WNT–β-catenin signalling — a versatile player in kidney injury and repair

Stefan J. Schunk[1], Jürgen Floege[2], Danilo Fliser[1] and Thimoteus Speer[2]✉

Abstract | The WNT–β-catenin system is an evolutionary conserved signalling pathway that is of particular importance for morphogenesis and cell organization during embryogenesis. The system is usually suppressed in adulthood; however, it can be re-activated in organ injury and regeneration. WNT-deficient mice display severe kidney defects at birth. Transient WNT–β-catenin activation stimulates tissue regeneration after acute kidney injury, whereas sustained (uncontrolled) WNT–β-catenin signalling promotes kidney fibrosis in chronic kidney disease (CKD), podocyte injury and proteinuria, persistent tissue damage during acute kidney injury and cystic kidney diseases. Additionally, WNT–β-catenin signalling is involved in CKD-associated vascular calcification and mineral bone disease. The WNT–β-catenin pathway is tightly regulated, for example, by proteins of the Dickkopf (DKK) family. In particular, DKK3 is released by 'stressed' tubular epithelial cells; DKK3 drives kidney fibrosis and is associated with short-term risk of CKD progression and acute kidney injury. Thus, targeting the WNT–β-catenin pathway might represent a promising therapeutic strategy in kidney injury and associated complications.

Since its identification in 1982 by Nusse and colleague[1], the WNT–β-catenin signalling pathway has been the subject of intensive research, leading to a steadily growing body of evidence on its regulation and its role in health and disease. WNT is a conjugation of the two terms *Wingless* (gene in drosophila) and *Int1* (homologue of *Wingless* in vertebrates). WNT signalling drives the organization of tissues during embryogenesis and maintains tissue structure during human life. In addition to embryogenesis, the WNT pathway also has a fundamental role in growth during normal adolescence and also during pathological growth processes such as cancer development[2]. Accordingly, *WNT* genes are highly conserved across different species, from sponges to humans. In the mammalian genome, 19 genes encoding WNT have been identified. Although several *WNT* genes share common functions, they also exert specific functions during distinct developmental processes. Accordingly, knockout mice of individual *Wnt* genes display diverse phenotypes, pointing to a highly complex regulatory network[2].

In kidney development, WNT4 and WNT9B are of particular importance[3]. They regulate the transition of mesenchymal into epithelial cells, and promote nephron elongation and differentiation[4]. Accordingly, *Wnt4*-knockout mice are characterized by severe kidney defects at birth[3]. WNT signalling is also involved in various pathological processes during adulthood such as kidney fibrosis in the course of chronic kidney disease (CKD), podocyte injury and proteinuria, acute kidney injury (AKI), cystic kidney diseases, and CKD-associated vascular damage and mineral bone disease (CKD-MBD). This Review discusses advances in the understanding of WNT–β-catenin signalling and its regulation during kidney injury, as well as its potential diagnostic and therapeutic implications.

WNT–β-catenin signalling pathways

WNT signalling pathways can be categorized into canonical, that is, β-catenin-dependent, and non-canonical, that is, β-catenin-independent signalling pathways.

Canonical WNT signalling pathways. β-Catenin serves as a transcription factor that induces activation of T cell factor (TCF)–lymphoid enhancer factor (LEF)-dependent gene expression upon its translocation to the nucleus (FIG. 1). In steady state, β-catenin is inactivated by a 'destruction complex' comprising the proteins glycogen synthase kinase 3β (GSK3β), adenomatous polyposis coli (APC), casein kinase 1 (CK1) and axin. This protein complex mediates phosphorylation of β-catenin, which is then ubiquitinylated and subsequently degraded in

[1]*Department of Internal Medicine IV, Nephrology and Hypertension, Saarland University, Homburg/Saar, Germany.*

[2]*Department of Nephrology and Clinical Immunology, University Hospital, Rheinisch Westfälische Technische Hochschule (RWTH), Aachen, Germany.*

✉*e-mail: timo.speer@uks.eu*

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Key points

- WNT–β-catenin is key regulator of embryogenesis and tissue organization.
- WNT–β-catenin can be re-expressed in adult life in a variety of disease conditions.
- Transient WNT–β-catenin signalling induces repair and regeneration during acute kidney injury, whereas sustained (uncontrolled) WNT–β-catenin signalling promotes kidney fibrosis, podocyte damage and mineral bone disease associated with chronic kidney disease.
- Dickkopf 3 is a WNT–β-catenin modulator that drives kidney fibrosis and represents a novel marker for chronic kidney disease progression and acute kidney injury.
- Several WNT–β-catenin signalling inhibitors are in development, of which so far only the sclerostin-inhibiting antibody is available for treatment of osteoporosis.
- Future studies will improve understanding of the regulation of WNT–β-catenin and set the stage for specific therapies.

proteasomes[5]. WNT ligands induce a spatial interaction between the receptors Frizzled proteins (FZD) and lipoprotein receptor-related protein 5 (LRP5) and LRP6 to initiate β-catenin-dependent signalling. Upon binding of WNT to this receptor complex, Dishevelled (DVL) protein is recruited and prevents degradation of β-catenin[5,6]. Subsequently, β-catenin translocates into the nucleus, where it forms a complex with the TCF–LEF transcription factors to trigger WNT-dependent gene expression[7].

Non-canonical WNT signalling pathways. In addition to canonical WNT signalling, two non-canonical signalling pathways exist. FZD proteins can directly induce WNT–Ca^{2+} signalling via phospholipase-dependent production of inositol triphosphate–diacylglycerol or cyclic GMP-phosphodiesterase and p38-MAPK[8]. Another non-canonical pathway is WNT–planar cell polarity signalling, which is of importance for directional information transfer and communication between epithelial cells and mesenchymal cells undergoing structural changes[9].

Secretion of WNT proteins. The activation of the WNT receptors FZD and LRP5 and LRP6 on the cell surface by WNT proteins requires their secretion into the extracellular space. The secretory route of WNT proteins is determined by post-translational palmitoylation (that is, lipidation) mediated by the enzyme porcupine in the endoplasmic reticulum (FIG. 2). Binding of WNT proteins to the chaperone Wntless (WLS) directs their trafficking through the Golgi complex[10–13]. The hydrophilicity of palmitoylated WNT proteins is low, which explains why they circulate incorporated in exosomes, or coupled to lipoproteins or other carrier proteins, or attach to the plasma membrane[14–17]. TABLE 1 gives an overview of proteins involved in WNT–β-catenin signalling.

