular EMT with the transdifferentiation of cultured tubular cells into myofibroblasts [68]. Treatments targeting hypoxic tubulointerstitial damage involve EPO, as anemia is a risk factor for renal failure, with the subsequent improvement in oxygenation of the kidney.

Inflammation is the third suspected actor on the scene. As mentioned above, multiple acute and chronic injuries induce chemotaxis of inflammatory cells within the kidney. Consequent production of pro-fibrotic mediators acts directly on resident fibroblasts, pericytes, endothelial and epithelial cells with induction of the EMT process.

Molecular pathways relevant in kidney disease

As described above, multiple pathways can be activated during kidney diseases in the various types of renal and inflammatory cells. A strategic choice has to be made in to give priority to management in terms of research resource allocation. Most pharmaceutical investigators are likely to target AKI pathways, which can be easily validated in clinical settings, despite the fact that most patients have CKD. Pathophysiology of CKD involves alternative mechanisms, such as the loss of a function (e.g. of glomerular filtration) or the alteration of a specific cell type (e.g. podocytes). Multiple and non-exclusive pathogenetic mechan-

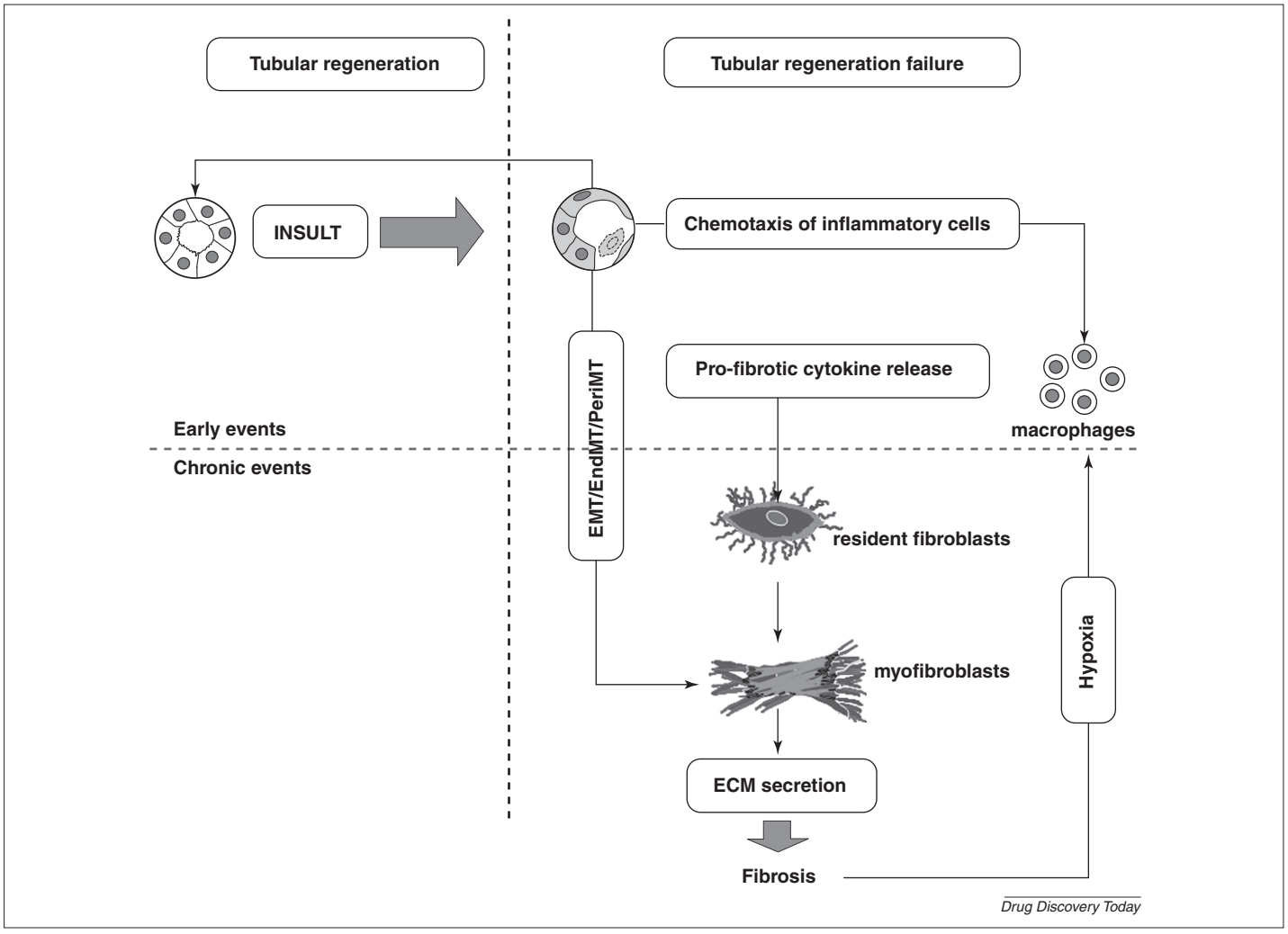

FIGURE 3

Targeting CKD is a matter of time. Multiple and nonexclusive pathogenetic mechanisms can be identified in space and time. For certain elements in this scheme, a clear role still has to be determined (in space and time; e.g. for EMT, Endothelial to-mesenchymal transition (EndMT) and Pericytes to-mesenchymal transition (PeriMT)). Targeting upstream events, such as tubule preservation, inflammation and macrophage profibrotic activity, could result in a successful approach to targeting CKD. Currently, there is no scientific consensus regarding the role of hypoxia in CKD onset. In fibrosis pathogenesis, consensus has been reached only regarding the activation of resident fibroblasts and their modulation toward myofibroblasts.

TABLE 1

Relevant targets in CKD.

Target(s)	Approach	Drug development status	Refs
RAAS, Aldo	Control of systemic blood pressure	In clinical practice	[69–72,74–77,79,80,82,83]
Endothelin receptor A	Inflammation	In clinical practice	[53,64,196,197]
Endothelin receptor B	Inflammation	In clinical practice	[53,64,196,197]
M1/M2 switch	Inflammation	Preclinical level	[117–119]
HIF-1	Hypoxia	Preclinical level	[122–125]
Actin polymerization	Direct inhibition of fibrotic cells	Phase I	[21,155,156]
Myofibroblast viability	Direct inhibition of fibrotic cells	Preclinical level	[162]
MMPs	ECM remodeling	Preclinical level	[167,168]
PAI-1	ECM remodeling	Phase I, II	[169–174]
LOXL2	ECM remodeling	Phase I	[169,175]
DDRs	ECM remodeling	Preclinical level	[169,176–178]
Snail, Twist and Slug	Phenotypical modulation	Preclinical level	[126]
Wnt/β-catenin signaling	Phenotypical modulation	Phase I	[127–130]
TGF-β1	Phenotypical modulation	Phase I, II	[131,132]
Smads	Phenotypical modulation	Phase I	[131,135–137]
αvβ6 integrin	Phenotypical modulation	Phase II	[138,139]
BMP-7	Phenotypical modulation	Phase I	[140–143]
Kielin/chordin-like protein	Phenotypical modulation	Preclinical level	[144]
USAG-1	Phenotypical modulation	Preclinical level	[145,146]
Gremlin	Phenotypical modulation	Preclinical level	[147,148]
ALK-3	Phenotypical modulation	Preclinical level	[149–151]
HGF	Phenotypical modulation	Phase I	[152–154]
Cannabinoid receptors	Phenotypical modulation	Preclinical level	[157,158]
MeCP2	Phenotypical modulation	Preclinical level	[159,161]
PPAR-γ	Phenotypical modulation	In clinical practice	[160]

isms can be identified as described above, and targeted (Table 1): (i) RAAS; (ii) inflammation; (iii) hypoxia; (iv) phenotypical modulation processes; (v) direct inhibition of effector cells; and (vi) ECM remodeling (Fig. 3). In all cases, more insight can be gained from results obtained in other organs, such as liver and lungs.

RAAS

RAAS has a pivotal role in many of the pathophysiological mechanisms involved in the progression of chronic renal disease [69]. RAAS has become more complex in recent years, with the discovery of alternative ways of AngII formation in addition to angiotensin-converting enzyme (ACE) such as, for example, the serine protease chymase, the discovery of a second form of ACE, the ACE2, and of novel peptides, such as angiotensin 1-9 (Ang1-9), angiotensin 1-7 (Ang1-7), angiotensin III (AngIII) and angiotensin IV (AngIV), which exhibit biological activity. AngII has emerged as a multifunctional cytokine exhibiting many non-hemodynamic properties, such as acting as a growth factor and a profibrogenic and proinflammatory cytokine. In addition, increased aldosterone (Aldo) levels contribute to renal injury, independent of blood pressure or AngII.

In the classic view of the RAAS, renin is secreted from the juxtaglomerular apparatus of the kidney and acts on the circulating precursor angiotensinogen, which is produced as a glycoprotein in the liver, to generate angiotensin I (AngI) [70]. This molecule has little effect on blood pressure and is converted by the high ACE activity of the lungs to the active octapeptide AngII. This molecule acts on the heart and the kidneys by binding to the G protein-coupled receptors type 1 (AT_1) and type 2 (AT_2). The AT_1 receptor mediates the more deleterious effects of AngII (i.e. vasoconstriction and cardiac and vessel hypertrophy). In addition, AngII binds to specific receptors in the adrenal cortex, resulting in the release of Aldo as a main effector of this systemic hormone system. In addition to the conversion of AngI to AngII, ACE inactivates the vasodilator peptide bradykinin.

It is now generally appreciated that, as well as ACE, the serine protease chymase can generate AngII from AngI, whereas ACE2 cleaves AngI into the inactive Ang1–9, which is converted by ACE into the vasodilator and anti-proliferative Ang1–7 [70]. Both chymase and ACE2 are produced in the normal kidney and have been demonstrated to be upregulated in renal diseases. Chymase upregulation was shown in mesangial cells and vascular smooth muscle cells in diabetic nephropathy [71]; in addition, ACE2 neoexpression was found in glomerular and peritubular capillary endothelium in renal biopsies from patients with diverse renal diseases [72]. Interestingly, ACE inhibitor treatment neither reduced chymase activity [73] nor altered ACE2 expression in renal diseases [72].

