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Developmental signalling pathways in renal fibrosis: the roles of Notch, Wnt and Hedgehog

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Abstract

Kidney fibrosis is the histological manifestation of functional decline in the kidney. Fibrosis is a reactive process that develops in response to excessive epithelial injury and inflammation. Here, we describe how three key developmental signalling pathways—Notch, Wnt and Hedgehog—are reactivated in response to kidney injury. Although transient activation of these pathways is needed for repair of injured tissue, their sustained activation promotes fibrosis. Excessive Wnt and Notch expression prohibit epithelial differentiation whereas increased Wnt and Hh expression induce fibroblast proliferation and myofibroblastic transdifferentiation. Notch, Wnt and Hedgehog are fundamentally different signalling mechanisms, but their choreographed activation seems to be just as important for fibrosis as it is for embryonic kidney development. Decreasing the activity of Notch, Wnt, or Hh signalling could potentially be a new therapeutic strategy to ameliorate the development of chronic kidney disease.

Introduction

Nearly 9% of the world's population has chronic kidney disease (CKD) defined as either a 40% reduction in filtering capacity of the kidney or its abnormal leakiness for plasma albumin[1]. Fibrosis is the histological manifestation of CKD[2]. Strategies that can effectively slow, or even revert, renal fibrosis in patients with CKD could have a marked clinical impact in preventing end-stage kidney disease, a condition that can only be treated with dialysis or transplantation and is associated with at least a threefold increase in mortality compared to that of the general population[3].

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Author contributions

M.E. S. H. and G.R. researched data for the article. M.E. and K.S. discussed the article's content, after which M.E., S.H., G.R. and K.S. wrote the manuscript. H.P. reviewed the manuscript.

Competing interests

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Fibrotic changes can occur in the glomerulus—referred to as glomerulosclerosis—or in the tubules—referred to as tubulointerstitial fibrosis [4,5]. Of note, glomerulosclerosis and tubulointerstitial fibrosis involve similar cellular changes involving the loss of epithelial cells and their vascular capillary bed and the accumulation of activated myofibroblasts, matrix, and inflammatory cells[6]. Matrix build-up, which results from fibroblast transdifferentiation and proliferation, is the *sine qua non* of fibrosis. Myofibroblast activation is a reactive process that develops in response to epithelial injury, paracrine signalling and cytokine production[7]. In the tubulointerstitium activated myofibroblasts mostly originate from resident fibroblasts or pericytes[8,9]. In the glomerulus, mesangial cells take on myofibroblast-like characteristics including matrix secretion, proliferation and transdifferentiation[4]. While myofibroblast activation gives the critical histological characteristics of fibrosis the functional decline in the kidney is caused by a loss of differentiated epithelial cells.

Genome-wide transcriptome analysis of kidneys from patients with CKD and fibrosis showed that genes belonging to the Notch, Wnt and Hh signalling pathways are differentially expressed between patients with renal fibrosis and healthy control individuals (Woroniecka et al. 2011)[10]. These three pathways act through different signalling mechanisms, but they all have a critical role in kidney development. This Review discusses how these three pathways are reactivated in response to renal injury and describes their involvement in the development of renal fibrosis.

Mechanisms of renal fibrosis

Epithelial damage and inflammation

Animal model studies indicate that uncontrolled epithelial damage and inflammation are the primary processes that cause fibrosis in the kidney. Several factors can induce excessive epithelial cell injury, including high levels of glucose free fatty acids, advanced glycation end products, transforming growth factor β, and physical stretch[4]. When the injury cannot be repaired, epithelial cells die via apoptosis, necrosis, autophagy, mitotic catastrophe or oncosis [11,1213–15]. In addition to these cell death pathways, viable epithelial cells can be cultured from the urine of patients with CKD, indicating that detachment also contributes to epithelial cell loss [16]. A decrease in nutrient availability caused by capillary loss often exacerbates epithelial damage [517].

The direct role of epithelial cell loss in fibrosis development is best demonstrated in the glomerulus[18], as podocytes cannot proliferate or be replaced readily[19]. When podocyte loss exceeds 20%, changes become irreversible causing the entire glomerulus to scar even if the original injury is no longer present [20,21]. The death of epithelial cells triggers a complex cellular and molecular response. The remaining epithelial cells grow in size and number to cover any open basement membrane [22–2425]. Levels of paracrine factors, such as VEGF, secreted by epithelial cells will decrease causing further endothelial damage and ultimately leading to capillary loss[6,2627]. Epithelial cell injury also triggers the influx of inflammatory cells. These immune cells have an essential role in clearing dead cell remnants and regenerating damaged tubule epithelial cells, leading to repair and resolution [28] (Figure 1).

Paradoxically, the inflammatory response associated with CKD exacerbates epithelial injury and contributes to fibrosis development[29–31]. Histological analysis of human and animal models has shown that the degree of inflammatory cell infiltration strongly correlates with the severity of kidney fibrosis[32]. The increased cytokine production that accompanies fibrosis is mostly controlled by the activity of the transcription factors NF-κB and STAT. Pharmacological inhibition of NF-κB signalling by pyrrolidine dithiocarbamate significantly diminished kidney injury and ameliorated tubular degeneration, tubular necrosis and tubulointerstitial inflammation in models of kidney fibrosis induced by folate or adenine overload in mice[33,34]. Similarly, genetic deletion of STAT has been shown to ameliorate kidney fibrosis in mouse models[29,30,35]. These studies support the key role of NF-κB, STAT and inflammation in the development of fibrosis.

Although inflammation mostly occur secondary to epithelial damage, uncontrolled inflammatory reactions, for example as seen in the context of viral or bacterial infections, in transplant recipients and in patients with autoimmune disease, can also be the primary cause of fibrosis[36]. Under these conditions epithelial damage occurs secondary to the inflammatory reaction. Further studies are needed to dissect the role and mechanism of the inflammatory response in fibrosis and to understand inflammatory processes that result in repair and those that contribute to fibrosis [17].

Fibroblast activation is a reactive process

The scarring that is characteristic of fibrosis develops because of an increase in the number of activated myofibroblasts, which lay down an excessive amount of extracellular matrix (ECM)[5]. Activated myofibroblasts are usually identified by the expression of α smooth muscle actin and vimentin but also usually express desmin and secrete collagen. The activation and proliferation of myofibroblasts are reactive processes. The number of myofibroblasts in the human kidney tissue samples can predict future functional decline in patients with CKD[37] and correlates strongly with epithelial injury. The association between myofibroblast number and epithelial injury in animal models is so strong that epithelial cells have been proposed to directly transdifferentiate into myofibroblasts[38]. Lineage tagging experiments performed in mice using Six2 as a marker of tubule epithelial cells and FoxD1 as a marker of resident fibroblasts indicated that most activated myofibroblasts in fibrotic kidneys in fact originate from resident fibroblasts, as they were the progeny of FoxD1 positive cells[9,39].

In fibrosis, fibroblasts change their characteristics, expressing different ECM proteins and synthesizing greater amounts of collagens, and proteoglycans (fibronectin, laminin and tenascin)[39,40]. The new ECM composition, in turn, causes alterations in the existing epithelial cells and fibroblasts[40,41] (Figure 1). The excessive amount of fibrillary collagen raises the stiffness of the organ, which causes further myofibroblast activation and epithelial damage[42]. Increased stiffness and matrix is likely needed to make up for the lost epithelial cells. For example in wound healing, myofibroblasts can pull the wound edges together and the increased matrix can reduce the active energy that is needed to keep these sides together [42]. Paracrine factors released by epithelial cells including Tgf-β, Ctgf, Fgf, Pdgf, Il-1, Tnf, angiotensin II and aldosterone, cause fibroblast activation[2,6,43–46]. Tgf-β is thought

to be the master regulator of myofibroblast activation and believed to be the key culprit in fibrosis. In rodent models of fibrosis, inhibition of Tgf-β signalling decreased the extent of ECM production[47]. However, Tgf-β also interferes with immune cells, and mice lacking Tgf-β are at increased risk of developing autoimmune disorders[48,49]. Given that fibroblast activation is a reactive process that occurs in response to epithelial injury, it would seem more appropriate to target the molecular alterations in epithelial cells rather than those involved in fibroblasts.

Notch in kidney development and homeostasis

The Notch signalling pathway is a phylogenetically conserved cell–cell communication mechanism with multiple different functions including cell fate decision, cell lineage specification and cell lineage stabilization [50]. Notch signalling helps to differentiate neighbouring cells through the asymmetric expression of the pathway's ligands and receptors. The 'signal receiving' cell expresses one of four transmembrane receptors: Notch1, Notch2, Notch3 or Notch4[51]. These receptors are presented on the plasma membrane of the 'signal receiving' cell after undergoing fringe-dependent glycosylation, cleavage by a furin-like convertase (S1 cleavage) and a second cleavage by the ADAM metalloprotease receptors (S2 cleavage). The Notch ligands Jagged1 (Jag1), Jagged2 (Jag2), Delta-like 1 (Dll1), Delta-like 3 (Dll3) or Delta-like 4 (Dll4) are expressed on the surface of the 'signal-sending' cells. Binding of the ligand to the receptor initiates a third cleavage of the receptor (S3), after which the intracellular domain of the Notch receptor is transported to the nucleus to act as a transcriptional regulator. The γ-secretase complex mediates this third cleavage step. In the nucleus, the Notch intracellular domain binds to different transcriptional regulators, such as Rbpj and MAML, to regulate expression of target genes, most of which belong to the hairy and enhancer of split (Hes1-7) and hes-related family bHLH transcription factor with YRPW motif (Hey1, Hey2 and HeyL) families [52](Figure 2).

Notch signalling plays a critical role in kidney development[53]. Mutations of the Notch ligand, JAGGED1, and Notch receptor, NOTCH2, in humans cause kidney developmental abnormalities[54]. Mouse model experiments showed that Notch2, but not Notch1, is critical for proximal tubular cell fate determination. Few if any proximal tubules and podocytes can be found in mice with a kidney-specific deletion of Notch2[55]. On the other hand, overexpression of Notch2 directs cells to take on a proximal tubule fate[56]. Genetic deletion of other Notch receptors (Notch3 and Notch4) and ligands (Jagged2 and Delta4) in mice did not induce observable renal defects[53,57].

As expected, the expression of Notch pathway proteins is largely suppressed once organ development is complete[58]. In most adult organs active Notch signalling is confined to the resident stem cell population. Here, Notch has an important role in maintaining self-renewal, enhancing proliferation and inhibiting differentiation. In addition, as Notch is important in asymmetric cell division, Notch activity is associated with the commitment of cells to specific lineages, for example of Goblet cells in the intestine[59] and of the proximal tubule cells in the kidney[55].

Studies over the past decade have identified cells with progenitor like properties in the adult mouse and human kidney[60,61]. Cells expressing high levels of Sox9 show progenitor cell-like characteristics including proliferation and differentiation capacity[62–64]. Renin-positive, $CD133^+$ or slowly cycling medullary cells have also been shown to have progenitor properties[65,66]. Although the relationship between Sox9 and renin expressing progenitor cells is unclear, they both have higher Notch expression compared to differentiated tubule epithelial cells [60,64].