Receptors. WNT proteins interact with a receptor complex on the cell surface comprising FZD and LRP5 and LRP6. FZD proteins are seven transmembrane receptors with a cysteine-rich domain responsible for the binding of WNT proteins[18]. LRP5 and LRP6 consist of one transmembrane domain and an intracellular part with distinct phosphorylation sites that are required to initiate cellular WNT–β-catenin signalling[6]. The importance of LRP5 and LRP6 is evidenced by *Lrp5*-knockout mice, which exhibit reduced bone mass and vascularization defects, whereas *Lrp6*-knockout mice are not viable[19]. Beyond LRP5 and LRP6, the receptor tyrosine kinase-like orphan receptor 1 (ROR1) and ROR2 also serve as WNT receptors and induce β-catenin-independent signalling cascades[20].

Several inhibitors of WNT-dependent signalling cascades have been identified, such as Dickkopf (DKK) proteins, sclerostin and WNT inhibitory factor 1 (WIF1). WIF1 directly binds WNT ligands and thereby impedes their receptor interaction[21,22], DKK proteins and sclerostin bind to LRP5 and LRP6 to suppress WNT–β-catenin signalling[23]. For instance, DKK1 binds directly to LRP6, inhibits its interaction with FZD and thereby inhibits WNT–β-catenin signalling (FIG. 1). Moreover, expression of DKK proteins can be induced by WNT–β-catenin signalling itself as a negative feedback loop to limit uncontrolled WNT–β-catenin signalling[24]. In this context, DKK proteins might serve as a surrogate of WNT–β-catenin signalling activation[25].

In addition to LRP5 and LRP6, the (pro)renin receptor (PRR) modulates cellular WNT–β-catenin signalling. PRR has emerged as an amplifier of canonical WNT signalling[26]. PRR expression is induced by activation of the WNT–β-catenin system and in parallel PRR is required for signal transduction by the FZD–LRP5 or FZD–LRP6 (REF.[26]) receptor complex. *PRR*-deficient mice are not viable, underscoring the importance of this co-receptor[27,28].

Cellular effects in the kidney

The role of the WNT–β-catenin signalling pathway in the interaction between tubular epithelial cells, fibroblasts and macrophages is summarized in FIG. 3.

Tubular epithelial cells. Tubular epithelial cells represent a major source of WNT proteins in the kidney. These WNT proteins dictate the tubular phenotype in an autocrine manner and control the phenotype of fibroblasts in a paracrine fashion[29–31]. Notably, transient activation of the WNT–β-catenin system must be distinguished from sustained activation.

Transient stimulation of tubular epithelial cells in vitro with WNT6 promotes tubular cell regeneration by inducing de novo tubulogenesis and inhibiting epithelial cell dedifferentiation[32]. Similarly, WNT1 or constitutive activation of β-catenin prevents tubular cell apoptosis and induces a pro-survival pathway by phosphorylation of AKT and reduced expression of p53 and BAX[33–35]. In proximal tubular epithelial cells, β-catenin signalling augments the activity of the transcription factor FOXO3 to prevent tubular cell apoptosis by targeting cystathionine γ-lyase[36].

By contrast, sustained activation of tubular epithelial cells by WNT proteins such as WNT9A induces tubular senescence, characterized by increased expression of p16, p19, p53 and p21 (REF.[37]). Moreover, by engaging non-canonical WNT–β-catenin signalling, WNT5A induces the expression of matrix metalloproteinase 2 (MMP2) in tubular epithelial cells, leading to disruption of the basal membrane[38].

These studies illustrate that transient activation of the WNT-β-catenin system preserves the integrity of