In addition to AngII, other Ang peptides might exert clinically relevant vasoactive actions. As described previously, Ang1–7, a heptapeptide derived from AngI and/or from AngII, might antagonize AngII, especially in situations of an overactive RAAS, such as during sodium restriction, an effect that results in renal vasodilation and increased natriuresis. In addition, digestion of AngI and AngII by angiotensinases, peptidases, aminopeptidases, carboxypeptidases, or endopeptidases, results in different peptide fragments with functions distinct from those of AngI and AngII. Thus, AngII is metabolized in the kidney by aminopeptidase A into AngIII and further into AngIV [74]. AngIV (the 3–8 peptide) binds the AT_4 receptor selectively and stimulates plasminogen activator inhibitor-1 release (PAI-1) [75]. Emerging evidence suggests that AngII is not only a vasoactive peptide, but also a true cytokine that regulates cell growth, inflammation and fibrosis [70]. Non-hemodynamic effects of angiotensin include increased production of reactive oxygen species; upregulation of cytokines, cell adhesion molecules and profibrotic growth factors; induction of TGF-β expression; increased synthesis of extracellular matrix proteins; stimulation of PAI-1 production by endothelial and vascular smooth muscle cells; and macrophage activation and infiltration.

Therefore, inhibition of the RAAS is particularly effective in preventing glomerulosclerosis and tubulointerstitial fibrosis. The benefit seen with such drugs is in addition to that expected from their antihypertensive effects alone. Drugs acting on the RAAS include agents that inhibit the synthesis and release of renin or AngII directly (i.e. renin and ACE inhibitors), drugs that antagonize the receptor effects of AngII (i.e. AngII receptor blockers; ARBs), the Aldo-receptor antagonists, and a new class of combined ACEI and neutral endopeptidase inhibitors, called vasopeptidase inhibitors (VPIs). Renin inhibitors (e.g. aliskiren, enalkiren and zalkiren) are promising drugs for combating renal fibrosis, as shown in severely hypertensive rats transgenic for genes encoding human renin and angiotensinogen [76]. However, clinical effects on renal fibrosis remain to be determined. The recently discovered receptor for renin and prorenin [(pro)renin receptor; (P)RR] was linked to glomerular fibrosis [77]. The specific (P)RR activates intracellular signaling and enhances receptor-bound (pro)renin catalytic activity on the cell surface. Experimental evidence regarding the role of (P)RR in renal damage is accumulating and suggests that blocking (P)RR represents a new therapeutic target for tissue protection. (P)RR blockers might be more potent than renin inhibitors, because renin inhibitors do not block renin or prorenin binding to, and activation of, (P)RR. VPIs (e.g. AVE7688 and omapatrilat) block both ACE and neutral endopeptidase (NEP). NEP catalyzes the hydrolysis of atrial natriuretic peptide (ANP), brain natriuretic peptide and C-type natriuretic peptide [78]. Simultaneous inhibition of the RAAS and NEP system by VPIs results in vasodilation, natriuresis and diuresis, and decreases peripheral vascular resistance and blood pressure to a greater extent than does ACE inhibition alone [53]. In an animal model of Alport syndrome with progressive renal fibrosis, AVE7688 demonstrated nephroprotection with antifibrotic, anti-inflammatory, and antiproteinuric effects [79]. Finding that AVE7688 corrected the imbalanced ratio between the vasoconstrictor endothelin 1 (ET-1) and the vasodilators NO and ANP, could explain the superior renoprotection of vasopeptidase over ACE inhibitors [80]. At present, several VPIs are in various stages of development. Omapatrilat has undergone the most extensive clinical development. However, the high incidence of angioedema compared with enalapril (as reported in the Omapatrilat Cardiovascular Treatment Assessment Versus Enalapril trial) might limit its use [81]. Combination therapy of ACEI and ARB [82] or high-dosage of ACEI [83] showed potential in experimental studies to stop or even regress renal fibrosis, at least in the early stages.

Combined therapy, such as RAAS inhibitors associated with statin, Vitamin D or Rho kinase inhibitors, has been widely described in literature and demonstrated to be effective in reducing glomerulosclerosis and inflammation. Statins, or 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, independent of their ability to lower cholesterol, have pleiotropic properties, including enhanced endothelial function with increased endothelial nitric oxide synthase (eNOS) expression and decreased ET-1 expression, anti-oxidant, anti-inflammatory and immunomodulatory actions as well as antihypertensive and antiproliferative effects [84]. Indeed, statins interfere with the prenylation of Ras and Rho family small GTP98 binding proteins, thereby blocking the activation of signaling pathways and transcription factors, which regulate inflammatory and fibrogenic genes that are crucial to renal disease progression [85,86]. In rats with severe passive Heymann nephritis, the addition of statin to a chronic background of ACE inhibition and ARB induced remission of proteinuria and conferred complete protection of the kidney [87]. In a more recent study, the effects of maximal RAAS inhibition by ACEI plus ARB in combination with statin were assessed in rats with overt diabetic nephropathy [88]. Dual RAAS blockade significantly reduced proteinuria with respect to vehicle, and the addition of statin further lowered proteinuria to control levels. Glomerulosclerosis was ameliorated by RAS inhibitors or statin, and regression was achieved by the addition of statin. Finally, tubular damage, interstitial inflammation and the expression of the fibrotic markers TGF-β1 and phosphorylated Smad 2/3 in tubuli were demonstrated to be significantly reduced by the triple regimen.

Analogs of vitamin D have been demonstrated to attenuate renal injury in several models of kidney disease [89,90]. The activity of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], the active hormonal form of vitamin D, is mediated by the vitamin D receptor (VDR), a member of the nuclear receptor superfamily [91], which is highly expressed in the kidney [92]. The most well-known function of the vitamin D endocrine system is to maintain calcium and phosphorus homoeostasis. However, previous studies unveiled a range of so-called 'non-calcemic' activities, including regulation of the RAAS and of the nuclear factor (NF)-κB pathway [92]. $1,25(OH)_2D_3$ and vitamin D analogs were shown to inhibit renin expression in animals [93,94] by suppressing renin gene transcription, as demonstrated by *in vitro* experiments [95]. In addition, $1,25(OH)_2D_3$ was demonstrated to suppress activation of NF-κB [96,97], a family of transcription factors that regulates a range of genes involved in inflammation, proliferation and fibrogenesis, and which is known to have a key role in kidney disease [98]. Consistently, plasma vitamin D status was found to be inversely associated with increased renal inflammation in patients with a variety of kidney diseases [99]. Moreover, vitamin D ana-

logs, alone or in combination with RAAS inhibitors, were demonstrated to prevent or attenuate proteinuria, glomerulosclerosis and tubulointerstitial fibrosis in several experimental models of kidney diseases [100–102]. Finally, two recent animal studies using the UUO model reported an attenuation of renal fibrosis with vitamin D analog [103] and in VDR-null mice [89]. Together, these data enforce the suggestion that the $1,25(OH)_2D_3$–VDR system has a crucial renoprotective role in pathophysiological conditions. Whether vitamin D analogs could further optimize existing therapies in human renal disease is currently under investigation [90,104]. Results from clinical trials that have used vitamin D analogs in patients with CKD [105] and in renal transplant recipients [106] are expected in the near future.

Animal studies have reported that Rho-kinase inhibition has renoprotective effects in different experimental models, such as UUO [107], hypertension [108,109] and diabetes [110]. During the 1990s, Rho-kinase was identified as one of the main downstream effectors of the small G protein RhoA [111,112]. Rho-kinase is activated by Rho and mediates some of its biological effects. Rho functions as a molecular switch, in that Rho is inactive in its guanosine diphosphate (GDP)-bound form and active in its guanosine triphosphate (GTP)-bound form [113], and it participates in various cellular functions, including formation of stress fibers and focal adhesions, regulation of calcium ion sensitivity, production of cytokines and regulation of G1 to S in cell cycle progression [114]. The discovery of specific Rho-kinase inhibitors advanced the knowledge of the Rho–Rho-kinase pathway *in vitro* and *in vivo* [115]. Recently, combination therapy including an ACEI (imidapril) and a Rho-kinase inhibitor (fasudil) was demonstrated to be more effective than either monotherapy for the prevention of renal interstitial fibrosis induced by UUO, with reduction of TGF-β and/or collagen production, monocyte and/or macrophage infiltration, myofibroblast differentiation and oxidative stress generation [116]. Thus, Rho-kinase inhibitor could serve as a novel strategy for the treatment of renal fibrosis. However, further translational studies in humans are needed to substantiate these findings.

Inflammatory cells and myofibroblasts

An exciting field for target discovery in kidney disease is the crosstalk between inflammatory cells and myofibroblasts. Rephrasing the dialog between these partners might lead to the development of new therapies. This could be the case for infiltrating macrophages, which are classically recognized as active players in progressive renal scarring but might also have a protective role by preserving renal architecture and limiting progressive renal scarring, as demonstrated in the late stage of an experimental kidney fibrosis model after UUO (reviewed in [117]). These apparently paradoxical findings might reflect the differential regulation of inflammation and fibrosis mediated by two functionally distinct macrophage phenotypes. Thus, classically activated (M1) and alternatively activated (M2) macrophages [117–119] mediate contrasting and complementary functions in tissue fibrosis. Whereas M2 macrophages produce large amounts of TGF-β and induce myofibroblast proliferation, promoting ECM synthesis and fibrosis development, M1 macrophages produce matrix metalloproteinases (MMPs) by themselves and induce myofibroblasts to produce MMPs, promoting ECM degradation and fibrosis resolution. Therefore, new therapeutic agents that specifically target M1, induce the transformation of M2 to M1 or interfere with M1 and/or M2 and myofibroblast crosstalk, could represent a selective approach for renal fibrosis treatment.