Although the renewal of human tubule epithelial cells is slow in healthy human kidneys it is greatly accelerated following acute kidney injury, whereby resident cells expand by proliferation and progenitor cells give rise to new daughter cells. For example, in response to tubule epithelial cell injury, $Sox9^+$ cells proliferate and differentiate into different tubule segments [64]. Increasing Notch expression in $Sox9^+$ cells accelerated epithelial repair in a mouse model of folic acid-induced kidney injury[64]. Consistent with this finding, inhibition of Notch with a γ-secretase inhibitor (GSI) delayed recovery in a mouse model of acute kidney injury [67], suggesting that Notch might be involved in cell proliferation and the production of new daughter cells following injury. In contrast to the above findings, however, GSI-mediated inhibition of Notch has also been shown to be protective in AKI models[68,71], and genetic overexpression of Notch has been shown to aggravate injury[69]. The reasons for these contradictory results are not clear but it is possible that that Notch likely exert a compartment or temporal-specific roles in acute kidney injury and epithelial regeneration (Table 1), with differential effects on proliferation, regeneration and repair depending on the timing and location of Notch activation.

Notch in chronic kidney injury

Both transcript and protein levels of members of the Notch signalling pathway are strongly increased in the glomeruli and tubuli of patients with different forms of CKD, including diabetic nephropathy, lupus nephritis and focal segmental glomerulosclerosis (FSGS)[70] (TABLE 1). Notch1 expression in renal tubules correlated with tubulointerstitial fibrosis and decreased kidney function in patients with CKD [70]. Increased Notch receptor, ligand and target gene expression was also reported in mouse models of fibrosis[71].

The functional role of Notch signalling in CKD has been examined using mouse models[71]. Conditional inducible cell-specific expression of cleaved Notch1 in epithelial tubular cells resulted in increased epithelial cell proliferation and tubule epithelial dedifferentiation. The renal histology was characterized by tubule atrophy, dilatation and fibrosis, including matrix, myofibroblast and inflammatory cell accumulation[71]. Notch signalling was both sufficient and necessary to induce fibrosis. In mice, genetic deletion of Rbpj, the central transcriptional mediator of Notch signaling in proximal tubular epithelial cells, significantly reduced folic acid-induced tubulointerstitial fibrosis[71]. Pharmacological inhibition of Notch signalling by injection of a GSI diminished the extent of fibrosis in mice both in folic acid injection or unilateral ureteral obstruction (UUO) models[71]. Treatment with the GSI significantly reduced transcript levels of the fibrosis markers collagen, fibronectin and vimentin, and decreased the expression of the myofibroblast protein α-SMA[71]. Although this study

strongly supports a role for Notch signalling in the development of fibrosis it did not establish the ligand and receptor that are responsible for fibrosis development.

A more recent study from 2012 revealed that Notch3 is the likely critical isoform involved in kidney fibrosis[57]. Genetic deletion of Notch3 protected mice from tubulointerstitial fibrosis induced by UUO. In Notch3-knockout mice, the number of α-SMA-positive cells was significantly decreased, indicative of reduced myofibroblast activation[57]. Immunostaining analysis and cell culture studies indicated that Notch3 is expressed in tubule epithelial cells [57]. As this study used global Notch3-knockout mice it was not able to define the cell types in which Notch3 has a role. Total Notch1 and Notch2 knockouts are lethal, so segment-specific deletions will be necessary to investigate the effects of these molecules.

The mechanism by which Notch signalling contributes to the development of fibrosis is not fully understood. Expression of Notch receptors in tubular epithelial cells *in vitro* can drive transdifferentiation of epithelial cells into activated myofibroblasts (epithelial-to-mesenchymal transition [EMT])[71]. Notch is a strong regulator of the master transcription factors of EMT: *Snai1* and *Snai2*[72,73]. *Snai1* expression in tubule epithelial cells has a key role in the development of kidney fibrosis[74,75]. As mentioned earlier, linage-tracing studies do not support the role of EMT in kidney fibrosis development; however, some studies[74,75] now suggest that epithelial cells undergo partial EMT, which seems to be an analogous process to that of dedifferentiation. Notch is important in this process as gene expression studies performed in transgenic animals with tubule-specific expression of Notch indicate that Notch promotes the proliferation of epithelial cells and inhibits the expression of tubule epithelial differentiation markers, such as certain solute carriers [71]. This role is very similar to the role of Notch in the stem cell niche[76] and is likely to be important in replacing lost epithelial cells following injury[53,77,78]. The re-expression of Notch in the context of kidney injury and fibrosis is therefore not surprising as regeneration uses pathways similar to those that are active in development in other systems such as in skin [79]. While it is likely that this wave of proliferation of undifferentiated cells is critical for replacing lost tubule cells, the block that Notch places on differentiation on the other hand impedes the function of the organ.

Another important issue to consider is how epithelial Notch signalling is transmitted to fibroblasts to induce their proliferation and transdifferentiation. As an important cell–cell communication pathway, Notch signalling alone could be directly responsible for transmitting these signals or these signals could be transmitted via other paracrine signalling pathways. Increased epithelial expression of Notch was associated with increased levels of Tgf-β, which suggests that Tgf-β could be a soluble mediator that stimulates myofibroblast activation following epithelial injury[71,80]. As discussed in further detail below, the potential interaction of Notch with Wnt and Hh signaling might also have a role in the development of fibrosis[47,81,82].

Notch signalling and podocytes—In addition to their increased expression in tubular epithelial cells, increased expression of Notch proteins has also been observed in glomerular epithelial cells of patients with CKD, including those with diabetic kidney disease and FSGS[83]. The expression of cleaved Notch1 in podocytes and glomeruli correlated with

albuminuria and glomerulosclerosis, whereas glomerular expression of Jagged1 and activated Notch2 was associated with proteinuria alone[70]. Notch expression was also increased in podocytes of mouse models of diabetic kidney disease[83].

Mechanistic studies showed that conditional inducible expression of the intracellular domain of Notch1 in podocytes of mice resulted in albuminuria, glomerulosclerosis, tubulointerstitial fibrosis and death of the animals[83]. The histological lesions in these animals resembled FSGS. The timing of Notch expression had a slight effect of the histological FSGS subtype; early (developmental) expression induced collapsing FSGS like lesions whereas expression on adult cells resulted in classic FSGS lesions[84]. Notch seems to not only be sufficient to induce sclerosis of the glomerulus, but also necessary, as genetic deletion of Rbpj from podocytes reduced albuminuria in diabetic mouse models [83]. On a mechanistic level, Notch functions to inhibit podocyte differentiation [83,85], which similar to its role in tubule cells. Expression of Notch in developing podocytes also induces proliferation; however, mature podocytes cannot proliferate and Notch expression causes a mitotic catastrophe resulting in podocyte death[83].

Notch1 and Notch2 might have different roles in podocytes. A 2014 study reported a positive linear correlation between the number of podocytes expressing activated Notch2 and the number of residual podocytes in human nephrotic syndrome specimens[86]. This study also showed that administration of a Notch2 agonistic monoclonal antibody ameliorated proteinuria and glomerulosclerosis in a mouse model of nephrosis and FSGS. *In vitro*, knockdown of Notch2 increased apoptosis of damaged podocytes, while Notch2 agonistic antibodies enhanced activation of Akt and protected damaged podocytes from apoptosis[87]. Our group has performed genetic studies to specifically study the role of Notch1 and Notch2 in podocytes[88]. We found that genetic deletion of Notch1 in podocytes resulted in marked amelioration of diabetic kidney disease. By contrast, podocyte-specific genetic deletion of Notch2 had no effect on albuminuria and mesangial expansion. Notch1-null podocytes were protected from apoptosis and dedifferentiation *in vitro*, likely explaining the protective phenotype *in vivo*. Deletion of Notch1 in podocytes also resulted in an increase in Notch2 expression, indicating an interaction between the receptors. At the same time, transgenic overexpression of Notch2 in podocytes did not induce phenotypic changes[88]. These results indicate that Notch1 has a dominant damaging role in the glomerulus.

More recently, Notch3 was also proposed to be a player in glomerular disease development[89]. Increased Notch3 expression was described in glomerular podocytes of patients with lupus nephritis or focal segmental glomerulosclerosis, as well as in animal models of rapidly progressive glomerulonephritis. Mice lacking Notch3 expression were protected as they exhibited less proteinuria and structural damage than wild-type mice. *In vitro* studies showed that podocytes expressing active Notch3 reorganize their cytoskeleton toward a proliferative/migratory and inflammatory phenotype. Antisense oligodeoxynucleotides targeting Notch3 ameliorated kidney injury and inflammatory infiltration in the kidney, together with fewer fibrin deposits in glomeruli and decreased peritubular inflammation[89]. These studies indicate that Notch1, Notch2 and Notch3 not only have differential roles during development, but also exert differential effects in the adult human kidney depending on the injury model studied.

Wnt in kidney development and homeostasis

Wnt signalling was first recognized as having an important role in carcinogenesis, but this pathway has since been known to have key functions in embryonic development[90,91] and in the stem cell compartment[92–94]. At present, 19 Wnt ligands and 15 receptors and co-receptors have been identified, which makes it difficult to understand the complex molecular processes involved in and regulated by Wnt signalling[92]. Wnt proteins are a group of secreted lipid-modified glycoproteins, many of which act through the canonical Wnt pathway, in which β-catenin is a key mediator. In the steady state, the so-called 'destruction complex', which consists of glycogen synthase kinase 3β (GSK3β), the scaffolding protein axin, casein kinase 1α, and the adenomatous polyposis coli protein, phosphorylates cytoplasmic β-catenin at the N-terminal residue, which leads to its ubiquitin-mediated proteasomal degradation. However, when Wnt proteins are present, they bind to Frizzled (Fzd) and LRP5/6 receptor proteins, promoting the inactivation of GSK3β and disassembly of the 'destruction complex'. Non-phosphorylated β-catenin accumulates in the cytoplasm and translocates into the nucleus, where it binds to T-cell factor (TCF) and lymphoid enhancer factor (LEF) to regulate Wnt target genes[92,94] (Figure 3). Wnt molecules can also signal independent of LRP5/6 and β-catenin in a so-called 'non-canonical' manner, mostly acting via the planar cell polarity pathway[95,96].

Wnt4 and Wnt9b are important in kidney development. During development, Wnt proteins stimulate uncommitted mesenchymal cells to differentiate into epithelial cells (mesenchymal-to-epithelial transition [MET])[97]. In addition, reciprocal Wnt signals between kidney stromal cells and renal epithelial cells are critical in regulating nephron elongation and differentiation[98,99].

Using reporter mouse cell lines, a high-level of Wnt activity has been observed in the renal medulla in adult mouse kidneys. Findings from a 2004 study indicated that these medullary cells might act as stem or progenitor cells in the kidney[100] (TABLE 2). A later report confirmed that Wnt-responsive cells are likely to be progenitor or stem cells and contribute to kidney regeneration after injury, and used a different reporter strain mice to show that these Wnt-responsive cells are scattered along the renal tubules and concentrated in the medulla[101].

A concerted upregulation of multiple Wnt ligands (Wnt2, Wnt2b, Wnt4, Wnt5a, Wnt7b and Wnt10b) has been described in mouse models of acute ischaemia–reperfusion injury[99,102,103]. Since Wnt proteins are located in the stem cell compartment, their increased expression in response to epithelial injury is not surprising, and is indicative of their role in cell regeneration. Wnt4, in particular, contributes to the regeneration of tubular epithelial cells by regulating the cell-cycle proteins cyclinD1 and cyclinA[99,104]. Macrophages that accumulate in regenerating kidneys express Wnt ligands, including Wnt4, Wnt7b, Wnt10a and Wnt10b, on their surface[99,102]. High Wnt ligand expression in isolated macrophages and an increased Wnt signalling response in epithelial cells have been observed following ischaemia–reperfusion injury in mice[102]. Ablation of macrophages *in vivo* in mice led to reduced expression of Wnt indicating that Wnt is derived from this compartment. Macrophage-specific deletion of Wnt7b resulted in impaired epithelial cell regeneration and

increased epithelial injury[102]. This study thereby demonstrates that the influx of macrophages has a critical role in orchestrating the repair of epithelial cells by secreting Wnt7b. Consistent with these studies genetic deletion of β-catenin from renal tubule epithelial cells exacerbates acute kidney injury[105]. The effects of Wnt inhibitors in the context of kidney injury are summarized in TABLE 3.