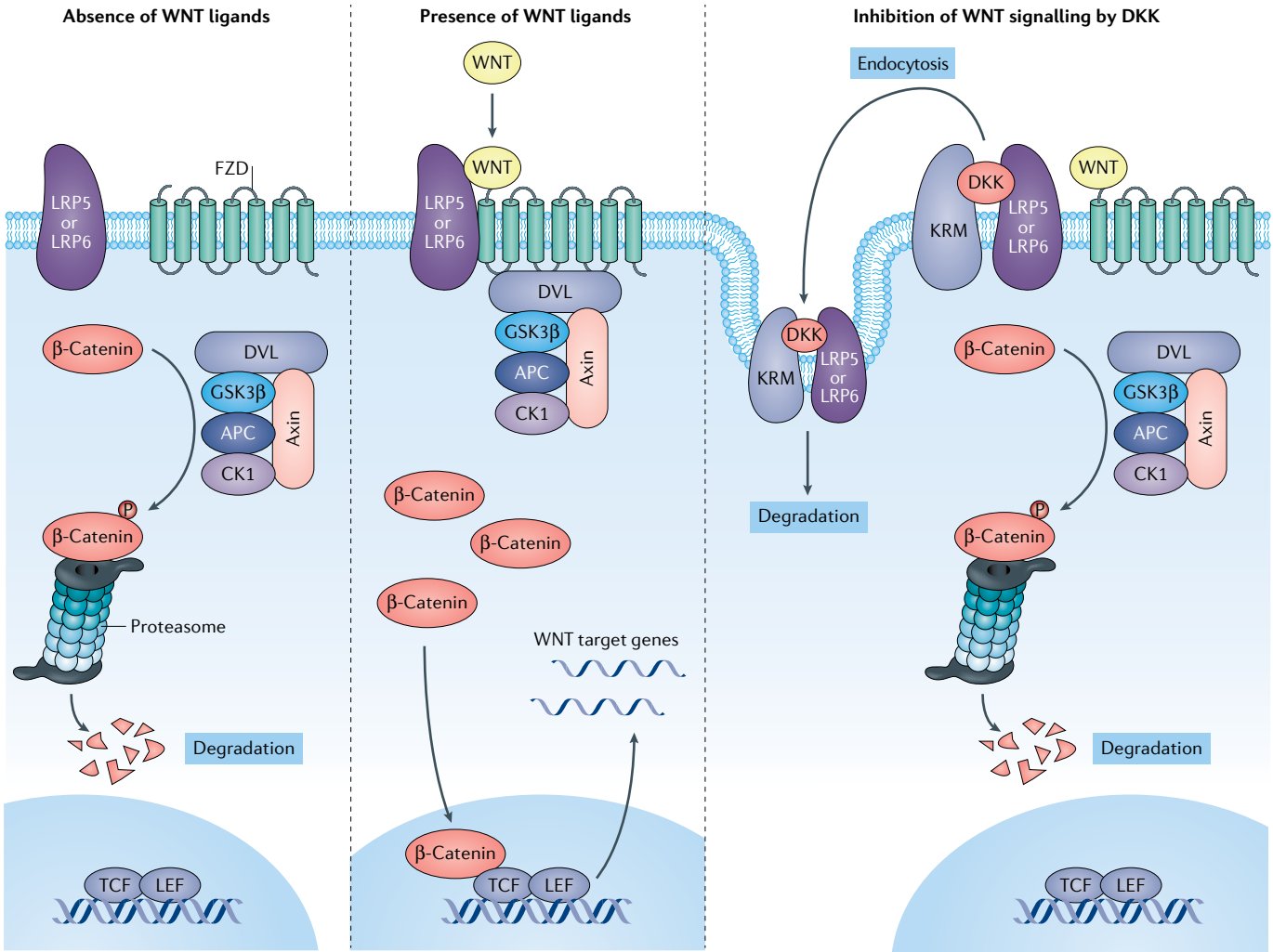

Fig. 1 | **Canonical WNT signalling in the absence or presence of WNT ligands and its inhibition by DKK proteins.** In the absence of WNT ligands, a protein complex comprising Dishevelled protein (DVL), glycogen synthase kinase 3β (GSK3β), adenomatous polyposis coli (APC), casein kinase 1 (CK1) and axin mediates phosphorylation of β-catenin. Phosphorylated β-catenin is then ubiquitinylated and subsequently subjected to proteasomal degradation. WNT ligands interact with a receptor complex consisting of Frizzled protein (FZD) and lipoprotein receptor-related protein 5 (LRP5) or LRP6 on the cell surface, which leads to recruitment of DVL and the axin–GSK3β–APC–CK1 complex to the receptor. This step suppresses the phosphorylation of β-catenin, which accumulates in the cytoplasm. Subsequently, β-catenin translocates into the nucleus, where it activates T cell factor (TCF) and lymphoid enhancer factor (LEF)-dependent gene expression. Dickkopf (DKK) proteins serve as inhibitors of WNT–β-catenin signalling. DKK binds to LRP6 and thereby prevents its interaction with FZD. In addition, together with Kremen (KRM), DKK induces endocytosis of the LRP6–DKK complex, which is subjected to degradation.

the tubular epithelium, whereas sustained activation drives tubular damage.

Fibroblasts. Fibroblasts constitute a main driver of renal scarring after injury. Sustained activation of fibroblasts leads to the secretion of extracellular matrix components such as collagen, proteoglycans and fibronectin, which results in the development of kidney fibrosis[39]. Fibroblasts can be activated in a paracrine manner by exogenous WNT ligands, which mainly originate from tubular epithelial cells via epithelial–fibroblast crosstalk[37,40]. In a study using tubular epithelial-specific or fibroblast-specific *Wls*-knockout mice, Wntless deficiency (that is, the inability to secrete WNT ligands) in tubular epithelial cells prevented fibroblast activation and fibrosis in vivo, whereas a fibroblast-specific *Wls* knockout had no effect on fibrosis[40]. These findings highlight kidney fibroblasts as an effector cell type of WNT ligands secreted by tubular epithelial cells upon injury. Activation of the WNT–β-catenin pathway in fibroblasts induces fibroblast proliferation[40] as well as their differentiation towards myofibroblasts, which is characterized by secretion of fibronectin and increased expression of α-smooth muscle actin[37]. Moreover, tubular cell-derived WNT ligands directly interact with interstitial myofibroblast progenitors to induce interstitial fibrosis without inflammation[30]. Accordingly, mice with constitutively active canonical WNT–β-catenin signalling in interstitial pericytes and fibroblasts develop spontaneous myofibroblast differentiation in the absence of injury[31].

The interaction between tubular epithelial cells and fibroblasts, however, is not unidirectional. β-Catenin deficiency in fibroblasts leads to reduced tubular cell apoptosis and increased tubular regeneration, which is accompanied by reduced inflammation after kidney injury[41]. These findings point to a complex regulatory network between tubular epithelial cells and fibroblasts, which is regulated by autocrine and paracrine WNT–β-catenin signalling[29–31].

Macrophages. Besides controlling adaptive T lymphocyte mediated immunity, M2 macrophages particularly promote kidney fibrosis at least partially by differentiating directly into myofibroblasts[42]. Within the kidney, macrophages represent a source and recipient of WNT ligands alike[43]. Upon injury, macrophages release WNT7B, which acts upon interstitial and epithelial cells to induce repair and regeneration[43]. Vice versa, tubular cell-derived WNT ligands induce pro-inflammatory activation of renal macrophages during fibrosis[44,45].

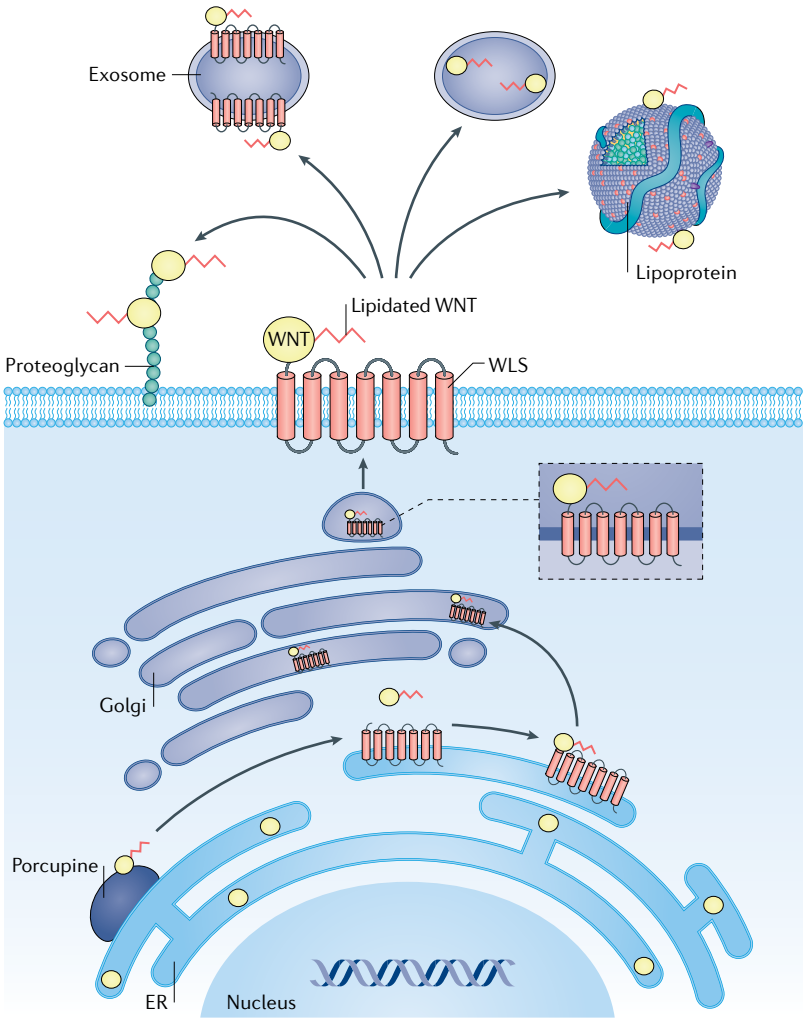

Fig. 2 | **The secretory route of WNT proteins in the cell.** In the endoplasmic reticulum (ER), WNT proteins are post-translationally lipidated via palmitoylation by porcupine. Subsequently, WNT proteins bind to the chaperone Wntless (WLS), which guides them through the Golgi complex. Owing to their hydrophobicity they are either transported in exosomes, bound to lipoproteins or they attach to the cell membrane via binding to proteoglycans.