Hypoxia

Hypoxia has been documented to have a role in chronic renal diseases of different etiologies [e.g. 5/6 nephrectomy, diabetic nephropathy, immunoglobulin A (IgA) nephropathy and UUO]. Retrospective [120] and prospective [121] studies suggested that improvement of anemia, and thus improvement of renal hypoxia, by treatment with EPO delayed the progression of renal failure. Treatments with hypoxia-inducible factor (HIF), one of the most important factors in the cellular response to hypoxia, and/or with VEGF, an angiogenic growth factor, might be new strategies against ischemia-induced tubulointerstitial fibrosis, investigated currently only in animal models [122,123]. However, experimental evidence indicated that hypoxia, via HIF activation, might either accelerate [124] or attenuate [125] renal fibrogenesis. Therefore, this field of pharmaceutical research appears premature and many relevant points still have to be addressed by basic research.

Phenotypical modulation processes

Targeting phenotypical modulation processes enables potentially multiple levels of intervention. Thus, inhibition of early tubular events, such cell detachment, can be achieved by blocking transcriptional factors (e.g. Snail, Twist or Slug), which regulate expression of relevant adhesion molecules, such as E-cadherins, or by targeting enzymes (e.g. glycogen synthase kinase-3 β; GSK-3β) that regulate at the transcriptional factor level within tubular cells: activation of the Snail gene (*SNAI1*) has been demonstrated *in vitro* to suppress fibrosis via the direct repression of the kidney differentiation factor hepatocyte nuclear factor (HNF)-1β. GSK-3β-conditional-KO mice have elevated collagen production, elevated levels of profibrotic α-SMA and increased myofibroblast formation during wound healing, and elevated expression and production of ET-1 compared with control mice [126]. Antagonizing ET-1 was demonstrated, in the same study, to reverse the phenotype of Gsk3b-CKO mice, suggesting that targeting the GSK-3β pathway or ET-1 is of benefit in controlling tissue repair and fibrogenic responses *in vivo*.

Advanced phenotypical transformation of epithelial, endothelial cells or pericytes toward a mesenchymal phenotype can also be controlled by targeting the Wnt/β-catenin signaling pathway. Wnt proteins transmit their signal across the plasma membrane through interactions with serpentine receptors, the Frizzled (Fzd) family of proteins, and co-receptors, members of the low-density lipoprotein (LDL) receptor-related protein (e.g. LRP5/6). Upon binding to their receptors, Wnt proteins induce a series of downstream signaling events, inducing stimulation of the transcription of Wnt target genes, such as those encoding fibronectin [127] and MMP-7 [128], which are correlated with mesenchymal phenotype and invasiveness. Kidney disease states characterized by renal fibrosis exhibit overexpression of multiple Wnt proteins in glomeruli and interstitium [129]. Therefore, targeting Wnt/β-catenin signaling might be an effective strategy to hinder the progression of renal interstitial fibrosis. Thus, in a mouse model of obstructive nephropathy, delivery of the Wnt antagonist Dickkopf-1 (*DKK1*)

gene was demonstrated to reduce significantly renal β-catenin accumulation and suppress Wnt/β-catenin target genes in fibrotic kidney, leading to a reduction in renal matrix deposition and fibrosis [130].

Several cytokines have a crucial role in cell phenotypical modulation in the kidney but a vast body of literature (reviewed in [131]) assigns the most prominent role in fibrosis to TGF-β. This molecule contributes to key phenomena and its fibrogenic potential is uniquely powerful because of three simultaneous actions: (i) stimulation of matrix synthesis; (ii) inhibition of matrix degradation; and (iii) modulation of matrix receptor expression to facilitate cell–matrix interactions [132]. Thus, gene delivery of TGF-β1 to the normal rat kidney stimulates ECM production, by increasing the synthesis of ECM proteins on the one hand and by inhibiting their degradation on the other one, and directly mediates transformation of resident fibroblast or tubular epithelial cells to myofibroblasts [132]. Therefore, all possible strategies that directly, or indirectly, counteract TGF-β activity were tested in experimental CKD; anti-TGF-β antibodies, TGF-β receptor antagonists and short-interfering RNAs (siRNAs) were used with success in various experimental diabetic and non-diabetic proteinuric nephropathies (reviewed in [133]). In humans, however, the first evaluation of a systemically administered and repeatedly dosed anti-TGF-β1 drug showed no evidence of efficacy in patients with systemic sclerosis [134].

Different indirect targets of TGF-β activity have also been tested. Thus, several studies in experimental nephrology have shown that TGF-β receptor downstream factors, such as Smads, are crucial in CKD (reviewed in [135]). TGF-β-mediated renal fibrosis is positively regulated by Smad2/3, but negatively regulated by Smad7. Targeting blockade of TGF-β–Smad signaling by expression of Smad7 might therefore provide a new therapeutic potential tool in renal fibrosis [135]. Thus, in a rat UUO model, treatment with inducible Smad7, resulting in increased Smad7 expression and complete inhibition of Smad2 and Smad3 activation, was demonstrated to abrogate tubulointerstitial fibrosis [136,137].

Physiological levels of TGF-β are required for normal development, tissue repair, maintenance of organ functions and immunomodulation. Therefore, a more interesting approach is to target TGF-β1 at the lesion site. One of these approaches is to target the integrin αvβ6, a heterodimeric matrix receptor which is preferentially expressed at sites of epithelial remodeling and which binds and activates latent TGF-β1 [138]. Two different groups, using β6 integrin-null mice [138] and αvβ6-blocking mAbs [139], respectively, showed that interruption of the αvβ6-mediated TGF-β1 activation can protect against tubulointerstitial fibrosis [139]. This approach is currently in Phase II clinical trials and remains to be validated in the clinical setting.

Another promising approach is the stimulation of TGF-β antagonizing pathways in the kidney. For instance, since its first identification in 1990 [140], bone morphogenetic protein-7 (BMP-7), a factor belonging to the TGF-β superfamily and involved in bone formation, has engaged the curiosity of many scientists. BMP-7 acts physiologically as a major and essential morphogen and survival factor in renal development, as demonstrated by the following: (i) homozygous null mice exhibited arrested renal development and dysplastic kidneys, and died of renal failure soon after birth [141,142]; and (ii) recombinant BMP-7 (rBMP-7) reduced the severity of injury after acute and chronic organ failure by counteracting TGF-β1-mediated profibrotic effects [143]. It is now generally assumed that a balance between TGF-β1 and BMP-7, both biologically active, exists in normal tissue, with a shift toward TGF-β1 during inflammation and fibrogenesis. This balance is further modulated by several extracellular proteinogenic BMP-7 modifiers, but the overall regulation of this network is still unknown. Thus, kielin/chordin-like protein (KCP), a potent paracrine enhancer of BMP signaling, has an important role in the attenuation of the initiation and progression of fibrotic disease after renal injury, as demonstrated in Kcp-null mice [144]. By contrast, the product of uterine sensitization–associated gene-1 (USAG-1), the expression of which in adults is confined to the kidneys, binds to and inhibits the biological activity of BMP-7, and might contribute to the pathogenesis of renal deterioration, as demonstrated using mice lacking USAG-1 [145,146]. Other BMP-7 antagonists (e.g. Gremlin [147,148]) or agonists (e.g. direct agonism of the BMP-7 receptor ALK-3 [149–151]) are other interesting pharmaceutical strategies for enhancing BMP-7 signaling and thus to counteract profibrotic TGF-β1 activity. BMP-7 therapy in patients has, however, not yet been reported, but the results of ongoing studies are eagerly awaited.

In addition to TGF-β, other cytokines have a role in renal fibrogenesis. Thus, extensive studies have demonstrated that HGF, a potent antifibrotic factor, prevents the onset and progression of a variety of experimental CKDs [152,153]. When anti-HGF IgG was administered to mice with chronic renal failure, renal dysfunction and fibrosis were accelerated, whereas administration of HGF or HGF gene therapy improved renal fibrosis and dysfunction. Therefore, supplementation with HGF might represent a new approach to inhibit or improve chronic renal failure (reviewed in [154]).

Direct inhibition of effector cells

Direct inhibition of effector cells responsible for specific changes is another possible valuable strategy. Thus, the targeting of myofibroblasts, involved in renal fibrosis, or of mesangial cells and podocytes involved in glomerular diseases, has been tested.

Pioneering experiments have shown that modulation of myofibroblasts is achievable by intracellular delivery of the α-SMA NH_2-terminal peptide Ac-EEED *in vitro* and *in vivo*, as demonstrated with topical administration on rat wound granulation tissue [20]. AcEEED inhibits myofibroblasts contractile activity and exerts an antifibrotic activity probably through its capacity to displace α-SMA from stress fibers. Although systemic delivery of this peptide has not yet been tested, and could cause concern in the long term, this approach suggests a new antifibrotic strategy based on direct control of cell cytoskeleton. Relevance of cytoskeleton-related targets has also been pointed out by Fogo *et al.*, who identified thymosin β-4, an ubiquitously expressed G-actin-binding protein, as being upregulated in sclerotic glomeruli [155] as well as in myofibroblasts in the fibrotic interstitium (Potthoff and Fogo, unpublished data). More recently, Delaroche *et al.* showed that some cell-penetrating peptides, such as (R/W)16, were able to compete with the actin-binding protein thymosin β4 [156], and therefore might be attractive targets for exploring the possible regression of sclerosis.

The modulation of myofibroblasts through TGF-β1 inhibition has also been achieved by targeting several relevant proteins, such as cannabinoid receptors [157,158] and methyl-CpG binding protein 2 (MeCP2) [159], as demonstrated in liver fibrosis, or peroxisome proliferator-activated receptor gamma (PPAR-γ) [160,161]. PPAR-γ abrogates TGF-β-induced myofibroblast transdifferentiation *in vitro* [160], and pretreatment with a PPAR-γ agonist was demonstrated to attenuate renal interstitial fibrosis and inflammation in the model of UUO through reduction of TGF-β expression [161], suggesting that administration of synthetic PPAR-γ agonists, such thiazolidinediones (TZDs), which are widely used as insulin-sensitizing agents in patients with type 2 diabetes, could represent a novel approach to limit the progression of fibrotic CKD.