Wnt signalling in chronic kidney injury

Increased Wnt expression is not only limited to acute kidney injury models as Wnt expression is also increased in kidneys of animal models of CKD. The expression of most of the 19 Wnt proteins (except Wnt5b, Wnt8b and Wnt9b) and 10 Fzd receptors (except Fzd4 and Fzd5) were increased in renal tubular cells in the UUO model of kidney fibrosis[106] (Table 3). Mechanistic studies indicate that Wnt and β-catenin are not only regulated in CKD, but also contribute to fibrosis development. Genetic overexpression of active β-catenin in tubular cells induces some features of fibrosis, including epithelial dedifferentiation and EMT in mice[105,107].

In line with this finding, a reduction in Wnt/β-catenin activation is associated with significantly improved outcomes in renal fibrosis models. The outcomes of several strategies that interfere with Wnt signalling at different levels have been published. Dickkopf1 (Dkk1) is a Wnt antagonist that binds to the LRP5/6 receptor and inhibits the canonical Wnt pathway. Injection of a vector encoding Dkk1 into mouse models of kidney fibrosis reduced β-catenin accumulation and fibrosis, as evidenced by reduced collagen deposition, decreased interstitial expansion and reduced levels of the α-SMA protein[106]. Secreted frizzled-related protein 1 (Sfrp1) acts as a biphasic modulator of Wnt signalling, counteracting Wnt-induced effects at high concentrations and promoting them at lower concentrations[108]. Mice lacking Sfrp1 had an increased level of fibrosis with increased vimentin and α-SMA expression levels following UUO injury[109]. Deletion of Dapper3, another Wnt antagonist, resulted in accumulation of dishevelled 2 and β-catenin, activation of Wnt-targeted profibrotic genes and an exacerbated fibrotic phenotype in the UUO model[110].

In addition to genetic manipulation, small molecules that interfere with Wnt signalling also modulate the development of fibrosis and could therefore represent a new therapeutic avenue for the treatment of fibrosis[111]. The vitamin D analogue paricalcitol have been proposed to inhibit Wnt/β-catenin signalling by competing with TCF-4 for β-catenin (TABLE 3) and thereby has a protective role against the development of fibrosis[99,112]. The small molecule Wnt/ß-catenin inhibitor ICG-001 also reduced fibrosis development[113].

Although these studies indicate that Wnt is an essential modulator of fibrosis development, the conditional, tubule-specific deletion of β-catenin in mice had no effect on renal fibrosis development in a UUO-induced injury model[107]. These findings potentially indicate that tubular cells might not be the target of Wnt signalling in fibrosis. In support of this notion, lineage-tracing studies revealed that Wnt4 expression was high in activated myofibroblasts, especially in the medullary interstitium following UUO or ischemia–reperfusion injury in mice[114]. *In vitro*, in mouse kidney cells administration of exogenous Wnt4 drove myofibroblast differentiation, but conditional deletion of Wnt4 in interstitial cells did not reduce myofibroblast proliferation, cell numbers, or expression of myofibroblast specific

genes during fibrosis (TABLE 2). A mouse model with constitutive activation of canonical Wnt4/β-catenin signalling in interstitial pericytes and fibroblasts exhibited spontaneous myofibroblast differentiation even in the absence of injury[114]. These results indicate that Wnt4/β-catenin in fibroblasts is sufficient, but Wnt4 is not required, for fibrosis development, and potentially suggest that Wnt signalling could be a communication mechanism between injured tubular epithelial cells and fibroblasts. Lastly we should also note that the effect of Wnts could also be independent of β-catenin, mediated through the non-canonical Wnt signalling pathway. For example Wnt11, an important profibrotic mediator of Tgf-β that drives the expression of mesenchymal genes, exerts its action independent of β-catenin[115].

Wnt signalling and podocytes—Increased Wnt activity has been observed in glomeruli of mouse models of CKD[116,117]. Wnt expression was increased in podocytes of mice treated with adriamycin, a substance that causes podocyte injury with features similar to that of FSGS. Hydrodynamic-based tail vein injection of exogenous Wnt1 into BALB/c mice exacerbated podocyte dysfunction, which was evidenced by decreased protein levels of Nephrin. Blocking Wnt signalling with DKK1 in adriamycin-injected mice ameliorated albuminuria and counteracted the decrease in Nephrin levels[117]. Furthermore, podocyte-specific deletion of β-catenin protected mice from adriamycin-induced podocyte injury, including albuminuria and podocyte foot process effacement[117]. ICG-001 was similarly effective in ameliorating proteinuria in the adriamycin-induced glomerular injury model. The authors propose that the effect of Wnt on proteinuria is mediated by regulating the expression of the renin–angiotensin system as they describe beta catenin binding to the promoter of renin and the angiotensin receptors [113].

The effect of Wnt signalling seems to be context dependent. Studies from our laboratory showed that genetic deletion of β-catenin from podocytes did not protect mice from developing diabetic nephropathy[116]. Indeed, we found that mice with podocyte-specific deletion of β-catenin had increased albuminuria and glomerulosclerosis. Genetic overexpression of β-catenin in podocytes resulted in low-grade albuminuria and minor alterations in the basement membrane. *In vitro* experiments in mouse podocytes showed that increased Wnt/β-catenin activation in podocytes induced loss of expression of markers associated with differentiated podocytes. Decreased Wnt/β-catenin signalling was necessary for full differentiation of podocytes, but these ‘highly’ differentiated cells showed an increased susceptibility to apoptosis[116].

Hedgehog in kidney development

The Hedgehog (Hh) pathway has a fundamental role in tissue patterning, cell growth and differentiation[118,119]. In adult tissues, Hh signalling is important for tissue maintenance of the stem cell compartment[120,121]. Aberrant Hh signalling has been reported in many cancer types, including those of the basal cell, prostate, mammary glands, and lungs[122,123]. The three Hh ligands, Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert hedgehog (Dhh), are lipid-modified proteins capable of acting over short and long distances from the source of secretion. In humans, Hh molecules initiate signalling by binding to its receptor Patched1 (PTCH1), which results in ciliary localization of the G-protein coupled receptor-like protein

Smoothened (SMO). SMO mediates the translocation of full-length GLI proteins to the nucleus, therein activating expression of Hh target genes, including GLI1, PTCH1, CCND1, N-MYC, JAG2, BCL2 and SNAI1[118,122]. In the absence of Hh proteins, the ciliary localization of SMO is inhibited, which leads to sequestration of GLI proteins. Following proteolytic cleavage, the C-terminal-shortened GLI repressor binds to DNA, inhibiting target gene transcription[118,120] (Figure 4).

Three GLI transcription factors are important in mediating Hh signalling. GLI2 and GLI3 are thought to have activator and repressor functions, whereas GLI1 only acts as an activator[124]. In patients with Pallister–Hall-Syndrome, frameshift mutations have been described in the *GLI3* gene, which produce an abbreviated protein with similarities to the GLI3 repressor. Hydronephrosis, renal hypoplasia and renal dysplasia characterize the associated autosomal dominant disorder[118]. Genetic deletion of GLIS2 (GLI-similar 2) is associated with nephronophthisis and interstitial fibrosis in humans[125]. Glis2 seems to oppose Gli1 activity by binding cis-acting regulatory sequences in the 5′ flanking regions of Snai1 and Wnt4, thereby inhibiting de-differentiation of tubular cells[126]. Although these genetic studies indicate that Hh signalling has key role in kidney development, its role in kidney homeostasis or its potential role in maintaining the resident stem cell niche has not been studied.

Hedgehog signalling in renal fibrosis

The functional role of Hh in healthy and diseased kidneys has just begun to be understood. It is hard to detect the expression of Hh pathway associated genes in healthy human and mouse kidneys as the expression level of these proteins are low. Linage tracing indicates that Shh expression is localized to aquaporin 2-positive collecting ducts[127]. Proximal tubule cells, on the other hand, seem to express Ihh. The Hh receptor Ptch1 and effector gene Gli are expressed on interstitial cells[127]. One group reported increased Shh expression in injured tubule epithelial cells in mouse models of UUO and ischaemia–reperfusion injury-induced kidney fibrosis[128]. However, another study did not confirm the increased Shh expression in the UUO mouse model system[127]. On the other hand, there was a consensus by both studies that Gli expression is increased in interstitial fibroblasts in different mouse fibrosis models[127,128].

Mechanistic studies indicate that the Hh pathway has an important functional role in fibrosis development in the kidney and the heart. *In vitro* studies suggest that Hh promotes myofibroblastic transdifferentiation and proliferation of interstitial fibroblasts. Rat kidney fibroblasts treated with Shh in vitro showed increased expression of α smooth muscle actin and cell cycle-associated gene expression [129] On the other hand Shh treatment did not alter the phenotype of cultured tubule epithelial cells [128]. Inhibition of Hh signalling by cyclopamine, a potent inhibitor of SMO, significantly attenuated kidney fibrosis development in mouse models of ischaemia–reperfusion injury and obstructive nephropathy[128,129]. The source of Shh seems to the tubule epithelial cells as expression of Shh on tubule cells *in vivo* was sufficient to induce myofibroblast proliferation and fibrosis development [128].

Studies over the past couple of years have defined a critical role for Gli and Gli expressing fibroblasts in mediating fibrotic response to Hh signalling[130]. A subset of myofibroblasts express high levels of Gli1; these Gli^+ cells also exhibit stem cell characteristics *in vitro* and *in vivo*. Genetic ablation of $Gli1^+$ cells reduced kidney damage in mouse models of kidney fibrosis[129]. Gli^+ cells seem to mediate fibrosis not only in the kidney; in the heart, for example, genetic ablation of $Gli1^+$ cells, even in the setting of actively established cardiac fibrosis, resulted in reversal of cardiac fibrosis and improved heart function[131]. These studies suggest that the Hh signalling pathway has a key role in the development of renal fibrosis, mostly through secretion of Hh ligands from epithelial cells and Gli1 upregulation in myofibroblasts.

Interaction of developmental pathways

Notch and Wnt signalling strongly interact with each other in both synergistic and antagonistic manners depending on the microenvironment. Crosstalk between Notch and Wnt signalling has initially been described in the development and patterning of the *Drosophila* wing[82]. The two signalling pathways primarily synergize to regulate growth of the early wing tissue; later in development, Notch signalling promotes *wingless* (the *Drosophila* Wnt orthologue) expression at the wing margin. Conversely, *wingless* regulates expression of the Notch ligands Serrate (the *Drosophila* Jagged orthologue) and Delta to coordinate the detailed patterning of the wing. Notch has also been found to downregulate *wingless* by establishing a complex with β-catenin and promoting β-catenin degradation in *Drosophila*[82].

During kidney development, the sequential activation of Wnt signalling is needed to form a mesenchymal stem cell pool from epithelial cells through a process of MET. Induction by Wnt9b directs cells to exit the stem cell niche and express Wnt4, which is both necessary and sufficient for the formation of epithelia. In the absence of Notch or Wnt signalling, MET fails to occur, nephrons do not form and newborn mice die owing to kidney failure. Notch2 activation follows Wnt activation and is needed for determination of proximal–distal tubular cell fate [97]. Notch activation can bypass the need for Wnt activation in the developing mouse kidneys, whereas overexpression of Wnt3a can bypass the need for Notch signalling, as shown in chick kidney developmental models[132]. A positive feedback loop between Notch2 and Wnt4 also exists, with Notch 2 promoting proximal tubule fate by inducing cell differentiation and thereby depleting the progenitor cell population[56].