Upon injury, resident kidney macrophages acquire a developmental state with upregulation of canonical WNT–β-catenin signalling[46]. In these macrophages, activation of the WNT–β-catenin system (for example, by WNT3A or WNT5A) drives their proliferation by upregulation of cyclin D1 (REF.[47]) and their polarization towards an alternative (M2) phenotype induced by treatment with IL-4 and TGFβ1, which is mediated by phosphorylation of STAT3 (REFS[48,49]).

WNT–β-catenin signalling and kidney injury

Of the 19 WNT ligands identified in humans, 16 are expressed in the kidney in response to a variety of distinct types of injury (reviewed elsewhere[3,50]). The effects of WNT–β-catenin signalling on kidney injury are shown in FIG. 4.

Acute kidney injury. Early experiments in AKI rat models demonstrated that tubular WNT4 expression increases after acute ischaemia–reperfusion injury (IRI)[51]. Whereas moderate IRI leads to transient WNT–β-catenin activation, severe IRI promotes a long-lasting WNT–β-catenin response[52]. WNT1 overexpression 5 days after IRI drives progression of AKI to CKD, mainly by activation of renal fibroblasts as an example of sustained activation of the WNT–β-catenin cascade[52]. Notably, WNT–β-catenin signalling is not only involved in the initiation of AKI and its progression to CKD, but might also be of importance for regeneration after AKI. The extracellular matrix glycoprotein tenascin-C accumulates in injured kidneys, where it recruits WNT ligands, creating a micromilieu for tubular repair[53].

Podocyte injury and proteinuria. WNT–β-catenin signalling directly interferes with podocyte function[54–57]. In vivo, overexpression of WNT1 enhances proteinuria[54]. In addition, upregulation of WNT1 was detected in human biopsy samples from patients with diabetic nephropathy and focal segmental glomerulosclerosis, and WNT1 directly induced podocyte dysfunction in vitro[56]. Podocyte dysfunction results from WNT–β-catenin signalling affecting podocyte adhesion, differentiation and survival[58].

Oxidative stress has an important role in activating podocytic WNT–β-catenin pathways. Mice with podocyte-specific β-catenin deficiency are protected against podocyte damage and proteinuria after administration of advanced oxidation protein products[59] and administration of angiotensin-II[60]. By contrast, paricalcitol ameliorates proteinuria and kidney damage by inhibiting WNT–β-catenin signalling[61]. Besides podocytic injury, activation of WNT–β-catenin signalling triggers renal inflammation such as kidney infiltration with macrophages, release of pro-inflammatory cytokines and expression of cell adhesion molecules during proteinuric nephropathy[45].

Polycystic kidney diseases. Autosomal-dominant polycystic kidney disease (ADPKD), caused by mutations in the *PKD1* or *PKD2* genes, represents the most common form of polycystic kidney disease[62]. The ciliary protein polycystin 1, which is encoded by *PKD1*, modulates

Table 1 | **Important proteins in WNT–β-catenin signalling**

Abbreviated name	Full name	Function
WNT	Wingless and INT1	Family of 19 glycoproteins that activate WNT–β-catenin signalling
β-Catenin	β-Catenin	Cytoplasmic protein that translocates into the nucleus and mediates TCF–LEF-dependent gene expression
Porcupine	Porcupine	Enzyme mediating palmitoylation of WNT proteins in the endoplasmic reticulum
WLS	Wntless	Chaperone mediating trafficking of WNT proteins through the Golgi complex
FZD	Frizzled proteins	Part of cell surface receptor complex for WNT ligands together with LRP5 and LRP6
LRP5 and LRP6	Lipoprotein receptor-related proteins 5 and 6	Part of cell surface receptor complex for WNT ligands together with FZD
ROR1 and ROR2	Receptor tyrosine kinase-like orphan receptors 1 and 2	Cell surface receptor that activates β-catenin-independent WNT signalling
GSK3β	Glycogen synthase kinase 3β	Components of the 'destruction complex', which induces degradation of β-catenin in the absence of WNT ligands
APC	Adenomatous polyposis coli	
CK1	Casein kinase 1	
Axin	Axin	
DVL	Dishevelled protein	Protein recruited to the FZD–LRP5 and FZD–LRP6 complex in the presence of WNT ligands
TCF–LEF	T cell factor–lymphoid enhancer factor	Downstream transcription factors of the WNT–β-catenin pathway
DKK	Dickkopf	Proteins that serve as modulators and/or inhibitors of WNT–β-catenin signalling
Klotho	Klotho	
Sclerostin	Sclerostin	
sFRP	Soluble frizzled-related protein	
WIF1	WNT inhibitory factor 1	
TGFβ	Transforming growth factor-β	Cytokine that has an important role in the regulation of extracellular matrix production
SNAIL	SNAIL	WNT–β-catenin target genes that are involved in kidney injury
MMP7	Matrix metalloproteinase 7	
TRPC6	Transient receptor potential channel 6	
HGF	Hepatocyte growth factor	
RAS	Renin–angiotensin system	
PAI1	Plasminogen activator inhibitor 1	
PRR	(Pro)renin receptor	

WNT–β-catenin signalling, and ectopic WNT–β-catenin activation directly induces cyst formation[63,64].

Non-canonical WNT signalling might also be important in the development of polycystic kidney diseases. Polycystin 1 has been identified as a WNT (co)receptor, and defective WNT–Ca^{2+}-dependent signalling has emerged as a cause of cystic kidney disease[65]. WNT–planar cell polarity signalling regulates tubular branching and elongation during nephrogenesis and several studies have documented that abnormal WNT–planar cell polarity signalling might also drive the development of polycystic kidney diseases[66]. For instance, patients with mutations in the protein inversin, which shifts canonical to WNT–planar cell polarity signalling, develop forms of autosomal-recessive polycystic kidney disease[67,68]. Mutations in other genes involved in WNT–planar cell polarity signalling, such as *VANGL1, VANGL2, CELSR1, CELSR2* and *CELSR3*, might also be of importance during cystogenesis[66].

Inhibition of WNT signalling might thus serve as a therapeutic strategy to slow or even prevent cystogenesis. Canonical WNT inhibitors have been shown to ameliorate cyst formation in a mouse model of ADPKD[69].

Kidney fibrosis and progressive CKD. Kidney fibrosis represents the common final pathway of most pathological processes leading to CKD. Kidney fibrosis is characterized by the production of extracellular matrix proteins, such as collagen, which accumulate within the injured kidney.