Finally, specific targeting of proteins whose expression is restricted to activated myofibroblasts might be another therapeutic strategy to reduce renal fibrosis. Thus, Douglass *et al.* [162] recently demonstrated that specific targeting of myofibroblast apoptosis using the C1-3 scAb, a recombinant human monoclonal single-chain antibody (scAbs) directed against synaptophysin (a membrane protein whose expression is restricted to hepatic stellate cell-derived myofibroblasts) reduced liver fibrosis.

An additional approach could involve targeting pathways specifically active in defined cells such as podocytes. Thus, activation of the Notch pathway in mature podocytes has been linked to the pathogenesis of a variety of glomerular diseases [163,164]. This pathway could be a new therapeutic target, as suggested by *in vivo* studies showing that pharmacological inhibition of the Notch pathway was protective in rats with proteinuric kidney diseases [165]. However, recent results [166] suggest a more complex regulation of this pathway in that the severity of glomerular disorders depends on the Notch-regulated balance between podocyte death and regeneration provided by renal progenitors. Indeed, it was demonstrated that Notch expression, which was absent in the glomeruli of healthy adult kidneys, was strongly upregulated not only in podocytes in patients affected by glomerular disorders, but also in renal progenitors. These cells are localized to the inner surface of the Bowman's capsule and replace mature podocytes, which cannot proliferate and therefore die if injured. Thus, inhibition of the Notch pathway in experimental nephropathy appeared not only to reduce podocyte loss during the initial phases of glomerular injury, but also to reduce renal progenitor cell proliferation during the regenerative phases of chronic glomerular injury, worsening proteinuria and glomerulosclerosis.

ECM remodeling

Remodeling of ECM by MMPs (reviewed in [167]), which were discovered almost 40 years ago, is still pharmaceutically not mature. Indeed, MMPs are structurally similar and biologically complex, given that, as in the case of MMP-9, they have both antifibrotic (cleaving type-IV collagen as well as denatured collagens that result from the action of interstitial collagenases) and profibrotic (activating TGF-β, and endothelin-1 and favoring EMT through collagen degradation) effects [168].

Other ECM remodeling enzymes, such as plasminogen activator inhibitor (PAI)-1 [169] and lysyl oxidase-like-2 (LOXL2) are more promising candidates for CKD targeting. Absent from normal kidneys, PAI-1 is frequently expressed in injured kidneys. Studies on experimental CKD models (UUO) in genetically engineered mice have shown that PAI-1 expression is associated with significant fibrosis [170,171]. By contrast, models with genetic PAI-1 deficiency have been protective, including diabetic nephropathy, 5/6 nephrectomy, crescentic anti-GBM nephritis, protein-overload nephropathy and spontaneous renal fibrosis developing in TGF-β overexpressing mice [172–174]. LOXL2, whose expression in healthy tissues is limited, is overexpressed in fibrotic lung and liver tissues. Targeting LOXL2 with an inhibitory monoclonal antibody [175] has recently been shown to reduce fibrosis and could represent a new therapeutic approach with broad applicability in fibrotic diseases. Finally, discoidin domain receptor 1 (DDR1), the only collagen receptor displaying a direct intracellular signaling activity through activation of P38MAP/kinase, might be another target for fibrosis therapy. Indeed, it has been suggested in several experimental studies that DDR1 has an important role in the regulation of renal fibrosis and in the development of inflammation in several tissue types, including renal tissues: DDR1 deficiency is protective in mouse models of hypertension-induced and Alport diseases [176–178]. Moreover, DDR1 has recently been put forward as a novel susceptibility gene for childhood IgA nephropathy [179].

Tools for drug discovery

In vitro *models*

All epithelial phenotypes present in the kidney are available in terms of stabilized cell lines. Proximal tubular cell lines (PTCs) of murine, porcine (LLC-PK1) and human (human kidney-2, HK-2) origins are available, as are well-characterized distal tubular cell lines of canine origin (Madin-Darby canine kidney cells; MDCK) and podocyte cell lines of murine and human origins.

As PTCs are responsible for the reabsorption of hormones, vitamins, small proteins and peptides filtered by the glomerulus, they are particularly relevant for drug discovery. The HK-2 cell line [180], although far from being perfect, has been shown to grow continuously in serum-free media retaining phenotypical [alkaline phosphatase$^+$, γ glutamyltranspeptidase$^+$, leucine aminopeptidase$^+$, acid phosphatase$^+$, cytokeratin$^+$, $\alpha_3\beta_1$ integrin$^+$, fibronectin$^+$; factor VHI-related antigen$^-$, 6.19 antigen–$^-$ and common acute lymphoblastic leukemia antigen (CALLA) endopeptidase$^-$], and functional (Na^+-dependent/phlorizin-sensitive sugar transport; and adenylate cyclase responsiveness to parathyroid, but not to antidiuretic, hormone) characteristics of well-differentiated PTCs. They might undergo EMT at low serum concentration (0.5% FCS or in serum-free medium [149]) when stimulated with TGF-β_1. All these results suggest that the HK-2 cell line could be used for pharmaceutical high-throughput screening (HTS). The porcine LLC-PK1 cell line [181] also has PTC characteristics. When forming a monolayer, this line expresses the multi-ligand binding receptor megalin [182] belonging to the LDL receptor family and present on absorptive epithelial cells. This cell line is an excellent instrument for *in vitro* studies of the transcellular transport of proteins and protein-bound molecules. MDCK cells, originally derived from the kidney of a normal cocker spaniel [183], have been used to investigate mechanisms of distal tubule transport and, more importantly, to study EMT extensively.

The podocyte, a terminal differentiated cell phenotype *in vivo*, is not supposed to replicate in culture conditions. However, both

TABLE 2

Animal models of CKD available for pharmaceutical development[a].

Animal model	Characteristics	Length	Features	Effect of SOC (ACEi/ARB)	Discovery phase	Refs
UUO (rat/mouse)	Fibrosis (inflammation)	4–7 days	↑TIF ↑BM fibrosis and inflammation ↑Infiltrates, fibroblast	Protective; enables add-on	Screening model; cpd profiling; cpd selection; cpd prioritization	[198,199]
Aldo/HS/Nx (rat, mouse)	HT nephropathy	3–6 weeks (short term)	↑↑BP, ↑UAE, ↑SCr, ↑GFR GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates	Protective; enables add-on	POC; CLS	[192]
Salt-induced nephropathy in DSS rats	HT nephropathy	3–6 weeks (short term)	↑↑BP, ↑UAE, ↑SCr, ↑GFR GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates	Protective; enables add-on	POC; CLS	[200]
5/6 Nx (rat, mouse)	Chronic renal failure	2–4 months (long term)	↑BP, ↑UAE, ↑SCr/↓GFR GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates	Protective; enables add-on	POC; CLS	[201]
ZSF1 (rat)	DN	4–6 months (long term)	↑BP, ↑UAE, GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates	Protective; enables add-on	POC; CLS	[201,202]
db/db/Nx (mouse)	DN	4–6 months (long term)	↑BP, ↑UAE, GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates	Protective; enables add-on	POC; CLS	[202,203]
eNOS KO/db/db (mouse)	DN	4–6 months (long term)	↑BP, ↑UAE, GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates ↑SCr	Protective; enables add-on	POC; CLS	[202,203]
ZDSD (rat)	DN	4–6 months (long term)	↑BP, ↑UAE, GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates ↑SCr	Protective; enables add-on	POC; CLS	[202,203]
T2DN/Nx (rat)	DN	4–6 months (long term)	↑BP, ↑UAE, GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates ↑SCr	Protective; enables add-on	POC; CLS	[202,203]
STZ ± Nx (rat, mouse)	DN	4–6 months (long term)	↑BP, ↑UAE, GS, TIF ↑BM fibrosis and inflammation ↑Infiltrates ↑SCr	Protective; enables add-on	POC; CLS	[202–204]
Cyclosporin A nephropathy (mouse)	Fibrosis (microangiopathy)	2–3 weeks				[205]

[a] *Abbreviations*: BM, biomarkers; BP, blood pressure; CLS, clinical leader selection; cpd, compound; DSS, Dahl-salt sensitive; GS, glomerulosclerosis; HT, hypertension; Nx, nephrectomy; POC, proof of concept; STZ, streptozotocin; TIF, tubulointerstitial fibrosis; UAE, urinary albumin excretion.

murine and human cell lines, as SV-40 conditionally immortalized cells, are available for *in vitro* studies. These cells will probably be crucial for pharmaceutical studies. Although recently indicated as good candidate for HTS [184], both the murine and human SV-40 conditionally immortalized cell lines do not express the slit diaphragm proteins nephrin and podocin, which are podocyte markers *in vivo*. In addition, the actin-binding protein synaptopodin, which is highly expressed in podocytes *in vivo* [185] is detectable at the protein level (by immunofluorescence) only in the murine cell line. In the human cell line, synaptopodin can be assessed only at

the mRNA level. The unique possible phenotypic readout for drug testing in human podocytes therefore appears to be limited to F-actin reorganization.

Finally, kidney mesenchymal cells are also available. Glomerular mesangial human [186], mouse [187] and rat [188] SV-40 conditionally immortalized cell lines have been extensively used in studies of diabetic nephropathy. Renal interstitial cells are commercially available uniquely as primary cells rather than as a cell line. Therefore, to reduce cell variability, experiments on renal interstitial cells can be conducted on foreskin fibroblasts, such AG01523 (Coriell Institute for Medical Research, Camden, NJ, USA; http://www.coriell.org) or WS-1 (ATCC-LGC Standards, Teddington, UK; http://www.lgcstandards-atcc.org/). In basal conditions, these cell lines exhibit a low content of α-SMA, thereby representing an ideal setting for the induction of myofibroblast activation through TGF-β1 administration and evaluation of reporter activity for TGF-β1 signaling. Even if any of these *in vitro* renal cells show low levels of differentiation compared with original cells, this does not reduce the relevance of these tools for pharmaceutical research.