The Notch and Wnt pathways also interact in tissue-resident stem cells. For example, in the intestine, high Wnt activity has been observed in the $Lgr5^+$ stem cell population[133]. This high Wnt activity is necessary for maintaining the stemness of this population[134]. Notch and Wnt signalling interact but it is also involved in transit amplification and lineage decision of the intestinal cells[76,135]. A similar mechanism is likely to be present in the kidney, as Wnt-responsive, $Lgr5^+$ and Sox9+ cells showed stem or progenitor cell characteristics and transcriptome profiling studies revealed high Jagged1 expression in these cells[60]. These studies indicate that Wnt and Notch interact mostly synergistically in the stem cell and epithelial cell compartment[81,136]. Their effect seems to be similar; they are activated

following injury to enhance proliferation and inhibit differentiation. They contribute to fibrosis development by inhibiting epithelial differentiation.

These pathways also interact by promoting the transformation of fibroblasts into activated myofibroblasts in fibrosis. In vitro, Hh treatment results in myofibroblastic transdifferentiation and proliferatiom of resident fibroblasts[128,129]. The effect of transgenic expression of Wnt4, similarly resulted in myofibroblastic transdifferentiation and proliferation[114], indicating that on the phenotypic outcome of Wnt and Hh is overlapping, raising the possibility of synergy. Although Notch expression has not been studied in renal fibroblasts, studies indicate that interstitial expression of Notch contributes to fibrosis development in the lung [137]. TGF-β might also be involved in mediating the effects of these signalling pathways on renal fibrosis.

Thus, although Notch, Wnt and Hh are critical morphogens with important roles in kidney development, it seems that their choreographed expression is also critical for organ fibrosis.

Conclusions

Overall, we can observe similar patterns of activity among the Notch, Wnt and Hh pathways. In adult organs, the expression of the components of these pathways is fairly low and in most organs their expression is restricted to the stem/progenitor cell compartment. Early results indicate a similar expression pattern and functional role in the kidney, but further studies are needed to explore this possibility.

Following injury, the expression of Notch, Wnt and Hh ligands and receptors has been shown to be increased in mouse and in human kidney tissue samples [70,99,129]. Increased Wnt signalling in the progenitor or stem cell niche seems to initiate the proliferative response in stem cells[138]. Notch and Wnt signalling can maintain the proliferation wave in the transit-amplifying population. These pathways likely interact in this niche and Hh might also be important in the process. In an acute injury, transient activation of Wnt is likely to contribute to the regeneration of tubule epithelial cells; the role of transient Notch activation in tubule regeneration is less clear[47].

Sustained injury in CKD results in sustained activation of the Wnt and Notch pathways in epithelial cells. In addition, cells in which these pathways are active are likely to preferentially expand. Sustained high expression of members of the Wnt and Notch pathways is likely to prevent the terminal differentiation of epithelial cells. Therefore, inhibiting Wnt and Notch signalling probably enables terminal differentiation of epithelial cells, and could therefore lead to improved histological and functional outcomes.

Future research studies should also focus on defining similarities and differences between the various Notch ligands and receptors in health and in disease conditions. Indeed, despite their remarkable similarities, different Notch ligands and receptors have a non-overlapping role in kidney development, and, consequently, they probably also have different roles in kidney disease[70]. As Wnt, Notch and Hh are paracrine signalling pathways, it will be important to determine the direction of signalling and their precise role in mediating epithelial, fibroblastic, endothelial and immune cell interaction. For example, the Hh targets

Gli2 and Wnt4 are specifically expressed in myofibroblasts and are responsible for the myofibroblastic transformation and proliferation[114,139].

A tight balance between the activities of these signalling pathways is essential to maintain homeostasis. Although these pathways are necessary for organ maintenance, it seems that the development of fibrosis might be the inevitable downside of a cell-survival response.

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References

1. Jha V. Chronic kidney disease: global dimension and perspectives. Lancet. 2013; 382:260–272. DOI: 10.1016/S0140-6736(13)60687-X [PubMed: 23727169]
2. Liu Y. Renal fibrosis: new insights into the pathogenesis and therapeutics. Kidney Int. 2006; 69:213–217. DOI: 10.1038/sj.ki.5000054 [PubMed: 16408108]
3. Tonelli M, et al. Chronic kidney disease and mortality risk: a systematic review. J Am Soc Nephrol. 2006; 17:2034–2047. DOI: 10.1681/ASN.2005101085 [PubMed: 16738019]
4. Reidy K, Kang HM, Hostetter T, Susztak K. Molecular mechanisms of diabetic kidney disease. J Clin Invest. 2014; 124:2333–2340. DOI: 10.1172/JCI72271 [PubMed: 24892707]
5. Duffield JS. Cellular and molecular mechanisms in kidney fibrosis. J Clin Invest. 2014; 124:2299–2306. DOI: 10.1172/JCI72267 [PubMed: 24892703]
6. Liu Y. Cellular and molecular mechanisms of renal fibrosis. Nat Rev Nephrol. 2011; 7:684–696. DOI: 10.1038/nrneph.2011.149 [PubMed: 22009250]
7. Kaissling B, Lehir M, Kriz W. Renal epithelial injury and fibrosis. Biochim Biophys Acta. 2013; 1832:931–939. DOI: 10.1016/j.bbadis.2013.02.010 [PubMed: 23466594]
8. Grgic I, Duffield JS, Humphreys BD. The origin of interstitial myofibroblasts in chronic kidney disease. Pediatr Nephrol. 2012; 27:183–193. DOI: 10.1007/s00467-011-1772-6 [PubMed: 21311912]
9. Humphreys BD, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol. 2010; 176:85–97. DOI: 10.2353/ajpath.2010.090517 [PubMed: 20008127]
10. Woroniecka KI, et al. Transcriptome analysis of human diabetic kidney disease. Diabetes. 2011; 60:2354–2369. DOI: 10.2337/db10-1181 [PubMed: 21752957]
11. Susztak K, Raff AC, Schiffer M, Bottinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes. 2006; 55:225–233. [PubMed: 16380497]
12. Huber TB, et al. Emerging role of autophagy in kidney function, diseases and aging. Autophagy. 2012; 8:1009–1031. DOI: 10.4161/auto.19821 [PubMed: 22692002]
13. Lieberthal W, Levine JS. Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. Am J Physiol. 1996; 271:F477–488. [PubMed: 8853409]
14. Ueda N, Kaushal GP, Shah SV. Recent advances in understanding mechanisms of renal tubular injury. Adv Ren Replace Ther. 1997; 4:17–24. [PubMed: 9113237]
15. Lieberthal W, Koh JS, Levine JS. Necrosis and apoptosis in acute renal failure. Semin Nephrol. 1998; 18:505–518. [PubMed: 9754603]
16. Petermann A, Floege J. Podocyte damage resulting in podocyturia: a potential diagnostic marker to assess glomerular disease activity. Nephron Clin Pract. 2007; 106:c61–66. DOI: 10.1159/000101799 [PubMed: 17570931]
17. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest. 2011; 121:4210–4221. DOI: 10.1172/JCI45161 [PubMed: 22045571]

18. Hakroush S, et al. Extensive podocyte loss triggers a rapid parietal epithelial cell response. J Am Soc Nephrol. 2014; 25:927–938. DOI: 10.1681/ASN.2013070687 [PubMed: 24335975]
19. Eng DG, et al. Glomerular parietal epithelial cells contribute to adult podocyte regeneration in experimental focal segmental glomerulosclerosis. Kidney Int. 2015; 88:999–1012. DOI: 10.1038/ki.2015.152 [PubMed: 25993321]
20. Wharram BL, et al. Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. J Am Soc Nephrol. 2005; 16:2941–2952. DOI: 10.1681/ASN.2005010055 [PubMed: 16107576]
21. Rutkowski JM, et al. Adiponectin promotes functional recovery after podocyte ablation. J Am Soc Nephrol. 2013; 24:268–282. DOI: 10.1681/ASN.2012040414 [PubMed: 23334396]
22. Inoki K, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. J Clin Invest. 2011; 121:2181–2196. DOI: 10.1172/JCI44771 [PubMed: 21606597]
23. Godel M, et al. Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. J Clin Invest. 2011; 121:2197–2209. DOI: 10.1172/JCI44774 [PubMed: 21606591]
24. Wiggins JE, et al. Podocyte hypertrophy, “adaptation,” and “decompensation” associated with glomerular enlargement and glomerulosclerosis in the aging rat: prevention by calorie restriction. J Am Soc Nephrol. 2005; 16:2953–2966. DOI: 10.1681/ASN.2005050488 [PubMed: 16120818]
25. Pagtalunan ME, et al. Podocyte loss and progressive glomerular injury in type II diabetes. J Clin Invest. 1997; 99:342–348. DOI: 10.1172/JCI119163 [PubMed: 9006003]
26. Wolf G, Chen S, Ziyadeh FN. From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy. Diabetes. 2005; 54:1626–1634. [PubMed: 15919782]
27. Dimke H, et al. Tubulovascular Cross-Talk by Vascular Endothelial Growth Factor A Maintains Peritubular Microvasculature in Kidney. J Am Soc Nephrol. 2014
28. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol. 2005; 15:599–607. DOI: 10.1016/j.tcb.2005.09.002 [PubMed: 16202600]
29. Gu L, et al. Deletion of podocyte STAT3 mitigates the entire spectrum of HIV-1-associated nephropathy. AIDS. 2013; 27:1091–1098. DOI: 10.1097/QAD.0b013e32835f1ea1 [PubMed: 23343908]
30. Dai Y, et al. Podocyte-specific deletion of signal transducer and activator of transcription 3 attenuates nephrotoxic serum-induced glomerulonephritis. Kidney Int. 2013; 84:950–961. DOI: 10.1038/ki.2013.197 [PubMed: 23842188]
31. Tak PP, Firestein GS. NF-kappaB: a key role in inflammatory diseases. J Clin Invest. 2001; 107:7–11. DOI: 10.1172/JCI11830 [PubMed: 11134171]
32. Meng XM, Nikolic-Paterson DJ, Lan HY. Inflammatory processes in renal fibrosis. Nat Rev Nephrol. 2014; 10:493–503. DOI: 10.1038/nrneph.2014.114 [PubMed: 24981817]
33. Kumar D, Singla SK, Puri V, Puri S. The restrained expression of NF-kB in renal tissue ameliorates folic acid induced acute kidney injury in mice. PLoS One. 2015; 10:e115947. [PubMed: 25559736]
34. Okabe C, et al. NF-kappaB activation mediates crystal translocation and interstitial inflammation in adenine overload nephropathy. Am J Physiol Renal Physiol. 2013; 305:F155–163. DOI: 10.1152/ajprenal.00491.2012 [PubMed: 23657853]
35. Yukawa K, et al. STAT6 deficiency inhibits tubulointerstitial fibrosis in obstructive nephropathy. Int J Mol Med. 2005; 15:225–230. [PubMed: 15647835]
36. Friedman SL, Sheppard D, Duffield JS, Violette S. Therapy for fibrotic diseases: nearing the starting line. Sci Transl Med. 2013; 5:167sr161.
37. Meran S, Steadman R. Fibroblasts and myofibroblasts in renal fibrosis. Int J Exp Pathol. 2011; 92:158–167. DOI: 10.1111/j.1365-2613.2011.00764.x [PubMed: 21355940]
38. Hu B, Phan SH. Myofibroblasts. Curr Opin Rheumatol. 2013; 25:71–77. DOI: 10.1097/BOR.0b013e32835b1352 [PubMed: 23114586]
39. Kendall RT, Feghali-Bostwick CA. Fibroblasts in fibrosis: novel roles and mediators. Front Pharmacol. 2014; 5:123. [PubMed: 24904424]