Activation of WNT–β-catenin-dependent signalling has been observed in the unilateral ureter obstruction (UUO) model, a classical small animal model of kidney fibrosis[70]. UUO decreased the expression of the

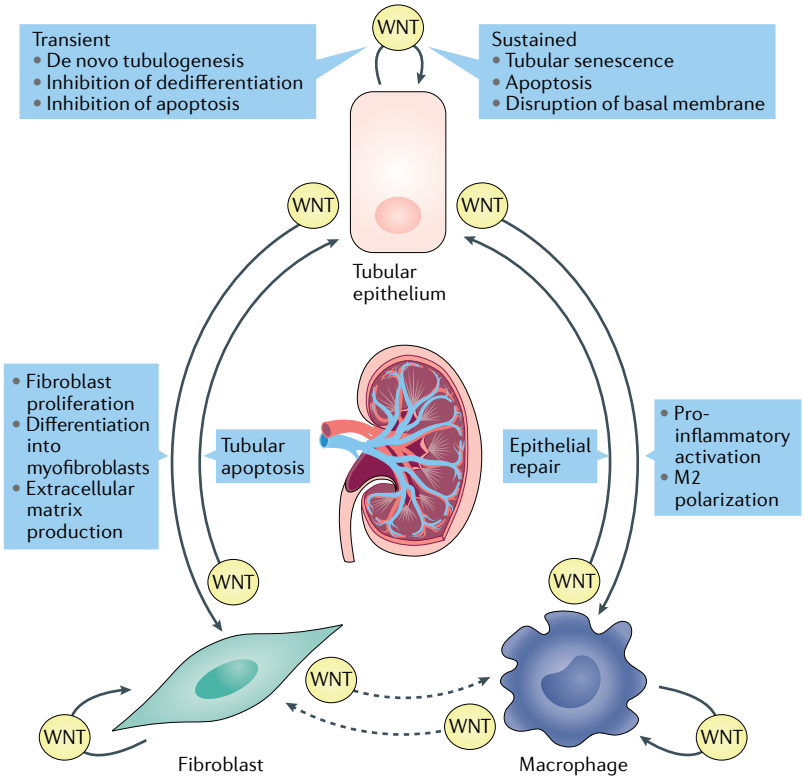

Fig. 3 | **The role of WNT–β-catenin in the regulation of the interaction between tubular epithelial cells, fibroblasts and macrophages.** WNT ligands can act in an autocrine or paracrine manner. In tubular epithelial cells, transient WNT–β-catenin activation exerts regenerative effects, whereas sustained WNT–β-catenin signalling induces tubular damage. In fibroblasts, epithelial-derived WNT ligands induce their activation and a profibrotic phenotype. In macrophages, activation of the WNT–β-catenin cascade induces pro-inflammatory activation as well as polarization towards the M2 phenotype. In turn, fibroblast-derived and macrophage-derived WNT ligands directly interact with tubular cells. A direct interaction between macrophages and fibroblasts mediated by the WNT system is highly likely but has not yet been proven.

WNT antagonist secreted frizzled-related protein 4 (sFRP4) and, vice versa, administration of sFRP4 in the same model suppressed the progression of kidney fibrosis[70]. Delivery of DKK1, another WNT antagonist, also substantially reduced the expression of WNT–β-catenin-dependent genes and matrix accumulation after UUO[71]. Similar results demonstrating the activation of WNT–β-catenin signalling have been obtained in other kidney injury models including IRI, angiotensin or adriamycin infusion, diabetic kidney disease, as well as during age-related kidney dysfunction[26,72,73]. Moreover, a trial has shown that heart failure induced by transverse aortic constriction promotes upregulation of WNT ligands and these circulating WNT ligands in turn promote proteinuria and kidney fibrosis[74].

WNT3A activates kidney macrophages and promotes their polarization towards an M2 phenotype, thus leading to transforming growth factor β1 (TGFβ1)-dependent extracellular matrix production[48]. These processes can be further amplified by activation of PRR, which potentiates β-catenin activation, aggravates kidney dysfunction and worsens renal tissue inflammation and fibrosis[26]. In a vicious circle, TGFβ itself can promote non-canonical WNT signalling to drive kidney fibrosis in vivo[75] and, in proximal tubular cells, TGFβ together with β-catenin exacerbates tubular injury in mouse models of CKD[76,77].

Several factors have been identified that protect the kidney from fibrosis by inhibiting the WNT pathway. In mice constitutively overexpressing Klotho together with nuclear β-galactosidase under control of the β-catenin-activated transgene promotor, tubulointerstitial fibrosis was reduced and WNT signalling was markedly lower than in wild-type mice[78]. Vice versa, loss of Klotho promoted kidney disease in various animal models by derepression of WNT–β-catenin signalling (that is, activation of WNT–β-catenin signalling by releasing it from a blocked state)[79]. Peroxisome proliferator-activated receptor-α interacts with LRP6 to inhibit WNT signalling, resulting in reduced oxidative stress[80]. Similar effects have been attributed to pigment epithelium-derived factor and the inhibition of porcupine-mediated WNT-*O*-acylation, both of which reduce kidney fibrosis by inhibiting WNT–β-catenin signalling[81,82]. In murine UUO, administration of ICG-001, a small molecule specifically disrupting β-catenin-mediated gene transcription, significantly reduced fibrosis[83].

These findings suggest that WNT–β-catenin signalling has a crucial role in kidney fibrosis. Therefore, the WNT–β-catenin pathway and its related components represent promising therapeutic targets to attenuate kidney fibrosis and to potentially halt CKD progression.

CKD-MBD and vascular calcification. CKD-MBD represents a common complication in patients with CKD and is characterized by abnormal bone turnover, mineralization and architecture, as well as CKD-associated cardiovascular disease including vascular stiffness and calcification[84]. WNT–β-catenin signalling modulates the function of osteoblasts, osteocytes and osteoclasts. Several WNT proteins, such as WNT1, WNT3A and WNT10B, induce differentiation of osteoblasts[85], resulting in increased bone mass[86] and suppressed bone resorption[87,88]. Vice versa, β-catenin deficiency in osteocytes leads to an increased number of osteoclasts and reduced bone mass[89].