In vivo *models*

Monkeys, dogs, sheep, rabbits and particularly rats and mice, have been used in experimental nephrology. Surgical, nephrotoxic, immunomediated or metabolic models exist in these species for AKI and CKD (reviewed in [189,190]). Owing to advantages of reproducibility, lower interanimal variation, and short time-course and easy performance, some models are more popular than others (e.g. the cisplatin model [191] for AKI and the UUO model [189] for fibrosis/CKD).

The cisplatin model is linked to toxic species arising from drug metabolism in tubular cells. Tubular nephrotoxicity, owing to cisplatin absorption and accumulation in proximal tubule cells, with apoptosis and necrosis of epithelial cells, is similar to the pathology and tubular dysfunction observed in oncology patients. This model is inducible in rats or mice by a single intraperitoneal injection of 6–20 mg cisplatin kg^{-1} body weight.

Induction of CKD by UUO, although not a common cause of kidney disease in humans, has proven to be a valuable tool for the study of tubulointerstitial fibrosis. The immediate consequence of urinary tract obstruction is an increase in pressure within the urinary tract, resulting in renal pelvis dilation, tubular dilation, tubular apoptosis, increased renin–angiotensin system activity followed by macrophage infiltration, leading to tubular atrophy and tubulointerstitial fibrosis. A major drawback of this model is the inability to measure renal function changes accurately because the remaining unobstructed kidney compensates for the obstructed kidney loss of function. Induction of tubulointerstitial fibrosis and glomerulosclerosis can also be also achieved by subtotal nephrectomy (i.e. 5/6 nephrectomy). These ligation and/or ablation models have shown good results in the rat, whereas those in rabbit and mouse have been less extensively studied and appear less reliable. The variability of the 5/6 nephrectomy model is, however, greater compared with UUO experiments.

Animal models for diabetic nephropathy share many features with human diabetic nephropathy, which is characterized by albuminuria, progressively declining glomerular filtration rate (GFR) and defined histopathological features, including thickening of the GBM, mesangial expansion often with nodular glomerulosclerosis, arteriolar hyalinosis and tubulointerstitial fibrosis. These animal models have been delineated by targeting proteinuria, glomerulosclerosis, glomerulonephritis, glomerular hypertrophy and tubulointerstitial nephritis (reviewed in [192]). For example, $eNOS^{-/-}$*db/db* mice [193,194] exhibit significant albuminuria and glomerular pathology that parallel the later phase of diabetic nephropathy (DN) in patients with type 2 diabetes, including arteriolar hyalinosis, mesangial expansion, thickening of GBM, and focal segmental and early nodular glomerulosclerosis. This model should prove useful for studying the role of endothelial dysfunction in the development of DN, and to develop new diagnostic tools and therapeutic interventions. (For a complete list of animal model available for CKD see Table 2.)

In general, although not yet perfect, animal models have provided valuable insights into the mechanisms of renal diseases, far more than in other disease areas. The main task of pharmaceutical scientist resides in the correct model adoption in relation to compound profile and target indication. What is still poorly used, and should, in our opinion, be used to a greater extent, is the acute-on-chronic approach animal model. As shown by epidemiological studies, this approach would take in account the fact that CKD is the most important predisposing risk factor for the development of AKI.

Conclusions

On the basis of the discussions above, it appears that approaching kidney disease is all but simple. Multiple processes are activated during renal diseases, and many cell types are involved. Moreover, insight from preclinical studies shows that not only resident renal cells, but also inflammatory cells have a crucial role in kidney disease progression. To make the scenario more complex, few, if any, reference drugs devoted to kidney disease exist on the market, with the exception of ARB and ACE, and clinical target validation could take several years in the case of CKD. In addition, chronic and long-term treatment, such as in the case of CKD, exposes patients to the unknown risks of off-target effects inhibiting key molecular pathways, such as for TGF-β.

As briefly highlighted in this review, it is still not clear which of the processes occurring during fibrosis onset (e.g. myofibroblast activation, ECM deposition, inflammation, hypoxia or ER-stress) is or are pivotal, and, if so, if this is translatable to patients. The microenvironment at the lesion site must be more complex so that the blockade of a single factor, although proved relevant in single-target knock-down experiments, would fail to slow down, inhibit or revert fibrosis in humans. Moreover the availability of a sound and clear panel of biomarkers for early CKD, which should enable researchers to explore the action of new compounds without waiting years for a functional response in patients, is still lacking.

By contrast, patients with CKD represent a relevant business case for pharma companies, as they currently account for more than 13% of the US population. Treatment of patients with CKD will also prove beneficial in reducing morbidity and mortality in high-risk cardiovascular patients, widening the business case even more [195].

From a theoretical perspective, a drug that is able to slow down the progression of chronic renal failure, on top of currently avail-

able treatments (i.e. ARB and ACEi), thus postponing the need for dialysis, will substantially change medical care in nephrology, increasing life expectancy and impacting dramatically on the quality of life of the patient. Seen from this perspective, such a result appears to be achievable owing to the range of targets available in kidney disease. Pathophysiological compound identification, biomarker discovery and accurate selection of clinical validation criteria appear, therefore, to be the three key elements needed to develop a successful innovative pharmaceutical approach to treating kidney diseases.

References

1 Tryggvason, K. and Wartiovaara, J. (2005) How does the kidney filter plasma? *Physiology (Bethesda)* 20, 96–101
2 Ali, T. *et al.* (2007) Incidence and outcomes in acute kidney injury: a comprehensive population-based study. *J. Am. Soc. Nephrol.* 18, 1292–1298
3 Hsu, C.Y. *et al.* (2007) Community-based incidence of acute renal failure. *Kidney Int.* 72, 208–212
4 Hou, S.H. *et al.* (1983) Hospital-acquired renal insufficiency: a prospective study. *Am. J. Med.* 74, 243–248
5 Coresh, J. *et al.* (2007) Prevalence of chronic kidney disease in the United States. *JAMA* 298, 2038–2047
6 Lameire, N. *et al.* (2005) Chronic kidney disease: a European perspective. *Kidney Int.* 99 (Suppl.), S30–S38
7 Venkatachalam, M.A. *et al.* (2010) Acute kidney injury: a springboard for progression in chronic kidney disease. *Am. J. Physiol. Renal Physiol.* 298, F1078–F1094
8 Lafrance, J.P. *et al.* (2010) Incidence and outcomes of acute kidney injury in a referred chronic kidney disease cohort. *Nephrol. Dial. Transplant.* 7, 2203–2209
9 Coca, S.G. *et al.* (2009) Long-term risk of mortality and other adverse outcomes after acute kidney injury: a systematic review and meta-analysis. *Am. J. Kidney Dis.* 53, 961–973
10 Azuma, H. *et al.* (1997) Cellular and molecular predictors of chronic renal dysfunction after initial ischemia/reperfusion injury of a single kidney. *Transplantation* 64, 190–197
11 Azuma, H. *et al.* (1997) Initial ischemia/reperfusion injury influences late functional and structural changes in the kidney. *Transplant. Proc.* 29, 1528–1529
12 Mackenzie, H.S. *et al.* (1995) Renal mass as a determinant of late allograft outcome: insights from experimental studies in rats. *Kidney Int. Suppl.* 52, S38–S42
13 Remuzzi, G. (1995) Abnormal protein traffic through the glomerular barrier induces proximal tubular cell dysfunction and causes renal injury. *Curr. Opin. Nephrol. Hypertens.* 4, 339–342
14 Tryggvason, K. *et al.* (2006) Nck links nephrin to actin in kidney podocytes. *Cell* 125, 221–224
15 Faul, C. *et al.* (2008) The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat. Med.* 14, 931–938
16 Leehey, D.J. *et al.* (2000) Role of angiotensin II in diabetic nephropathy. *Kidney Int. Suppl.* 77, S93–S98
17 Suzuki, T. *et al.* (2001) Role of atrophic tubules in development of interstitial fibrosis in microembolism-induced renal failure in rat. *Am. J. Pathol.* 158, 75–85
18 Gabbiani, G. *et al.* (1971) Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia* 27, 549–550
19 Tomasek, J.J. *et al.* (2002) Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* 3, 349–363
20 Hinz, B. *et al.* (2007) The myofibroblast: one function, multiple origins. *Am. J. Pathol.* 170, 1807–1816
21 Desmouliere, A. *et al.* (1995) Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am. J. Pathol.* 146, 56–66
22 Zeisberg, E.M. *et al.* (2008) Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J. Am. Soc. Nephrol.* 19, 2282–2287
23 Thiery, J.P. *et al.* (2009) Epithelial–mesenchymal transitions in development and disease. *Cell* 139, 871–890
24 Acloque, H. *et al.* (2009) Epithelial–mesenchymal transitions: the importance of changing cell state in development and disease. *J. Clin. Invest.* 119, 1438–1449
25 Zeisberg, E.M. *et al.* (2007) Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat. Med.* 13, 952–961
26 Zeisberg, M. and Duffield, J. (2010) Resolved: EMT produces fibroblasts in the kidney. *J. Am. Soc. Nephrol.* 21, 1247–1253
27 Yang, L. *et al.* (2010) Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. *Nat. Med.* 16, 535–543
28 Udo, K. *et al.* (2010) Adipose tissue explants and MDCK cells reciprocally regulate their morphogenesis in co-culture. *Kidney Int.* 78, 60–68
29 Sharma, M. *et al.* (2009) Analysis of stromal signatures in the tumor microenvironment of ductal carcinoma in situ. *Breast Cancer Res. Treat.*
30 Prunotto, M. and Moll, S. (2011) The emerging concept of a fibrotic microenvironment in CKD. *Kidney Int.* 79 (1), 135
31 Koesters, R. *et al.* (2010) Tubular overexpression of transforming growth factor-beta1 induces autophagy and fibrosis but not mesenchymal transition of renal epithelial cells. *Am. J. Pathol.* 177, 632–643
32 Schmidt, M. *et al.* (2003) Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. *J. Immunol.* 171, 380–389
33 Heidland, A. *et al.* (2001) Franz Volhard and Theodor Fahr: achievements and controversies in their research in renal disease and hypertension. *J. Hum. Hypertens.* 15, 5–16
34 Campese, V.M. *et al.* (2000) Is microalbuminuria a predictor of cardiovascular and renal disease in patients with essential hypertension? *Curr. Opin. Nephrol. Hypertens.* 9, 143–147
35 de Zeeuw, D. *et al.* (2004) Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: lessons from RENAAL. *Kidney Int.* 65, 2309–2320
36 Abbate, M. *et al.* (2002) Proximal tubular cells promote fibrogenesis by TGF-beta1-mediated induction of peritubular myofibroblasts. *Kidney Int.* 61, 2066–2077
37 Bertani, T. *et al.* (1986) Tubulo-interstitial lesions mediate renal damage in adriamycin glomerulopathy. *Kidney Int.* 30, 488–496
38 Bertani, T. *et al.* (1989) Age-related nephropathy and proteinuria in rats with intact kidneys exposed to diets with different protein content. *Lab. Invest.* 60, 196–204
39 Remuzzi, G. and Bertani, T. (1998) Pathophysiology of progressive nephropathies. *N. Engl. J. Med.* 339, 1448–1456
40 Baines, R.J. and Brunskill, N.J. (2008) The molecular interactions between filtered proteins and proximal tubular cells in proteinuria. *Nephron Exp. Nephrol.* 110, e67–e71
41 Eddy, A.A. and Giachelli, C.M. (1995) Renal expression of genes that promote interstitial inflammation and fibrosis in rats with protein-overload proteinuria. *Kidney Int.* 47, 1546–1557
42 Wang, Y. *et al.* (1997) Induction of monocyte chemoattractant protein-1 in proximal tubule cells by urinary protein. *J. Am. Soc. Nephrol.* 8, 1537–1545
43 Cybulsky, A.V. (2009) Endoplasmic reticulum stress in proteinuric kidney disease. *Kidney Int.* 77, 187–193
44 Markan, S. *et al.* (2009) Up regulation of the GRP-78 and GADD-153 and down regulation of Bcl-2 proteins in primary glomerular diseases: a possible involvement of the ER stress pathway in glomerulonephritis. *Mol. Cell. Biochem.* 324, 131–138
45 Ohse, T. *et al.* (2006) Albumin induces endoplasmic reticulum stress and apoptosis in renal proximal tubular cells. *Kidney Int.* 70, 1447–1455
46 Wu, X. *et al.* (2010) Albumin overload induces apoptosis in renal tubular epithelial cells through a CHOP-dependent pathway. *OMICS* 14, 61–73
47 Lindenmeyer, M.T. *et al.* (2008) Proteinuria and hyperglycemia induce endoplasmic reticulum stress. *J. Am. Soc. Nephrol.* 19, 2225–2236
48 Ulianich, L. *et al.* (2008) ER stress is associated with dedifferentiation and an epithelial-to-mesenchymal transition-like phenotype in PC Cl3 thyroid cells. *J. Cell Sci.* 121, 477–486
49 Seki, K. *et al.* (2003) Mouse Snail family transcription repressors regulate chondrocyte, extracellular matrix, type II collagen, and aggrecan. *J. Biol. Chem.* 278, 41862–41870
50 Prunotto, M. *et al.* (2010) Endocellular polyamine availability modulates epithelial-to-mesenchymal transition and unfolded protein response in MDCK cells. *Lab. Invest.* 90, 929–939
51 Dahan, K. *et al.* (2003) A cluster of mutations in the UMOD gene causes familial juvenile hyperuricemic nephropathy with abnormal expression of uromodulin. *J. Am. Soc. Nephrol.* 14, 2883–2893
52 Tang, S. *et al.* (2002) Role of complement in tubulointerstitial injury from proteinuria. *Kidney Blood Press. Res.* 25, 120–126
53 Boor, P. *et al.* (2007) Treatment targets in renal fibrosis. *Nephrol. Dial. Transplant.* 22, 3391–3407
54 Camussi, G. *et al.* (1982) In vitro alternative pathway activation of complement by the brush border of proximal tubules of normal rat kidney. *J. Immunol.* 128, 1659–1663