40. Klingberg F, Hinz B, White ES. The myofibroblast matrix: implications for tissue repair and fibrosis. J Pathol. 2013; 229:298–309. DOI: 10.1002/path.4104 [PubMed: 22996908]
41. Wight TN, Potter-Perigo S. The extracellular matrix: an active or passive player in fibrosis. Am J Physiol Gastrointest Liver Physiol. 2011; 301:G950–955. DOI: 10.1152/ajpgi.00132.2011 [PubMed: 21512158]
42. Olsen AL, et al. Hepatic stellate cells require a stiff environment for myofibroblastic differentiation. Am J Physiol Gastrointest Liver Physiol. 2011; 301:G110–118. DOI: 10.1152/ajpgi.00412.2010 [PubMed: 21527725]
43. Urban ML, Manenti L, Vaglio A. Fibrosis–A Common Pathway to Organ Injury and Failure. N Engl J Med. 2015; 373:95–96. DOI: 10.1056/NEJMc1504848#SA2
44. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest. 2007; 117:524–529. DOI: 10.1172/JCI31487 [PubMed: 17332879]
45. Lavoz C, et al. Angiotensin II contributes to renal fibrosis independently of Notch pathway activation. PLoS One. 2012; 7:e40490. [PubMed: 22792351]
46. Boor P, Floege J. Chronic kidney disease growth factors in renal fibrosis. Clin Exp Pharmacol Physiol. 2011; 38:441–450. DOI: 10.1111/j.1440-1681.2011.05487.x [PubMed: 21276040]
47. Chuang PY, Menon MC, He JC. Molecular targets for treatment of kidney fibrosis. J Mol Med (Berl). 2013; 91:549–559. DOI: 10.1007/s00109-012-0983-z [PubMed: 23179685]
48. Shull MM, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature. 1992; 359:693–699. DOI: 10.1038/359693a0 [PubMed: 1436033]
49. Kulkarni AB, et al. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci U S A. 1993; 90:770–774. [PubMed: 8421714]
50. Lai EC. Notch signaling: control of cell communication and cell fate. Development. 2004; 131:965–973. DOI: 10.1242/dev.01074 [PubMed: 14973298]
51. Ilagan MX, Lim S, Fulbright M, Piwnica-Worms D, Kopan R. Real-time imaging of notch activation with a luciferase complementation-based reporter. Sci Signal. 2011; 4:rs7. [PubMed: 21775282]
52. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell. 2009; 137:216–233. DOI: 10.1016/j.cell.2009.03.045 [PubMed: 19379690]
53. Sirin Y, Susztak K. Notch in the kidney: development and disease. J Pathol. 2012; 226:394–403. DOI: 10.1002/path.2967 [PubMed: 21952830]
54. Penton AL, Leonard LD, Spinner NB. Notch signaling in human development and disease. Semin Cell Dev Biol. 2012; 23:450–457. DOI: 10.1016/j.semcdb.2012.01.010 [PubMed: 22306179]
55. Cheng HT, et al. Notch2, but not Notch1, is required for proximal fate acquisition in the mammalian nephron. Development. 2007; 134:801–811. DOI: 10.1242/dev.02773 [PubMed: 17229764]
56. Fujimura S, Jiang Q, Kobayashi C, Nishinakamura R. Notch2 activation in the embryonic kidney depletes nephron progenitors. J Am Soc Nephrol. 2010; 21:803–810. DOI: 10.1681/ASN.2009040353 [PubMed: 20299358]
57. Djudjaj S, et al. Notch-3 receptor activation drives inflammation and fibrosis following tubulointerstitial kidney injury. J Pathol. 2012; 228:286–299. DOI: 10.1002/path.4076 [PubMed: 22806125]
58. Sharma S, Sirin Y, Susztak K. The story of Notch and chronic kidney disease. Curr Opin Nephrol Hypertens. 2011; 20:56–61. DOI: 10.1097/MNH.0b013e3283414c88 [PubMed: 21088575]
59. Carulli AJ, et al. Notch receptor regulation of intestinal stem cell homeostasis and crypt regeneration. Dev Biol. 2015; 402:98–108. DOI: 10.1016/j.ydbio.2015.03.012 [PubMed: 25835502]
60. Barker N, et al. Lgr5(+ve) stem/progenitor cells contribute to nephron formation during kidney development. Cell Rep. 2012; 2:540–552. DOI: 10.1016/j.celrep.2012.08.018 [PubMed: 22999937]

61. Pleniceanu O, Harari-Steinberg O, Dekel B. Concise review: Kidney stem/progenitor cells: differentiate, sort out, or reprogram. Stem Cells. 2010; 28:1649–1660. DOI: 10.1002/stem.486 [PubMed: 20652959]
62. Kumar S, et al. Sox9 Activation Highlights a Cellular Pathway of Renal Repair in the Acutely Injured Mammalian Kidney. Cell Rep. 2015; 12:1325–1338. DOI: 10.1016/j.celrep.2015.07.034 [PubMed: 26279573]
63. Reginensi A, et al. SOX9 controls epithelial branching by activating RET effector genes during kidney development. Hum Mol Genet. 2011; 20:1143–1153. DOI: 10.1093/hmg/ddq558 [PubMed: 21212101]
64. Kang HM, et al. Sox9-Positive Progenitor Cells Play a Key Role in Renal Tubule Epithelial Regeneration in Mice. Cell Rep. 2016; 14:861–871. DOI: 10.1016/j.celrep.2015.12.071 [PubMed: 26776520]
65. Gomez RA, Belyea B, Medrano S, Pentz ES, Sequeira-Lopez ML. Fate and plasticity of renin precursors in development and disease. Pediatr Nephrol. 2014; 29:721–726. DOI: 10.1007/s00467-013-2688-0 [PubMed: 24337407]
66. Bussolati B, et al. Isolation of renal progenitor cells from adult human kidney. Am J Pathol. 2005; 166(10):545–555. 62276–6. DOI: 10.1016/S0002-9440 [PubMed: 15681837]
67. Chen J, Chen JK, Conway EM, Harris RC. Survivin mediates renal proximal tubule recovery from AKI. J Am Soc Nephrol. 2013; 24:2023–2033. DOI: 10.1681/ASN.2013010076 [PubMed: 23949800]
68. Huang R, Zhou Q, Veeraragoo P, Yu H, Xiao Z. Notch2/Hes-1 pathway plays an important role in renal ischemia and reperfusion injury-associated inflammation and apoptosis and the gamma-secretase inhibitor DAPT has a nephroprotective effect. Ren Fail. 2011; 33:207–216. DOI: 10.3109/0886022X.2011.553979 [PubMed: 21332343]
69. Sorensen-Zender I, et al. Renal tubular Notch signaling triggers a prosenescent state after acute kidney injury. Am J Physiol Renal Physiol. 2014; 306:F907–915. DOI: 10.1152/ajprenal.00030.2014 [PubMed: 24573392]
70. Murea M, et al. Expression of Notch pathway proteins correlates with albuminuria, glomerulosclerosis, and renal function. Kidney Int. 2010; 78:514–522. DOI: 10.1038/ki.2010.172 [PubMed: 20531454]
71. Bielesz B, et al. Epithelial Notch signaling regulates interstitial fibrosis development in the kidneys of mice and humans. J Clin Invest. 2010; 120:4040–4054. DOI: 10.1172/JCI43025 [PubMed: 20978353]
72. Wang Z, Li Y, Kong D, Sarkar FH. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. Curr Drug Targets. 2010; 11:745–751. [PubMed: 20041844]
73. Saad S, Stanners SR, Yong R, Tang O, Pollock CA. Notch mediated epithelial to mesenchymal transformation is associated with increased expression of the Snail transcription factor. Int J Biochem Cell Biol. 2010; 42:1115–1122. DOI: 10.1016/j.biocel.2010.03.016 [PubMed: 20348013]
74. Grande MT, et al. Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. Nat Med. 2015; 21:989–997. DOI: 10.1038/nm.3901 [PubMed: 26236989]
75. Lovisa S, et al. Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. Nat Med. 2015; 21:998–1009. DOI: 10.1038/nm.3902 [PubMed: 26236991]
76. Lasagni L, et al. Notch activation differentially regulates renal progenitors proliferation and differentiation toward the podocyte lineage in glomerular disorders. Stem Cells. 2010; 28:1674–1685. DOI: 10.1002/stem.492 [PubMed: 20680961]
77. Chen L, Al-Awqati Q. Segmental expression of Notch and Hairy genes in nephrogenesis. Am J Physiol Renal Physiol. 2005; 288:F939–952. DOI: 10.1152/ajprenal.00369.2004 [PubMed: 15821257]
78. Barak H, Surendran K, Boyle SC. The role of Notch signaling in kidney development and disease. Adv Exp Med Biol. 2012; 727:99–113. DOI: 10.1007/978-1-4614-0899-4_8 [PubMed: 22399342]

79. Bielefeld KA, Amini-Nik S, Alman BA. Cutaneous wound healing: recruiting developmental pathways for regeneration. Cell Mol Life Sci. 2013; 70:2059–2081. DOI: 10.1007/s00018-012-1152-9 [PubMed: 23052205]
80. Xiao Z, et al. The Notch gamma-secretase inhibitor ameliorates kidney fibrosis via inhibition of TGF-beta/Smad2/3 signaling pathway activation. Int J Biochem Cell Biol. 2014; 55:65–71. DOI: 10.1016/j.biocel.2014.08.009 [PubMed: 25150830]
81. Takebe N, Harris PJ, Warren RQ, Ivy SP. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol. 2011; 8:97–106. DOI: 10.1038/nrclinonc.2010.196 [PubMed: 21151206]
82. Hayward P, Kalmar T, Arias AM. Wnt/Notch signalling and information processing during development. Development. 2008; 135:411–424. DOI: 10.1242/dev.000505 [PubMed: 18192283]
83. Niranjan T, et al. The Notch pathway in podocytes plays a role in the development of glomerular disease. Nat Med. 2008; 14:290–298. DOI: 10.1038/nm1731 [PubMed: 18311147]
84. Niranjan T, Murea M, Susztak K. The pathogenic role of Notch activation in podocytes. Nephron Exp Nephrol. 2009; 111:e73–79. DOI: 10.1159/000209207 [PubMed: 19293596]
85. Lin CL, et al. Modulation of notch-1 signaling alleviates vascular endothelial growth factor-mediated diabetic nephropathy. Diabetes. 2010; 59:1915–1925. DOI: 10.2337/db09-0663 [PubMed: 20522599]
86. Tanaka E, et al. Notch2 activation ameliorates nephrosis. Nat Commun. 2014; 5:3296. [PubMed: 24526233]
87. Arking DE, et al. Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. Nat Genet. 2014; 46:826–836. DOI: 10.1038/ng.3014 [PubMed: 24952745]
88. Sweetwyne MT, et al. Notch1 and Notch2 in Podocytes Play Differential Roles During Diabetic Nephropathy Development. Diabetes. 2015; 64:4099–4111. DOI: 10.2337/db15-0260 [PubMed: 26293507]
89. El Machhour F, Keuylian Z, Kavvadas P, Dussaule JC, Chatziantoniou C. Activation of Notch3 in Glomeruli Promotes the Development of Rapidly Progressive Renal Disease. J Am Soc Nephrol. 2015; 26:1561–1575. DOI: 10.1681/ASN.2013090968 [PubMed: 25421557]
90. Nusse R, Varmus H. Three decades of Wnts: a personal perspective on how a scientific field developed. EMBO J. 2012; 31:2670–2684. DOI: 10.1038/emboj.2012.146 [PubMed: 22617420]
91. Sokol SY. Spatial and temporal aspects of Wnt signaling and planar cell polarity during vertebrate embryonic development. Semin Cell Dev Biol. 2015; 42:78–85. DOI: 10.1016/j.semcdb.2015.05.002 [PubMed: 25986055]
92. Kahn M. Can we safely target the WNT pathway. Nat Rev Drug Discov. 2014; 13:513–532. DOI: 10.1038/nrd4233 [PubMed: 24981364]
93. Holland JD, Klaus A, Garratt AN, Birchmeier W. Wnt signaling in stem and cancer stem cells. Curr Opin Cell Biol. 2013; 25:254–264. DOI: 10.1016/j.ceb.2013.01.004 [PubMed: 23347562]
94. Guo Y, Xiao L, Sun L, Liu F. Wnt/beta-catenin signaling: a promising new target for fibrosis diseases. Physiol Res. 2012; 61:337–346. [PubMed: 22670697]
95. He X, Semenov M, Tamai K, Zeng X. LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. Development. 2004; 131:1663–1677. DOI: 10.1242/dev.01117 [PubMed: 15084453]
96. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. Nat Rev Genet. 2004; 5:691–701. DOI: 10.1038/nrg1427 [PubMed: 15372092]
97. Boyle SC, Kim M, Valerius MT, McMahon AP, Kopan R. Notch pathway activation can replace the requirement for Wnt4 and Wnt9b in mesenchymal-to-epithelial transition of nephron stem cells. Development. 2011; 138:4245–4254. DOI: 10.1242/dev.070433 [PubMed: 21852398]
98. Rosenberg SL, Chen S, McLaughlin N, El-Dahr SS. Regulation of kidney development by histone deacetylases. Pediatr Nephrol. 2011; 26:1445–1452. DOI: 10.1007/s00467-011-1796-y [PubMed: 21336812]
99. Kawakami T, Ren S, Duffield JS. Wnt signalling in kidney diseases: dual roles in renal injury and repair. J Pathol. 2013; 229:221–231. DOI: 10.1002/path.4121 [PubMed: 23097132]