In the bone, osteocytes represent the major source of the WNT inhibitors DKK1, secreted frizzled-related proteins (sFRPs) and sclerostin, which orchestrate the interplay between osteoblasts, osteoclasts and surrounding osteocytes to maintain bone homeostasis[90,91]. Sclerostin directly binds to LRP5 and LRP6 and thereby prevents activation of the WNT–β-catenin pathway[92]. Sclerostin-deficient mice are characterized by increased bone mass driven by increased WNT–β-catenin activation[93,94]. Moreover, sclerostin directly activates bone resorption by increasing the ratio of receptor activator of NF-κB ligand (RANKL) and osteoprotegerin[87,95]. DKK1 is expressed in osteocytes and osteoblasts alike[96]. It interacts with LRP5 and LRP6 to inhibit WNT–β-catenin-dependent signalling pathways, leading to reduced bone mass and bone formation[97]. The effects of the sFRPs on bone metabolism are similar to those of sclerostin and DKK1 (REF.[98]). The relevance of the WNT inhibitors sclerostin and DKK1 in CKD-MBD has been

underscored in small animal experiments, in which both proteins were antagonized by inhibiting antibodies resulting in increased bone volume and bone formation in animal models of fracture healing[99–104]. In diabetic mice subjected to mild kidney injury, neutralization of DKK1 stimulated bone formation and corrected osteodystrophy[104]. In the plasma as well as in the vascular tree of animals in various models of CKD, expression of the WNT inhibitors sclerostin, DKK1, sFRP1 and sFRP4 increases as kidney function deteriorates, which is linked to decreasing bone mass[25,104–106].

CKD is commonly associated with the development of secondary hyperparathyroidism characterized by elevated circulating parathyroid hormone (PTH) plasma levels. PTH drives bone resorption, resulting in reduced bone mass[107]. PTH directly interacts with LRP6 on the surface of osteoblasts, which induces β-catenin stabilization in the absence of a WNT ligand[108]. In healthy individuals, PTH thereby suppresses the production of sclerostin in osteocytes[109] and reduces DKK1 levels[110,111]. However, in patients with CKD, sclerostin levels are often elevated, despite hyperparathyroidism. Hyperphosphataemia increases the expression of WNT-inhibitory proteins, although the underlying mechanisms are only poorly understood[112]. Thus, although PTH is a well-characterized suppressor of sclerostin in health, CKD is proposed to lead to 'PTH resistance' in the regulation of sclerostin, resulting in both high PTH and high sclerostin levels[106].

Calcification of the vascular intimal and medial layers but also of heart valves represents a common feature of CKD-associated (cardio)vascular disease[84]. Owing to the similarities between calcification and osteogenesis, WNT–β-catenin signalling might plausibly have a crucial role in vascular calcification as well. In a clinical study of patients receiving dialysis, circulating levels of the WNT inhibitor sclerostin correlated with the extent of aortic and coronary artery calcification[113,114]. In line with this finding, the expression of WNT5A, WNT5B and WNT11 is higher in areas of calcified foci of aortic valves than in uncalcified valve tissue[115]. In vitro, WNT5A and WNT11 trigger calcification of human aortic valve interstitial cells[115]. Similarly, in vascular smooth muscle cells, high phosphate treatment activates the important calcification-promoting transcription factor RUNX2, β-catenin expression and, thereby, calcium deposition[116]. These findings indicate that CKD-associated hyperphosphataemia drives vascular calcification by altering WNT–β-catenin-dependent signalling pathways such as the production of MMP2

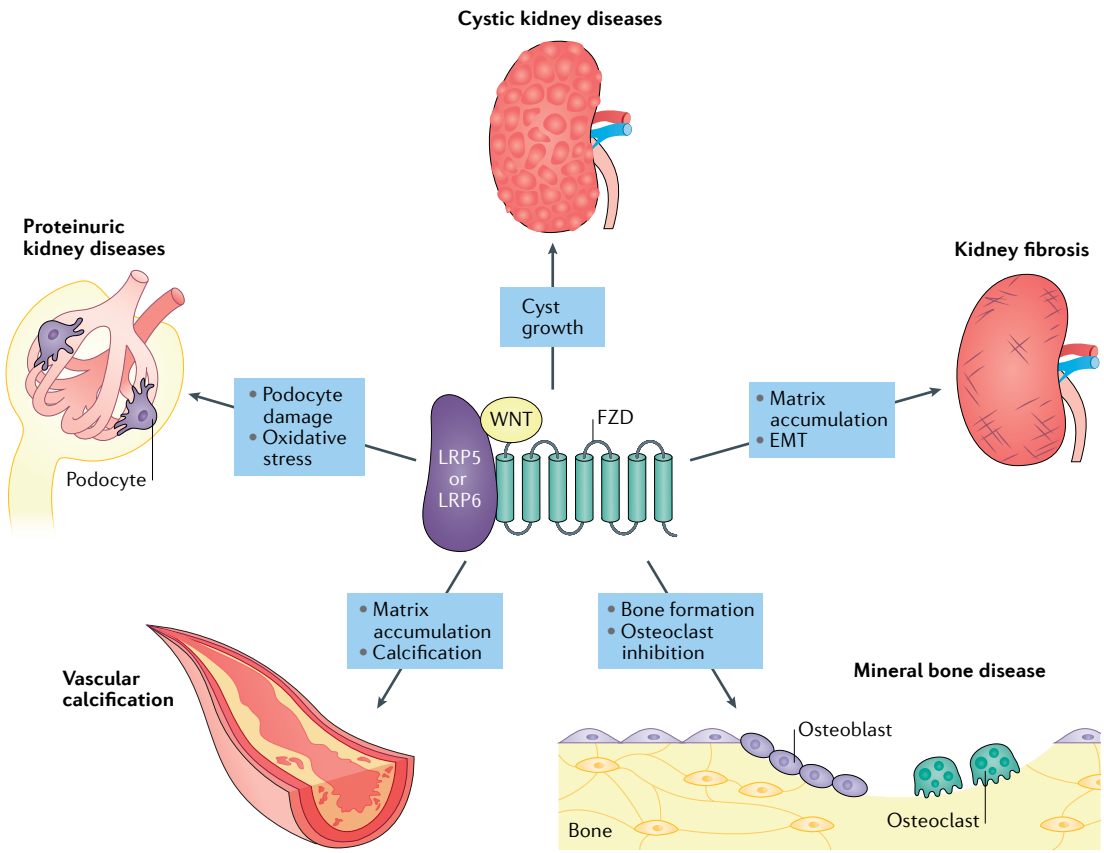

Fig. 4 | **Role of WNT–β-catenin signalling in kidney diseases.** WNT–β-catenin signalling promotes epithelial–mesenchymal transition (EMT) and matrix accumulation, leading to kidney fibrosis. Moreover, it drives cyst growth, promoting cystic kidney diseases. Podocyte damage and oxidative stress mediated by WNT–β-catenin signalling promote proteinuric kidney diseases. Finally, WNT–β-catenin signalling has an important role in chronic kidney disease-associated vascular calcification and mineral bone disease.

and MMP9 (REF.[117]). MSX2 is another important driver of CKD-associated vascular calcification that acts by increasing osteogenic differentiation of vascular smooth muscle cells. MSX2 activates WNT–β-catenin signalling by inducing the expression of WNT3A and WNT7A and by suppressing DKK1 (REF.[118]). Moreover, the pro-inflammatory cytokine TNF induces the expression of MSX2, WNT3A and WNT7A, thus linking WNT signalling and inflammation[119]. In addition, several uraemic toxins that accumulate in patients with CKD owing to reduced renal clearance, such as indoxyl sulfate, promote calcification by increasing the expression of WNT proteins (for example, WNT7B)[120].