55 Biancone, L. *et al.* (1994) Alternative pathway activation of complement by cultured human proximal tubular epithelial cells. *Kidney Int.* 45, 451–460
56 Nangaku, M. (2004) Mechanisms of tubulointerstitial injury in the kidney: final common pathways to end-stage renal failure. *Intern. Med.* 43, 9–17
57 Morita, Y. *et al.* (1997) The role of complement in the pathogenesis of tubulointerstitial lesions in rat mesangial proliferative glomerulonephritis. *J. Am. Soc. Nephrol.* 8, 1363–1372
58 Rangan, G.K. *et al.* (2005) C5b-9 does not mediate chronic tubulointerstitial disease in the absence of proteinuria. *Kidney Int.* 67, 492–503
59 Welch, T.R. *et al.* (2002) C5a is important in the tubulointerstitial component of experimental immune complex glomerulonephritis. *Clin. Exp. Immunol.* 130, 43–48
60 Boor, P. *et al.* (2007) Complement C5 mediates experimental tubulointerstitial fibrosis. *J. Am. Soc. Nephrol.* 18, 1508–1515
61 Bao, L. *et al.* (2007) Unrestricted C3 activation occurs in Crry-deficient kidneys and rapidly leads to chronic renal failure. *J. Am. Soc. Nephrol.* 18, 811–822
62 Spett, K. (1965) Pathological affect. *Neurol. Neurochir. Psychiatr. Pol.* 15, 573–576
63 Chatziantoniou, C. and Dussaule, J.C. (2005) Insights into the mechanisms of renal fibrosis: is it possible to achieve regression? *Am. J. Physiol. Renal Physiol.* 289, F227–F234
64 Abbate, M. *et al.* (1999) Nephrotoxicity of increased glomerular protein traffic. *Nephrol. Dial. Transplant.* 14, 304–312
65 Choi, Y.J. *et al.* (2000) Peritubular capillary loss is associated with chronic tubulointerstitial injury in human kidney: altered expression of vascular endothelial growth factor. *Hum. Pathol.* 31, 1491–1497
66 Norman, J.T. *et al.* (2000) Hypoxia promotes fibrogenesis in human renal fibroblasts. *Kidney Int.* 58, 2351–2366
67 Norman, J.T. *et al.* (1999) Hypoxia-induced changes in extracellular matrix metabolism in renal cells. *Exp. Nephrol.* 7, 463–469
68 Manotham, K. *et al.* (2004) Transdifferentiation of cultured tubular cells induced by hypoxia. *Kidney Int.* 65, 871–880
69 Remuzzi, G. *et al.* (2006) Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J. Clin. Invest.* 116, 288–296
70 Remuzzi, G. *et al.* (2005) The role of renin-angiotensin-aldosterone system in the progression of chronic kidney disease. *Kidney Int.* 68, S57–S65
71 Huang, X.R. *et al.* (2003) Chymase is upregulated in diabetic nephropathy: implications for an alternative pathway of angiotensin II-mediated diabetic renal and vascular disease. *J. Am. Soc. Nephrol.* 14, 1738–1747
72 Lely, A.T. *et al.* (2004) Renal ACE2 expression in human kidney disease. *J. Pathol.* 204, 587–593
73 Durvasula, R.V. *et al.* (2004) Activation of a local tissue angiotensin system in podocytes by mechanical strain. *Kidney Int.* 65, 30–39
74 Wolf, G. *et al.* (1997) Aminopeptidase A: a key enzyme in the intrarenal degradation of angiotensin II. *Exp. Nephrol.* 5, 364–369
75 Cesari, M. *et al.* (2002) Biological properties of the angiotensin peptides other than angiotensin II: implications for hypertension and cardiovascular diseases. *J. Hypertens.* 20, 793–799
76 Pilz, B. *et al.* (2005) Aliskiren, a human renin inhibitor, ameliorates cardiac and renal damage in double-transgenic rats. *Hypertension* 46, 569–576
77 Nguyen, G. (2007) The (pro)renin receptor: pathophysiological roles in cardiovascular and renal pathology. *Curr. Opin. Nephrol. Hypertens.* 16, 129–133
78 Weber, M.A. (2001) Vasopeptidase inhibitors. *Lancet* 358, 1525–1532
79 Gross, O. *et al.* (2005) Nephroprotection by antifibrotic and anti-inflammatory effects of the vasopeptidase inhibitor AVE7688. *Kidney Int.* 68, 456–463
80 Benigni, A. *et al.* (2004) Vasopeptidase inhibitor restores the balance of vasoactive hormones in progressive nephropathy. *Kidney Int.* 66, 1959–1965
81 Kostis, J.B. *et al.* (2004) Omapatrilat and enalapril in patients with hypertension: the Omapatrilat Cardiovascular Treatment vs. Enalapril (OCTAVE) trial. *Am. J. Hypertens.* 17, 103–111
82 Wolf, G. and Ritz, E. (2005) Combination therapy with ACE inhibitors and angiotensin II receptor blockers to halt progression of chronic renal disease: pathophysiology and indications. *Kidney Int.* 67, 799–812
83 Adamczak, M. *et al.* (2003) Reversal of glomerulosclerosis after high-dose enalapril treatment in subtotally nephrectomized rats. *J. Am. Soc. Nephrol.* 14, 2833–2842
84 Mason, J.C. (2005) The statins–therapeutic diversity in renal disease? *Curr. Opin. Nephrol. Hypertens.* 14, 17–24
85 Goldstein, J.L. and Brown, M.S. (1990) Regulation of the mevalonate pathway. *Nature* 343, 425–430
86 Etienne-Manneville, S. and Hall, A. (2002) Rho GTPases in cell biology. *Nature* 420, 629–635
87 Zoja, C. *et al.* (2002) How to fully protect the kidney in a severe model of progressive nephropathy: a multidrug approach. *J. Am. Soc. Nephrol.* 13, 2898–2908
88 Zoja, C. *et al.* (2010) Adding a statin to a combination of Ace inhibitor and Arb normalizes proteinuria in experimental diabetes which translates into full renoprotection. *Am. J. Physiol. Renal Physiol.* 299, F1203–F1211
89 Zhang, Y. *et al.* (2010) Vitamin D receptor attenuates renal fibrosis by suppressing the renin-angiotensin system. *J. Am. Soc. Nephrol.* 21, 966–973
90 Mirkovic, K. *et al.* (2010) Vitamin D in chronic kidney disease: new potential for intervention. *Curr. Drug Targets* doi:BSP/CDT/E-Pub/00158
91 Haussler, M.R. *et al.* (1998) The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J. Bone Miner. Res.* 13, 325–349
92 Li, Y.C. (2009) Renoprotective effects of vitamin D analogs. *Kidney Int.* 78, 134–139
93 Fryer, R.M. *et al.* (2007) Differential inhibition of renin mRNA expression by paricalcitol and calcitriol in C57/BL6 mice. *Nephron Physiol.* 106, 76–81
94 Li, Y.C. *et al.* (2002) 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J. Clin. Invest.* 110, 229–238
95 Yuan, W. *et al.* (2007) 1,25-dihydroxyvitamin D3 suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. *J. Biol. Chem.* 282, 29821–29830
96 Sun, J. *et al.* (2006) Increased NF-kappaB activity in fibroblasts lacking the vitamin D receptor. *Am. J. Physiol. Endocrinol. Metab.* 291, E315–E322
97 Deb, D.K. *et al.* (2009) 1,25-Dihydroxyvitamin D3 suppresses high glucose-induced angiotensinogen expression in kidney cells by blocking the NF-{kappa}B pathway. *Am. J. Physiol. Renal Physiol.* 296, F1212–F1218
98 Guijarro, C. and Egido, J. (2001) Transcription factor-kappa B (NF-kappa B) and renal disease. *Kidney Int.* 59, 415–424
99 Zehnder, D. *et al.* (2008) Reduction of the vitamin D hormonal system in kidney disease is associated with increased renal inflammation. *Kidney Int.* 74, 1343–1353
100 Makibayashi, K. *et al.* (2001) A vitamin D analog ameliorates glomerular injury on rat glomerulonephritis. *Am. J. Pathol.* 158, 1733–1741
101 Mizobuchi, M. *et al.* (2007) Combination therapy with an angiotensin-converting enzyme inhibitor and a vitamin D analog suppresses the progression of renal insufficiency in uremic rats. *J. Am. Soc. Nephrol.* 18, 1796–1806
102 Zhang, Z. *et al.* (2008) Combination therapy with AT1 blocker and vitamin D analog markedly ameliorates diabetic nephropathy: blockade of compensatory renin increase. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15896–15901
103 Tan, X. *et al.* (2006) Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. *J. Am. Soc. Nephrol.* 17, 3382–3393
104 Doorenbos, C.R. *et al.* (2009) Possible renoprotection by vitamin D in chronic renal disease: beyond mineral metabolism. *Nat. Rev. Nephrol.* 5, 691–700
105 Lambers Heerspink, H.J. *et al.* (2009) The selective vitamin D receptor activator for albuminuria lowering (VITAL) study: study design and baseline characteristics. *Am. J. Nephrol.* 30, 280–286
106 Thiem, U. *et al.* (2009) VITA-D: cholecalciferol substitution in vitamin D deficient kidney transplant recipients: a randomized, placebo-controlled study to evaluate the post-transplant outcome. *Trials* 10, 36
107 Nagatoya, K. *et al.* (2002) Y-27632 prevents tubulointerstitial fibrosis in mouse kidneys with unilateral ureteral obstruction. *Kidney Int.* 61, 1684–1695
108 Nishikimi, T. *et al.* (2004) Fasudil, a Rho-kinase inhibitor, attenuates glomerulosclerosis in Dahl salt-sensitive rats. *J. Hypertens.* 22, 1787–1796
109 Ishikawa, Y. *et al.* (2006) Long-term administration of rho-kinase inhibitor ameliorates renal damage in malignant hypertensive rats. *Hypertension* 47, 1075–1083
110 Peng, F. *et al.* (2008) RhoA/Rho-kinase contribute to the pathogenesis of diabetic renal disease. *Diabetes* 57, 1683–1692
111 Leung, T. *et al.* (1995) A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. *J. Biol. Chem.* 270, 29051–29054
112 Amano, M. *et al.* (1997) Formation of actin stress fibers and focal adhesions enhanced by Rho-kinase. *Science* 275, 1308–1311
113 Takai, Y. *et al.* (1995) Rho as a regulator of the cytoskeleton. *Trends Biochem. Sci.* 20, 227–231
114 Takai, Y. *et al.* (2001) Small GTP-binding proteins. *Physiol. Rev.* 81, 153–208
115 Uehata, M. *et al.* (1997) Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* 389, 990–994
116 Takeda, Y. *et al.* (2010) Beneficial effects of a combination of Rho-kinase inhibitor and ACE inhibitor on tubulointerstitial fibrosis induced by unilateral ureteral obstruction. *Hypertens. Res.* 33, 965–973
117 Nishida, M. and Hamaoka, K. (2008) Macrophage phenotype and renal fibrosis in obstructive nephropathy. *Nephron Exp. Nephrol.* 110, e31–e36
118 Ricardo, S.D. *et al.* (2008) Macrophage diversity in renal injury and repair. *J. Clin. Invest.* 118, 3522–3530
119 Song, E. *et al.* (2000) Influence of alternatively and classically activated macrophages on fibrogenic activities of human fibroblasts. *Cell. Immunol.* 204, 19–28