100. Oliver JA, Maarouf O, Cheema FH, Martens TP, Al-Awqati Q. The renal papilla is a niche for adult kidney stem cells. J Clin Invest. 2004; 114:795–804. DOI: 10.1172/JCI20921 [PubMed: 15372103]
101. Rinkevich Y, et al. In vivo clonal analysis reveals lineage-restricted progenitor characteristics in mammalian kidney development, maintenance, and regeneration. Cell Rep. 2014; 7:1270–1283. DOI: 10.1016/j.celrep.2014.04.018 [PubMed: 24835991]
102. Lin SL, et al. Macrophage Wnt7b is critical for kidney repair and regeneration. Proc Natl Acad Sci U S A. 2010; 107:4194–4199. DOI: 10.1073/pnas.0912228107 [PubMed: 20160075]
103. Xiao L, et al. Sustained Activation of Wnt/beta-Catenin Signaling Drives AKI to CKD Progression. J Am Soc Nephrol. 2015
104. Terada Y, et al. Expression and function of the developmental gene Wnt-4 during experimental acute renal failure in rats. J Am Soc Nephrol. 2003; 14:1223–1233. [PubMed: 12707392]
105. Zhou D, et al. Tubule-specific ablation of endogenous beta-catenin aggravates acute kidney injury in mice. Kidney Int. 2012; 82:537–547. DOI: 10.1038/ki.2012.173 [PubMed: 22622501]
106. He W, et al. Wnt/beta-catenin signaling promotes renal interstitial fibrosis. J Am Soc Nephrol. 2009; 20:765–776. DOI: 10.1681/ASN.2008060566 [PubMed: 19297557]
107. Zhou D, Tan RJ, Zhou L, Li Y, Liu Y. Kidney tubular beta-catenin signaling controls interstitial fibroblast fate via epithelial-mesenchymal communication. Sci Rep. 2013; 3:1878. [PubMed: 23698793]
108. Satoh W, Matsuyama M, Takemura H, Aizawa S, Shimono A. Sfrp1, Sfrp2, and Sfrp5 regulate the Wnt/beta-catenin and the planar cell polarity pathways during early trunk formation in mouse. Genesis. 2008; 46:92–103. DOI: 10.1002/dvg.20369 [PubMed: 18257070]
109. Matsuyama M, Nomori A, Nakakuni K, Shimono A, Fukushima M. Secreted Frizzled-related protein 1 (Sfrp1) regulates the progression of renal fibrosis in a mouse model of obstructive nephropathy. J Biol Chem. 2014; 289:31526–31533. DOI: 10.1074/jbc.M114.584565 [PubMed: 25253698]
110. Xue H, et al. Disruption of the Dapper3 gene aggravates ureteral obstruction-mediated renal fibrosis by amplifying Wnt/beta-catenin signaling. J Biol Chem. 2013; 288:15006–15014. DOI: 10.1074/jbc.M113.458448 [PubMed: 23580654]
111. Chen B, et al. Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol. 2009; 5:100–107. DOI: 10.1038/nchembio.137 [PubMed: 19125156]
112. Palmer HG, et al. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J Cell Biol. 2001; 154:369–387. [PubMed: 11470825]
113. Zhou L, et al. Multiple genes of the renin-angiotensin system are novel targets of Wnt/beta-catenin signaling. J Am Soc Nephrol. 2015; 26:107–120. DOI: 10.1681/ASN.2014010085 [PubMed: 25012166]
114. DiRocco DP, Kobayashi A, Taketo MM, McMahon AP, Humphreys BD. Wnt4/beta-catenin signaling in medullary kidney myofibroblasts. J Am Soc Nephrol. 2013; 24:1399–1412. DOI: 10.1681/ASN.2012050512 [PubMed: 23766539]
115. Zhang P, Cai Y, Soofi A, Dressler GR. Activation of Wnt11 by transforming growth factor-beta drives mesenchymal gene expression through non-canonical Wnt protein signaling in renal epithelial cells. J Biol Chem. 2012; 287:21290–21302. DOI: 10.1074/jbc.M112.357202 [PubMed: 22556418]
116. Kato H, et al. Wnt/beta-catenin pathway in podocytes integrates cell adhesion, differentiation, and survival. J Biol Chem. 2011; 286:26003–26015. DOI: 10.1074/jbc.M111.223164 [PubMed: 21613219]
117. Dai C, et al. Wnt/beta-catenin signaling promotes podocyte dysfunction and albuminuria. J Am Soc Nephrol. 2009; 20:1997–2008. DOI: 10.1681/ASN.2009010019 [PubMed: 19628668]
118. Cain JE, Rosenblum ND. Control of mammalian kidney development by the Hedgehog signaling pathway. Pediatr Nephrol. 2011; 26:1365–1371. DOI: 10.1007/s00467-010-1704-x [PubMed: 21161287]

119. Mao J, Kim BM, Rajurkar M, Shivdasani RA, McMahon AP. Hedgehog signaling controls mesenchymal growth in the developing mammalian digestive tract. Development. 2010; 137:1721–1729. DOI: 10.1242/dev.044586 [PubMed: 20430747]
120. Yao E, Chuang PT. Hedgehog signaling: From basic research to clinical applications. J Formos Med Assoc. 2015; 114:569–576. DOI: 10.1016/j.jfma.2015.01.005 [PubMed: 25701396]
121. Abidi A. Hedgehog signaling pathway: a novel target for cancer therapy: vismodegib, a promising therapeutic option in treatment of basal cell carcinomas. Indian J Pharmacol. 2014; 46:3–12. DOI: 10.4103/0253-7613.124884 [PubMed: 24550577]
122. Katoh Y, Katoh M. Hedgehog target genes: mechanisms of carcinogenesis induced by aberrant hedgehog signaling activation. Curr Mol Med. 2009; 9:873–886. [PubMed: 19860666]
123. Xie J, et al. Activating Smoothened mutations in sporadic basal-cell carcinoma. Nature. 1998; 391:90–92. DOI: 10.1038/34201 [PubMed: 9422511]
124. Bowers M, et al. Limb anterior-posterior polarity integrates activator and repressor functions of GLI2 as well as GLI3. Dev Biol. 2012; 370:110–124. DOI: 10.1016/j.ydbio.2012.07.017 [PubMed: 22841643]
125. Attanasio M, et al. Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. Nat Genet. 2007; 39:1018–1024. DOI: 10.1038/ng2072 [PubMed: 17618285]
126. Li B, et al. Increased hedgehog signaling in postnatal kidney results in aberrant activation of nephron developmental programs. Hum Mol Genet. 2011; 20:4155–4166. DOI: 10.1093/hmg/ddr339 [PubMed: 21816948]
127. Fabian SL, et al. Hedgehog-Gli pathway activation during kidney fibrosis. Am J Pathol. 2012; 180:1441–1453. DOI: 10.1016/j.ajpath.2011.12.039 [PubMed: 22342522]
128. Zhou D, et al. Sonic hedgehog is a novel tubule-derived growth factor for interstitial fibroblasts after kidney injury. J Am Soc Nephrol. 2014; 25:2187–2200. DOI: 10.1681/ASN.2013080893 [PubMed: 24744439]
129. Ding H, et al. Sonic hedgehog signaling mediates epithelial-mesenchymal communication and promotes renal fibrosis. J Am Soc Nephrol. 2012; 23:801–813. DOI: 10.1681/ASN.2011060614 [PubMed: 22302193]
130. Kramann R, et al. Pharmacological GLI2 inhibition prevents myofibroblast cell-cycle progression and reduces kidney fibrosis. J Clin Invest. 2015
131. Kramann R, et al. Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis. Cell Stem Cell. 2015; 16:51–66. DOI: 10.1016/j.stem.2014.11.004 [PubMed: 25465115]
132. Schneider J, Arraf AA, Grinstein M, Yelin R, Schultheiss TM. Wnt signaling orients the proximal-distal axis of chick kidney nephrons. Development. 2015; 142:2686–2695. DOI: 10.1242/dev.123968 [PubMed: 26116665]
133. Haegebarth A, Clevers H. Wnt signaling, lgr5, and stem cells in the intestine and skin. Am J Pathol. 2009; 174:715–721. DOI: 10.2353/ajpath.2009.080758 [PubMed: 19197002]
134. Krausova M, Korinek V. Wnt signaling in adult intestinal stem cells and cancer. Cell Signal. 2014; 26:570–579. DOI: 10.1016/j.cellsig.2013.11.032 [PubMed: 24308963]
135. Vooijs M, Liu Z, Kopan R. Notch: architect, landscaper, and guardian of the intestine. Gastroenterology. 2011; 141:448–459. DOI: 10.1053/j.gastro.2011.06.003 [PubMed: 21689653]
136. van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol. 2009; 71:241–260. DOI: 10.1146/annurev.physiol.010908.163145 [PubMed: 18808327]
137. Cao Z, et al. Targeting of the pulmonary capillary vascular niche promotes lung alveolar repair and ameliorates fibrosis. Nature medicine. 2016; 22:154–162. DOI: 10.1038/nm.4035
138. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature. 2005; 434:843–850. DOI: 10.1038/nature03319 [PubMed: 15829953]
139. Kramann R, et al. Pharmacological GLI2 inhibition prevents myofibroblast cell-cycle progression and reduces kidney fibrosis. The Journal of clinical investigation. 2015; 125:2935–2951. DOI: 10.1172/JCI74929 [PubMed: 26193634]