Versatile role of WNT–β-catenin

On the basis of the findings described above, WNT–β-catenin signalling has a dual role in kidney diseases. Although short-term activation of the WNT–β-catenin pathway represents a prerequisite for regeneration and repair after kidney injury, sustained (uncontrolled) activation of this pathway leads to (irreversible) kidney fibrosis.

The expression of a variety of WNT ligands is upregulated after AKI[33,51,52]. In the kidney IRI mouse model, the mRNA expression of WNT ligands has been shown to depend on the duration of ischaemia[52]. A shorter period of renal artery clamping was associated with higher mRNA levels of WNT ligands, as well as higher WNT–β-catenin protein expression, which regressed 10 days after injury. By contrast, a longer period of renal ischaemia led to persistently high WNT ligand and β-catenin expression, which is associated with the progression of AKI into CKD (a process termed AKI–CKD transition)[52]. Short-term WNT–β-catenin activation has been shown to be 'tubulo-protective': it prevents tubular epithelial cell apoptosis and promotes proliferation[33,34,51]. Accordingly, tubular epithelial cell-specific β-catenin deletion is associated with substantially more pronounced damage after AKI[33].

Sustained activation of the WNT–β-catenin system, however, might have a pivotal role in the development of kidney fibrosis. As outlined above, activation of canonical WNT–β-catenin signalling represents a common feature of many different animal models of CKD or kidney fibrosis, independent of the type of injury. This observation suggests that the type and intensity of injury, its spatiotemporal pattern as well as its cellular interactome represent critical determinants differentiating between WNT–β-catenin-driven repair or regeneration and maladaptive remodelling after injury. Although different types of injury induce a similar WNT–β-catenin response, the exact molecular signals inducing the WNT ligand expression in the injured kidney are mostly unknown so far. Identification of these WNT–β-catenin-activating mediators could help in the identification of specific therapeutic approaches to modulating WNT–β-catenin signalling.

WNT–β-catenin target genes in kidney injury

To date, several hundreds of TCF–LEF target genes have been described. A variety of these target proteins have been documented to have a particular role in kidney pathology, and we discuss only the most relevant ones below (TABLE 1).

SNAIL. SNAIL belongs to the zinc finger 1 transcription factor family, which is involved in epithelial-to-mesenchymal transition[121–123]. SNAIL expression in the kidney is induced in a variety of injury models, including UUO, 5/6-nephrectomy (subtotal nephrectomy), oxygen deprivation, chronic hyperglycaemia and cadmium exposition[124]. At a cellular level, SNAIL impacts fatty acid metabolism, cell cycle control and inflammation[124]. SNAIL is directly linked to WNT–β-catenin signalling. GSK3β (a key enzyme in WNT–β-catenin signalling) has been shown to mediate phosphorylation of SNAIL to induce its proteolysis[125,126]. Vice versa, initiation of WNT–β-catenin signalling leads to inactivation of GSK3β and thereby to increased SNAIL expression in podocytes of nephrotic rats[127]. Interestingly, downregulation of the WNT signalling amplifier PRR also reduces SNAIL expression in high-glucose-treated podocytes[128]. Moreover, SNAIL might also be directly involved in WNT–β-catenin signalling as a positive feedback loop by synergizing with WNT ligands to induce nuclear translocation of β-catenin[129].

Matrix metalloproteinase 7. MMP7 is an endopeptidase that is involved in epithelial-to-mesenchymal transition by degrading collagen type IV, laminin and E-cadherin[130,131]. Moreover, it promotes apoptosis of renal fibroblasts[29,132]. Owing to its substrate collagen type IV, a component of the glomerular basal membrane, MMP7 is also involved in glomerular damage[133]. Evidence suggests that MMP7 triggers podocyte dysfunction upon tubular injury[134]. Tubular cell-derived MMP7 has been documented to induce glomerular nephrin depletion, impaired glomerular permeability and proteinuria. Accordingly, MMP7-deficient mice are protected from angiotensin II-induced proteinuria and glomerular damage[134]. Urinary MMP7 concentrations correlate with renal WNT–β-catenin activity in patients with various types of kidney disease[135] and might aid in the identification of patients with progression of IgA nephropathy, which is characterized by the presence of MMP7 in kidney biopsy samples[130,136].

By contrast, MMP7 also exerts protective effects on the kidney as an adaptive response during AKI. In several AKI animal models (IRI, cisplatin administration and folic-acid induced AKI), kidney damage was much more pronounced in MMP7-deficient mice[137]. In patients undergoing cardiac surgery, urinary MMP7 levels are associated with more severe AKI and poorer outcomes[138].

TRPC6. TRPC6 (short transient receptor potential channel 6), another transcriptional target of the WNT–β-catenin signalling cascade, is involved in the pathogenesis of a variety of kidney diseases. Mutations in the *TRPC6* gene locus cause familial forms of focal segmental glomerulosclerosis[139]. TRPC6-mediated influx of calcium into podocytes induces cytoskeletal destabilization, mitochondrial dysfunction and apoptosis[140]. In diabetic kidney disease, TRPC6 deficiency inhibits podocyte apoptosis, preserves podocin expression, and

ameliorates albuminuria and histological damage in animal models[139].

Renin–angiotensin system. The renin–angiotensin system (RAS) is tightly connected to the WNT–β-catenin system. All RAS genes contain binding sites for TCF–LEF in their promoter regions[141]. Moreover, components of the RAS system, such as angiotensin II, represent activators of the WNT–β-catenin system[60]. Thus, the WNT–β-catenin–RAS axis can be considered a vicious circle in promoting kidney damage. Indeed, pharmacological inhibition of β-catenin by the small-molecule inhibitor ICG-001 suppresses WNT–β-catenin-dependent signalling in parallel with blocking activation of the RAS system to reduce the activation of myofibroblasts, extracellular matrix accumulation, renal inflammation and, thereby, kidney fibrosis[141,142].