120 Jungers, P. *et al.* (2001) Beneficial influence of recombinant human erythropoietin therapy on the rate of progression of chronic renal failure in predialysis patients. *Nephrol. Dial. Transplant.* 16, 307–312

121 Kuriyama, S. *et al.* (1997) Reversal of anemia by erythropoietin therapy retards the progression of chronic renal failure, especially in nondiabetic patients. *Nephron* 77, 176–185

122 Kang, D.H. *et al.* (2001) Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. *J. Am. Soc. Nephrol.* 12, 1448–1457

123 Hofer, T. *et al.* (2002) Oxygen sensing, HIF-1alpha stabilization and potential therapeutic strategies. *Pflugers Arch.* 443, 503–507

124 Higgins, D.F. *et al.* (2007) Hypoxia promotes fibrogenesis in vivo via HIF-1 stimulation of epithelial-to-mesenchymal transition. *J. Clin. Invest.* 117, 3810–3820

125 Song, Y.R. *et al.* (2010) Activation of hypoxia-inducible factor attenuates renal injury in rat remnant kidney. *Nephrol. Dial. Transplant.* 25, 77–85

126 Kapoor, M. *et al.* (2008) GSK-3beta in mouse fibroblasts controls wound healing and fibrosis through an endothelin-1-dependent mechanism. *J. Clin. Invest.* 118, 3279–3290

127 Gradl, D. *et al.* (1999) The Wnt/Wg signal transducer beta-catenin controls fibronectin expression. *Mol. Cell. Biol.* 19, 5576–5587

128 Brabletz, T. *et al.* (1999) Beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. *Am. J. Pathol.* 155, 1033–1038

129 Hwang, I. *et al.* (2009) Wnt/beta-catenin signaling: a novel target for therapeutic intervention of fibrotic kidney disease. *Arch. Pharm. Res.* 32, 1653–1662

130 He, W. *et al.* (2009) Wnt/beta-catenin signaling promotes renal interstitial fibrosis. *J. Am. Soc. Nephrol.* 20, 765–776

131 Iwano, M. (2010) EMT and TGF-beta in renal fibrosis. *Front. Biosci.* 2, 229–238

132 Border, W.A. and Noble, N.A. (1998) Evidence that TGF-beta should be a therapeutic target in diabetic nephropathy. *Kidney Int.* 54, 1390–1391

133 Gagliardini, E. and Benigni, A. (2007) Therapeutic potential of TGF-beta inhibition in chronic renal failure. *Expert Opin. Biol. Ther.* 7, 293–304

134 Denton, C.P. *et al.* (2007) Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. *Arthritis Rheum.* 56, 323–333

135 Lan, H.Y. (2008) Smad7 as a therapeutic agent for chronic kidney diseases. *Front. Biosci.* 13, 4984–4992

136 Lan, H.Y. *et al.* (2003) Inhibition of renal fibrosis by gene transfer of inducible Smad7 using ultrasound-microbubble system in rat UUO model. *J. Am. Soc. Nephrol.* 14, 1535–1548

137 Terada, Y. *et al.* (2002) Gene transfer of Smad7 using electroporation of adenovirus prevents renal fibrosis in post-obstructed kidney. *Kidney Int.* 61 (Suppl. 1), S94–S98

138 Ma, L.J. *et al.* (2003) Transforming growth factor-beta-dependent and -independent pathways of induction of tubulointerstitial fibrosis in beta6(−/−) mice. *Am. J. Pathol.* 163, 1261–1273

139 Hahm, K. *et al.* (2007) Alphav beta6 integrin regulates renal fibrosis and inflammation in Alport mouse. *Am. J. Pathol.* 170, 110–125

140 Ozkaynak, E. *et al.* (1990) OP-1 cDNA encodes an osteogenic protein in the TGF-beta family. *EMBO J.* 9, 2085–2093

141 Dudley, A.T. *et al.* (1995) A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev.* 9, 2795–2807

142 Luo, G. *et al.* (1995) BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev.* 9, 2808–2820

143 Vukicevic, S. *et al.* (1998) Osteogenic protein-1 (bone morphogenetic protein-7) reduces severity of injury after ischemic acute renal failure in rat. *J. Clin. Invest.* 102, 202–214

144 Lin, J. *et al.* (2005) Kielin/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. *Nat. Med.* 11, 387–393

145 Tanaka, M. *et al.* (2010) Loss of the BMP antagonist U. S. A.G-1 ameliorates disease in a mouse model of the progressive hereditary kidney disease Alport syndrome. *J. Clin. Invest.* 120, 768–777

146 Tanaka, M. *et al.* (2008) Expression of BMP-7 and U. S. A.G-1 (a BMP antagonist) in kidney development and injury. *Kidney Int.* 73, 181–191