140. Kobayashi T, et al. Expression and function of the Delta-1/Notch-2/Hes-1 pathway during experimental acute kidney injury. Kidney Int. 2008; 73:1240–1250. DOI: 10.1038/ki.2008.74 [PubMed: 18418349]
141. Gupta S, et al. Effect of Notch activation on the regenerative response to acute renal failure. Am J Physiol Renal Physiol. 2010; 298:F209–215. DOI: 10.1152/ajprenal.00451.2009 [PubMed: 19828677]
142. Walsh DW, et al. Co-regulation of Gremlin and Notch signalling in diabetic nephropathy. Biochimica et biophysica acta. 2008; 1782:10–21. DOI: 10.1016/j.bbadis.2007.09.005 [PubMed: 17980714]
143. Simic D, et al. Determination of relative Notch1 and gamma-secretase-related gene expression in puromycin-treated microdissected rat kidneys. Gene Expr. 2013; 16:39–47. [PubMed: 24397211]
144. Surendran K, McCaul SP, Simon TC. A role for Wnt-4 in renal fibrosis. Am J Physiol Renal Physiol. 2002; 282:F431–441. DOI: 10.1152/ajprenal.0009.2001 [PubMed: 11832423]
145. Kuma A, et al. Role of WNT10A-expressing kidney fibroblasts in acute interstitial nephritis. PLoS One. 2014; 9:e103240. [PubMed: 25054240]
146. Kim MK, et al. The differential expression of TGF-beta1, ILK and wnt signaling inducing epithelial to mesenchymal transition in human renal fibrogenesis: an immunohistochemical study. Int J Clin Exp Pathol. 2013; 6:1747–1758. [PubMed: 24040439]
147. Wang D, Dai C, Li Y, Liu Y. Canonical Wnt/beta-catenin signaling mediates transforming growth factor-beta1-driven podocyte injury and proteinuria. Kidney Int. 2011; 80:1159–1169. DOI: 10.1038/ki.2011.255 [PubMed: 21832980]
148. Jiang L, et al. Calmodulin-dependent protein kinase II/cAMP response element-binding protein/Wnt/beta-catenin signaling cascade regulates angiotensin II-induced podocyte injury and albuminuria. J Biol Chem. 2013; 288:23368–23379. DOI: 10.1074/jbc.M113.460394 [PubMed: 23803607]
149. Ren S, et al. LRP-6 is a coreceptor for multiple fibrogenic signaling pathways in pericytes and myofibroblasts that are inhibited by DKK-1. Proc Natl Acad Sci U S A. 2013; 110:1440–1445. DOI: 10.1073/pnas.1211179110 [PubMed: 23302695]
150. Hao S, et al. Targeted inhibition of beta-catenin/CBP signaling ameliorates renal interstitial fibrosis. J Am Soc Nephrol. 2011; 22:1642–1653. DOI: 10.1681/ASN.2010101079 [PubMed: 21816937]
151. He W, Kang YS, Dai C, Liu Y. Blockade of Wnt/beta-catenin signaling by paricalcitol ameliorates proteinuria and kidney injury. J Am Soc Nephrol. 2011; 22:90–103. DOI: 10.1681/ASN.2009121236 [PubMed: 21030600]
152. Surendran K, Schiavi S, Hruska KA. Wnt-dependent beta-catenin signaling is activated after unilateral ureteral obstruction, and recombinant secreted frizzled-related protein 4 alters the progression of renal fibrosis. J Am Soc Nephrol. 2005; 16:2373–2384. DOI: 10.1681/ASN.2004110949 [PubMed: 15944336]

Key points

- Kidney fibrosis, the histological manifestation of functional decline in the kidney, is a reactive process that develops in response to excessive epithelial injury and inflammation
- In fibrosis, epithelial cells and their vascular capillary bed are lost, while activated myofibroblasts, matrix and inflammatory cells accumulate
- Tissue injury causes activation of developmental pathways, and several reports highlighted that fibrosis is associated with increased expression and activity of Notch, Wnt and Hedgehog (Hh) signalling
- Although activation of these pathways might be important for regeneration of the damaged organ, excessive stimulation contributes to fibrosis development
- Notch and Wnt signalling has been shown to have a role in epithelial dedifferentiation, and Wnt and Hh signalling can induce myofibroblast transformation and proliferation
- Decreasing the activity of Notch, Wnt, or Hh signalling could potentially be a new therapeutic strategy to ameliorate the development of chronic kidney disease

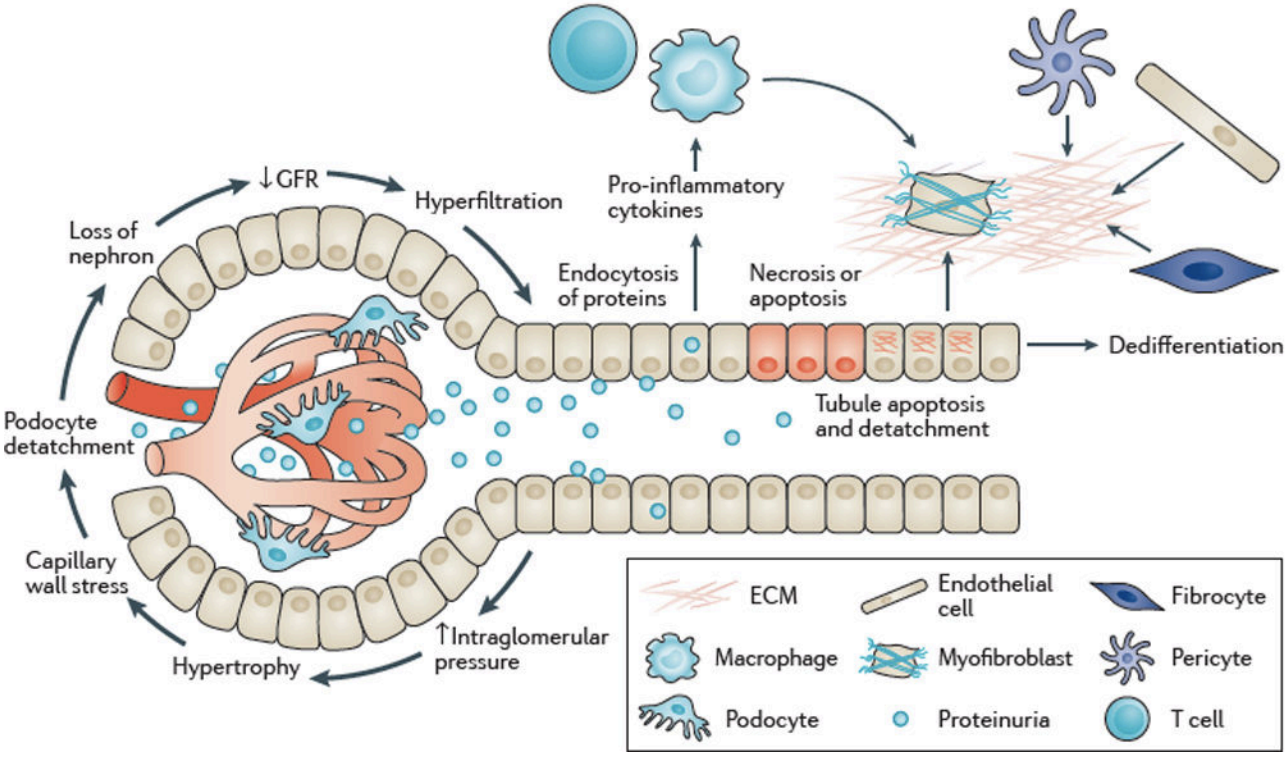

Figure 1. Development of kidney fibrosis

Glomerular hyperfiltration is associated with glomerular hypertrophy, which results in podocyte loss. Loss of podocytes causes albuminuria and glomerulosclerosis, and nephron loss. Hyperglycaemia, increased levels of fatty acids and proteinuria cause epithelial damage including apoptosis, detachment and dedifferentiation of epithelial cells, and increased expression of pro-inflammatory cytokines. Increased cytokine expression causes increased macrophage, T-cell, and mast-cell influx. Epithelial damage also induces myofibroblast transformation and production of extracellular matrix (ECM). GFR, glomerular filtration rate.

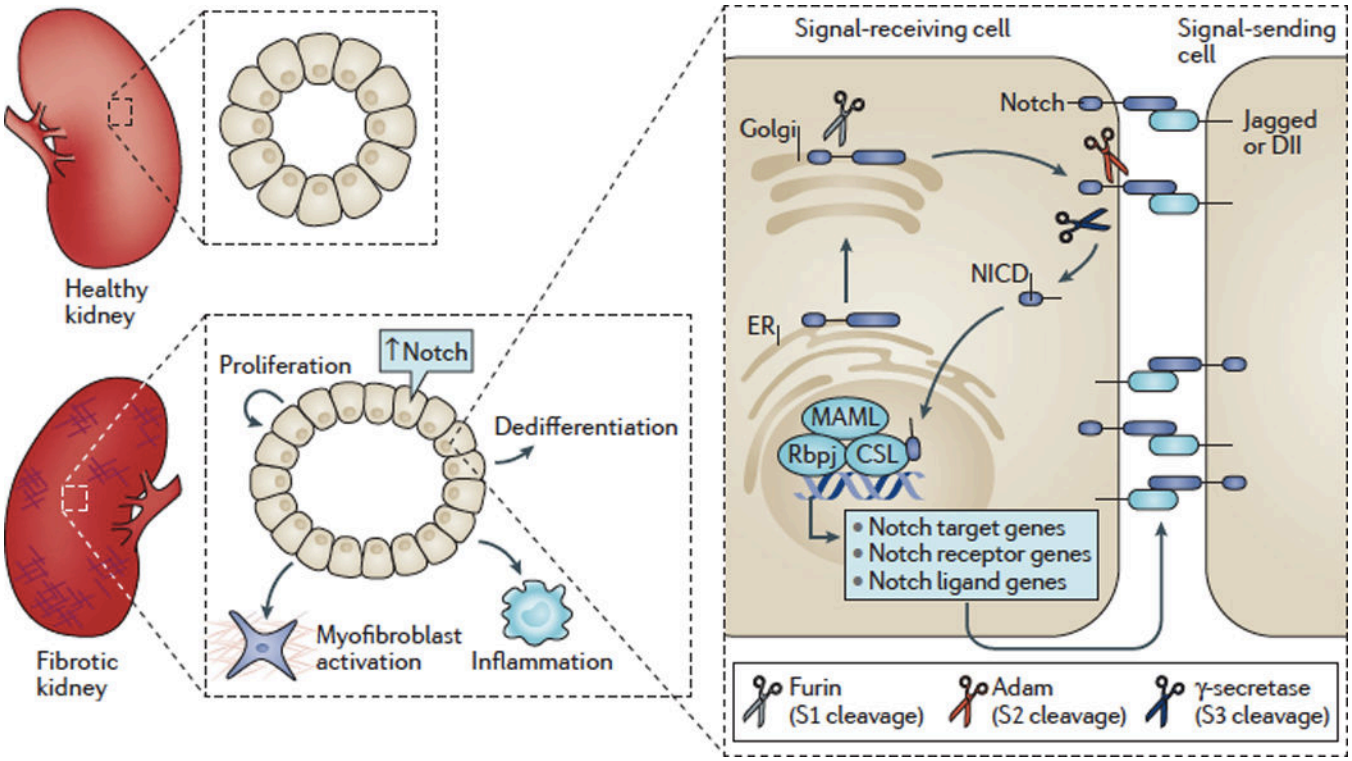

Figure 2. Notch signalling in renal fibrosis

In healthy adult kidneys, the activity of Notch signaling is low. Acute and chronic kidney disease is associated with increased epithelial expression of Notch signaling memebers. The Notch receptor is expressed on signal-receiving cells. Binding of the Notch ligand on the signal-sending cell to the receptor results in Notch cleavage (by the γ-secretase complex) and release of the Notch intracellular domain (NICD). In the nucleus, NICD binds to other transcriptional regulators such as Rbpj, and MAML for regulating gene expression. In chronic kidney disease, increased Notch activity can be observed in renal epithelial cells. Increased Notch receptor expression is associated with epithelial dedifferentiation, myofibroblast activation, matrix deposition, and the inflammatory response.