147 Carvajal, G. *et al.* (2008) Gremlin: a novel mediator of epithelial mesenchymal transition and fibrosis in chronic allograft nephropathy. *Transplant. Proc.* 40, 734–739

148 Dolan, V. *et al.* (2003) Gremlin – a putative pathogenic player in progressive renal disease. *Expert Opin. Ther. Targets* 7, 523–526

149 Zeisberg, M. *et al.* (2003) BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat. Med.* 9, 964–968

150 Archdeacon, P. and Detwiler, R.K. (2008) Bone morphogenetic protein 7 (BMP7): a critical role in kidney development and a putative modulator of kidney injury. *Adv. Chronic Kidney Dis.* 15, 314–320

151 Boor, P. *et al.* (2010) Renal fibrosis: novel insights into mechanisms and therapeutic targets. *Nat. Rev. Nephrol.* 6, 643–656

152 Iekushi, K. *et al.* Hepatocyte growth factor attenuates renal fibrosis through TGF-beta1 suppression by apoptosis of myofibroblasts. J. Hypertens. (2010) Sep 11

153 Dai, C. *et al.* (2010) Hepatocyte growth factor signaling ameliorates podocyte injury and proteinuria. *Kidney Int.* 77, 962–973

154 Mizuno, S. *et al.* (2008) HGF as a renotrophic and anti-fibrotic regulator in chronic renal disease. *Front. Biosci.* 13, 7072–7086

155 Ma, L.J. and Fogo, A.B. (2007) Modulation of glomerulosclerosis. *Semin. Immunopathol.* 29, 385–395

156 Delaroche, D. *et al.* (2010) Cell-penetrating peptides with intracellular actin-remodeling activity in malignant fibroblasts. *J. Biol. Chem.* 285, 7712–7721

157 Julien, B. *et al.* (2005) Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology* 128, 742–755

158 Wasmuth, H.E. and Trautwein, C. (2007) CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Hepatology* 45, 543–544

159 Mann, J. *et al.* (2010) MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. *Gastroenterology* 138, 705–714 714, e701–e704

160 Ghosh, A.K. *et al.* (2004) Disruption of transforming growth factor beta signaling and profibrotic responses in normal skin fibroblasts by peroxisome proliferator-activated receptor gamma. *Arthritis Rheum.* 50, 1305–1318

161 Kawai, T. *et al.* (2009) PPAR-gamma agonist attenuates renal interstitial fibrosis and inflammation through reduction of TGF-beta. *Lab. Invest.* 89, 47–58

162 Douglass, A. *et al.* (2008) Antibody-targeted myofibroblast apoptosis reduces fibrosis during sustained liver injury. *J. Hepatol.* 49, 88–98

163 Murea, M. *et al.* (2010) Expression of Notch pathway proteins correlates with albuminuria, glomerulosclerosis, and renal function. *Kidney Int.* 78, 514–522

164 Lasagni, L. *et al.* (2010) Notch activation differentially regulates renal progenitors proliferation and differentiation toward the podocyte lineage in glomerular disorders. *Stem Cells* 28 (9), 1674–1685

165 Niranjan, T. *et al.* (2008) The Notch pathway in podocytes plays a role in the development of glomerular disease. *Nat. Med.* 14, 290–298

166 Lasagni, L. *et al.* (2010) Notch activation differentially regulates renal progenitors proliferation and differentiation toward the podocyte lineage in glomerular disorders. *Stem Cells* 28, 1674–1685

167 Ronco, P. and Chatziantoniou, C. (2008) Matrix metalloproteinases and matrix receptors in progression and reversal of kidney disease: therapeutic perspectives. *Kidney Int.* 74, 873–878

168 Ronco, P. *et al.* (2007) Matrix metalloproteinases in kidney disease progression and repair: a case of flipping the coin. *Semin. Nephrol.* 27, 352–362

169 Bergheim, I. *et al.* (2006) Critical role of plasminogen activator inhibitor-1 in cholestatic liver injury and fibrosis. *J. Pharmacol. Exp. Ther.* 316, 592–600

170 Oda, T. *et al.* (2001) PAI-1 deficiency attenuates the fibrogenic response to ureteral obstruction. *Kidney Int.* 60, 587–596

171 Matsuo, S. *et al.* (2005) Multifunctionality of PAI-1 in fibrogenesis: evidence from obstructive nephropathy in PAI-1-overexpressing mice. *Kidney Int.* 67, 2221–2238

172 Eddy, A.A. and Fogo, A.B. (2006) Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action. *J. Am. Soc. Nephrol.* 17, 2999–3012

173 Huang, Y. *et al.* (2003) A mutant, noninhibitory plasminogen activator inhibitor type 1 decreases matrix accumulation in experimental glomerulonephritis. *J. Clin. Invest.* 112, 379–388

174 Huang, Y. *et al.* (2008) A PAI-1 mutant, PAI-1R, slows progression of diabetic nephropathy. *J. Am. Soc. Nephrol.* 19, 329–338

175 Barry-Hamilton, V. *et al.* (2010) Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. *Nat. Med.* 16, 1009–1017

176 Gross, O. *et al.* (2010) Loss of collagen-receptor DDR1 delays renal fibrosis in hereditary type IV collagen disease. *Matrix Biol.* 29, 346–356

177 Flamant, M. *et al.* (2006) Discoidin domain receptor 1 null mice are protected against hypertension-induced renal disease. *J. Am. Soc. Nephrol.* 17, 3374–3381

178 Lee, R. *et al.* (2004) Localization of discoidin domain receptors in rat kidney. *Nephron Exp. Nephrol.* 97, e62–e70

179 Hahn, W.H. *et al.* (2010) Linkage and association study of discoidin domain receptor 1 as a novel susceptibility gene for childhood IgA nephropathy. *Int. J. Mol. Med.* 25, 785–791

180 Ryan, M.J. *et al.* (1994) HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. *Kidney Int.* 45, 48–57

181 Hull, R.N. *et al.* (1976) The origin and characteristics of a pig kidney cell strain, LLC-PK. *In Vitro* 12, 670–677

182 Nielsen, R. *et al.* (1998) Characterization of a kidney proximal tubule cell line, LLC-PK1, expressing endocytotic active megalin. *J. Am. Soc. Nephrol.* 9, 1767–1776

183 Rindler, M.J. *et al.* (1979) Retention of differentiated properties in an established dog kidney epithelial cell line (MDCK). *J. Cell Biol.* 81, 635–648
184 Reiser, J. *et al.* (2010) Toward the development of podocyte-specific drugs. *Kidney Int.* 77, 662–668
185 Mundel, P. *et al.* (1997) Synaptopodin: an actin-associated protein in telencephalic dendrites and renal podocytes. *J. Cell Biol.* 139, 193–204
186 Sraer, J.D. *et al.* (1996) Stable cell lines of T-SV40 immortalized human glomerular mesangial cells. *Kidney Int.* 49, 267–270
187 MacKay, K. *et al.* (1988) Glomerular epithelial, mesangial, and endothelial cell lines from transgenic mice. *Kidney Int.* 33, 677–684
188 Nitta, K. and Horiba, N. (1997) An immortalized rat mesangial cell line. *In Vitro Cell. Dev. Biol. Anim.* 33, 156–157
189 Hewitson, T.D. *et al.* (2009) Small animal models of kidney disease: a review. *Methods Mol. Biol.* 466, 41–57
190 Heyman, S.N. *et al.* (2002) Animal models of acute tubular necrosis. *Curr. Opin. Crit. Care* 8, 526–534
191 Yao, X. *et al.* (2007) Cisplatin nephrotoxicity: a review. *Am. J. Med. Sci.* 334, 115–124
192 Balakumar, P. *et al.* (2008) Experimental models for nephropathy. *J. Renin Angiotensin Aldosterone Syst.* 9, 189–195
193 Zhao, H.J. *et al.* (2006) Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. *J. Am. Soc. Nephrol.* 17, 2664–2669
194 Nakagawa, T. (2009) A new mouse model resembling human diabetic nephropathy: uncoupling of VEGF with eNOS as a novel pathogenic mechanism. *Clin. Nephrol.* 71, 103–109
195 Astor, B.C. *et al.* (2008) Glomerular filtration rate, albuminuria, and risk of cardiovascular and all-cause mortality in the US population. *Am. J. Epidemiol.* 167, 1226–1234
196 Hocher, B. and Paul, M. (2000) Transgenic animal models for the analysis of the renal endothelin system. *Nephrol. Dial. Transplant.* 15, 935–937
197 Kohan, D.E. (2010) Endothelin, hypertension and chronic kidney disease: new insights. *Curr. Opin. Nephrol. Hypertens.* 19, 134–139
198 Truong, L.D. *et al.* (1996) Cell apoptosis and proliferation in experimental chronic obstructive uropathy. *Kidney Int.* 50, 200–207
199 Klahr, S. (2001) Urinary tract obstruction. *Semin. Nephrol.* 21, 133–145
200 Wang, P.X. and Sanders, P.W. (2005) Mechanism of hypertensive nephropathy in the Dahl/Rapp rat: a primary disorder of vascular smooth muscle. *Am. J. Physiol. Renal Physiol.* 288, F236–F242
201 Ma, L.J. and Fogo, A.B. (2003) Model of robust induction of glomerulosclerosis in mice: importance of genetic background. *Kidney Int.* 64, 350–355
202 Schlondorff, D. (2010) Choosing the right mouse model for diabetic nephropathy. *Kidney Int.* 77, 749–750
203 Brosius, F.C., III *et al.* (2009) Mouse models of diabetic nephropathy. *J. Am. Soc. Nephrol.* 20, 2503–2512
204 Tesch, G.H. and Allen, T.J. (2007) Rodent models of streptozotocin-induced diabetic nephropathy. *Nephrology* 12, 261–266
205 Kang, D.H. *et al.* (2001) Post-cyclosporine-mediated hypertension and nephropathy: amelioration by vascular endothelial growth factor. *Am. J. Physiol. Renal Physiol.* 280, F727–F736