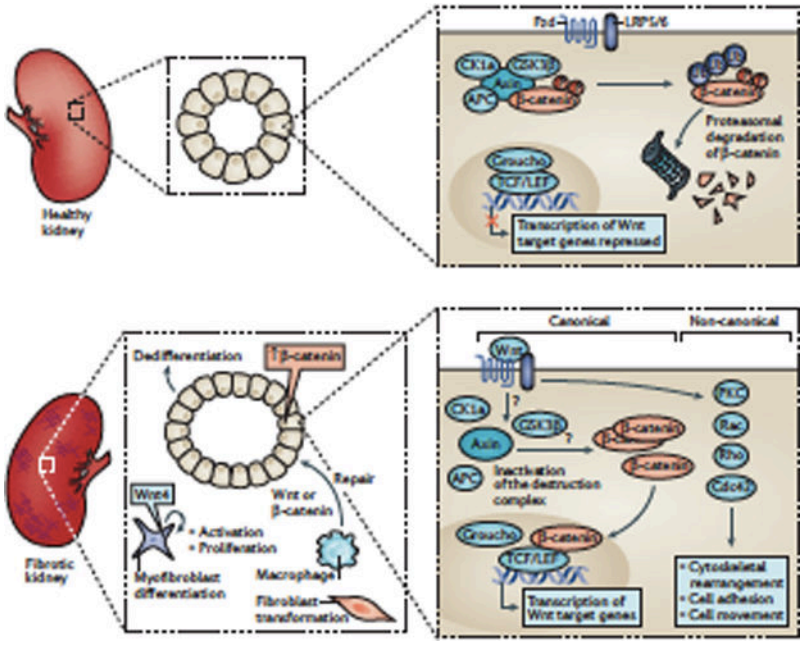

Figure 3. Dysregulation of Wnt signalling leads to the development of renal fibrosis

In the absence of the Wnt ligands (inactive Wnt signaling), cytoplasmic β-catenin is phosphorylated by the ‘destruction complex’ containing the glycogen synthase kinase 3b (GSK3b), the scaffolding protein axin, casein kinase 1a (CK1a), and the adenomatous polyposis coli protein (APC), which is followed by ubiquitin-mediated proteasomal degradation. Binding of Wnt to Frizzled (Fzd) and LRP5/6 receptor proteins inactivates GSK3b and disassembles the ‘destruction complex’. Accumulation of non-phosphorylated β-catenin in the cytoplasm leads to its translocation into the nucleus (canonical Wnt signaling). There, β-catenin replaces Groucho from TCF/LEF and activates Wnt target gene expression. Increased tubule-specific β-catenin expression has been described in chronic kidney disease (CKD). Myofibroblasts can express Wnt4, which induces proliferation of resident fibroblasts and their transformation into myofibroblasts. Wnt/β-catenin signalling in pericytes and interstitial fibroblasts exhibits spontaneous myofibroblast differentiation, the relevant step in fibrosis development. Macrophage expression of Wnt7b is associated with epithelial repair. The ß-catenin-independant (non-canonical) Wnt signaling pathway regulates cytoskeleton rearrangement, cell adhesion and cell movement via the kinases Rho, Rac and Cdc42.

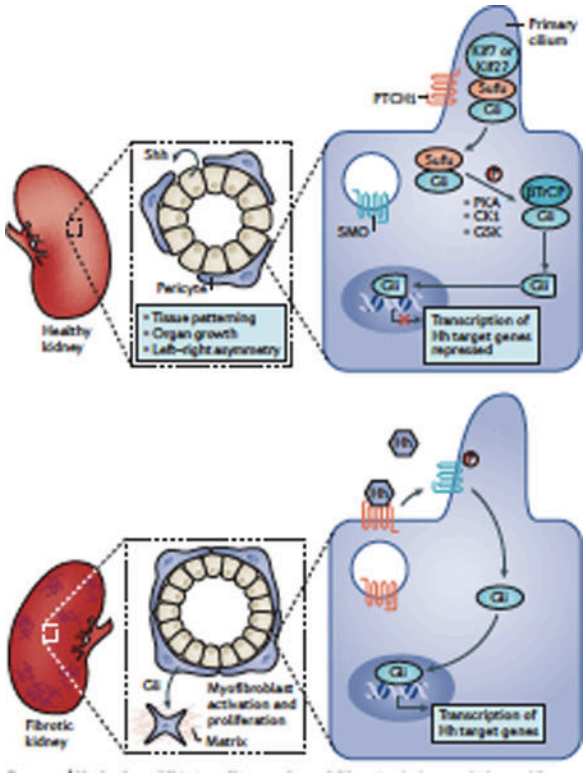

Figure 4. Hedgehog (Hh) signalling and renal fibrosis

In humans, Hh molecules bind to the receptor Patched1 (PTCH1), which leads to localization of SMO to the primary cilium. Ciliary SMO mediates the translocation of full-length GLI to the nucleus. GLI binds to DNA and activates expression of Hh target genes. In the *steady state* of Hh signaling GLI proteins are sequestered by Kif7/Kif27, Fused (Fu) and the Suppressor of Fused (Sufu) followed by phosphorylation by protein kinase A (PKA), casein kinase 1 (CK1) and glycogen synthase kinase 3b (GSK3b). This modification allows proteolytical cleavage of GLI by b-transducin repeat containing protein (bTrCP). The now C-terminal shortened GLI repressor binds to the DNA for inhibiting Hh target gene transcription Hh ligands are expressed in tubular epithelial cells, and interstitial cells respond to Hh ligands. Gli1-expressing pericytes have an important role in interstitial fibrosis, undergoing myofibroblast transformation and proliferation.

Table 1

Expression of Notch pathway members in acute and chronic kidney disease models.

Kidney injury	Expression of Notch receptors and ligands	References
Acute		
IRI	Notch2, Delta1, Hes1	Kobayashi *et al.*, 2008[77]
IRI	NICD1, Hes1	Sörensen-Zender *et al.*, 2014[65]
IRI	Notch2, Hes1	Huang *et al.*, 2011[64]
IRI	NICD1, NICD2	Gupta *et al.*, 2010[137]
Chronic		
DN	Jagged1, Hes1	Walsh *et al.*, 2008[138]
DN	Notch1, Notch2 and Jagged1 in podocytes, Notch1 in tubules	Murea et al., 2010[60]
FA	Notch1, Notch2, Notch3, Jagged1, Hes1 and HeyL, but not Dll1 and Dll4	Bielesz et al., 2010[61]
FSGS	Notch1 and Jagged1 in podocytes, Notch2, Jagged1 and Delta1 in tubules	Murea et al., 2010[60]
LN	Notch1 and Jagged1 in podocytes, Notch2 in tubules	Murea et al., 2010[60]
MCD	Notch1, Notch2, Jagged1 in podocytes, Notch2, Jagged1 and Delta1 in tubules	Murea et al., 2010[60]
Puromycin aminonucleosid treatment	No significant changes in the expression levels of Notch1, Hes1 and Presenilin1	Simic et al., 2013[139]
UUO	Notch1, Notch3, Notch4, NICD1, NCID2, NCID3, NCID4, Hes1, HeyL	Xiao et al., 2014[74]
UUO	Notch3, NICD3	Djudjaj et al., 2012[52]

DN, diabetic nephropathy; FA, folic acid; FSGS, focal segmental glomerulosclerosis; IRI, ischaemia–reperfusion injury; LN, lupus nephritis; MCD, minimal change disease; UUO, unilateral ureteral obstruction.

Table 2

Wnt proteins and their expression in the kidney.

Wnt proteins	Effects in the kidney	System	References
Wnt1	Podocyte dysfunction, albuminuria	Exogenous Wnt1 administration in adryamicin-induced nephropathy	Dai et al., 2009 [111]
Wnt4	Tubular epithelial destruction, increased number of interstitial cells	Cultured Wnt4-expressing fibroblasts were transferred to normal murine kidneys	Surendran et al., 2002[140]
Wnt4	Induced myofibroblast differentiation	Exogenous Wnt4 administration to cells from the 10T1/2 mesenchymal cell line	DiRocco et al., 2013[110]
	No effect on renal fibrosis induced by UUO	Wnt4 knockout in myofibroblasts of mice	DiRocco et al., 2013[110]
Wnt7b	Decreased stimulation of repair and regeneration, decreased cell-cycle progression in epithelial cells and decreased basement membrane repair	Mouse model with a Wnt7b knockout in macrophages	Lin et al., 2010[100]
Wnt10b	Enhanced expression of fibronectin	Cultured kidney fibroblasts from the COS-1 cell line	Kuma et al., 2014[141]
Wnt11	Mediated Tgf-β-dependent EMT	Immortalized transgenic kidney proximal tubule cells (TKPTS)	Zhang et al., 2012[109]
Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt8a, Wnt8b, Wnt9a, Wnt9b, Wnt10a, Wnt16	All were found at increased levels during kidney injury but a specific function for each ligand is not known yet	UUO Human fibrotic kidney IRI	He et al., 2009[102] Kim et al., 2013[142] Xiao et al., 2015[143]

EMT, epithelial-to-mesenchymal transition; UUO, unilateral ureteral obstruction.

Table 3

Specificity and effects of Wnt inhibitors on kidney injury.

Wnt inhibitors	Specificity	Effects in the kidney	System	References
Dapper3 gene	Not fully understood	Aggravated renal fibrosis in mice after UUO	Dapper3 gene knockout mouse	Xue et al., 2013[107]
Dkk1	Binds to LRP5/6	Reduced Tgf-β-induced proteinuria and the number of podocyte lesions	Dkk1 expression vector injected in mice	Wang et al., 2011[144]
		Reduced fibrosis	UUO	He et al., 2009[102]
		Attenuated podocyte injury	Angiotensin II-induced podocyte injury	Jiang et al., 2013[145]
		Reduced proteinuria	Adryamicin-induced nephropathy	Dai et al., 2009[111]
		reduced fibrosis	IRI	Ren et al., 2013[146]
Dkk2	Binds to LRP5/6, acting as either an inhibitor or and agonist of Wnt signalling	Dkk2-treated mice recovered better from ischemia-reperfusion than untreated mice	IRI	Kawakami et al., 2013[96]
ICG-001	A small-molecule antagonist of β-catenin/ TCF-mediated transcription	Reduced fibrosis	UUO	Hao et al., 2011[147]
Paricalcitol	Ligand-activated vitamin D receptor (VDR) competing with transcription factor TCF-4 for β-catenin binding	Ameliorated proteinuria and kidney injury	Adryamicin-induced nephropathy	He et al., 2011[148]
Sfrp1	Binds to both Wnt and Fzd	Mice lacking Sfrp1 had increased amounts of fibrotic lesions, and increased vimentin and α-SMA expression	UUO	Matsuyama et al., 2014[106]
Sfrp4	Binds to both Wnt and Fzd	Decreased number of myofibroblasts and fibrosis	UUO	Surendran et al., 2005[149]

α-SMA, α smooth muscle actin; Dkk, Dickkopf; Fzd, Frizzled; IRI, ischaemia–reperfusion injury; LRP5/6, low-density lipoprotein receptor-related protein; Sfrp, secreted frizzled-related protein; TCF-4, T-cell factor-4; Tgf-β, tumor growth factor- β; UUO, unilateral ureteral obstruction.