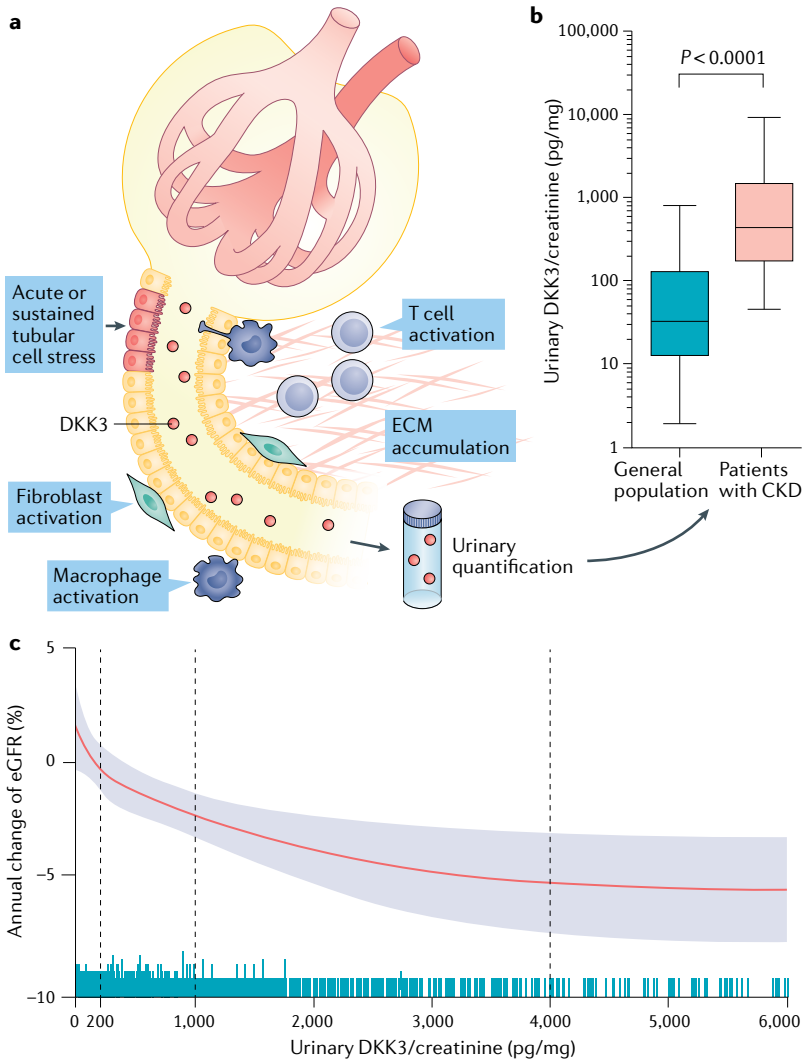

Fig. 5 | **Role of DKK3 as a tubular epithelial stress sensor. a** | Acute or sustained stress acting upon tubular epithelial cells promotes secretion of Dickkopf 3 (DKK3). DKK3 induces activation of T cells, macrophages and fibroblasts, leading to extracellular matrix (ECM) accumulation, finally driving kidney fibrosis. Secreted DKK3 can be detected in the urine and quantified by enzyme-linked immunosorbent assay. **b** | Urinary DKK3 levels in patients with chronic kidney disease (CKD) are substantially higher than in individuals from the general population. **c** | Urinary DKK3 levels are associated with short-term risk of CKD progression. The graph shows the association between urinary DKK3 levels and the annual change of estimated glomerular filtration rate (eGFR) ($N = 2,035$). Parts **b** and **c** adapted with permission from REF.[163], American Society of Nephrology.

Plasminogen activator inhibitor 1. Plasminogen activator inhibitor 1 (PAI1) has an important role in the haemostatic system. It acts as an inhibitor of urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA), and thereby possesses procoagulant properties[57]. A growing body of evidence suggests a role of PAI1 in kidney fibrosis[57]. In the UUO mouse model, PAI1 deficiency diminishes renal fibrosis and attenuates perirenal fat inflammation and nephropathy in obese mice on a high-fat diet[143,144]. Moreover, administration of a PAI1-inhibiting antibody retarded progression of diabetic kidney disease in mice[144,145], and a PAI1 inhibitor ameliorated podocyte damage and proteinuria[146].

DKK family of WNT signalling modulators

DKK proteins represent a family of four members (DKK1, DKK2, DKK3 and DKK4) that have emerged as modulators of WNT–β-catenin signalling. The term 'dickkopf' refers to the German word for big head or bullhead, as DKK proteins have been demonstrated to have an important role in embryogenesis. DKK1 overexpression in Xenopus induces abnormalities of cranial development[147]. DKK1, DKK2 and DKK4 directly bind to LRP6 and serve as WNT antagonists[23] (FIG. 1). Moreover, via interaction with the Kremen receptors, DKK1 and DKK2 induce endocytosis of LRP6, thereby suppressing WNT–β-catenin-dependent signalling[148]. Although the role of DKK1, DKK2 and DKK4 as antagonists of WNT–β-catenin signalling is well-established, the precise action of DKK3 on WNT signalling remains poorly understood. DKK3 differs from DKK1, DKK2 and DKK4 in several ways: the molecular mass of DKK3 (38 kDa) is higher than other members of the DKK family (DKK1 and DKK2, 28 kDa; DKK4, 24 kDa) and it shares lower DNA sequence similarity with DKK1, DKK2 and DKK4 (which are similar to each other)[147]. Moreover, *DKK1*, *DKK2* and *DKK4* genes are located within a chromosome 4, 5, 8 and 10 paralogy group, whereas *DKK3* is not[149,150]. These findings suggest that functional differences might exist between DKK3 and the other DKK family members. Indeed, DKK3 does not interact with Kremen to induce internalization of LRP6 (REFS[151,152]). Although some reports indicate that DKK3 antagonizes canonical WNT–β-catenin signalling[153], other reports suggest that DKK3 activates non-canonical WNT–β-catenin signalling[154]. Moreover, besides the WNT–β-catenin system, DKK3 binds to other receptors such as CXC-chemokine receptor 7 (CXCR7) to promote vascular regeneration[155].

DKK1. In mice, systemic delivery of DKK1 reduces renal pericyte activation, their transition to myofibroblasts and, subsequently, fibrogenesis, capillary rarefication and inflammation in the UUO model[156]. Moreover, administration of DKK1 has been shown to counteract kidney fibrosis induced by connective tissue growth factor[157]. By contrast, in hyperglycaemia-induced kidney injury, DKK1 promotes mesangial matrix accumulation and proteinuria[158]. Therefore, the role of DKK1 in kidney injury presently remains uncertain.

DKK3. DKK3 is not expressed in healthy human kidneys. However, several types of kidney injury induce de novo renal DKK3 expression[153]. In patients with ADPKD, SNPs within the *DKK3* gene locus are associated with a decline in glomerular filtration rate (GFR), pointing towards a potential role of DKK3 in the clinical course of ADPKD[159]. Moreover, DKK3 induces tubular cell death in proteinuric kidney disease[160] and alters the renal endothelial secretome, thus triggering fibroblast activation and epithelial-to-mesenchymal transition[161].

We have identified DKK3 as a major driver of kidney fibrosis in a variety of animal models including UUO and adenine-induced nephropathy[153,162]. DKK3 deficiency significantly ameliorated tubular damage and kidney fibrosis. In these models, we identified renal tubular cells as the major source of DKK3. DKK3 deficiency provoked an antifibrogenic T-cell response in the kidneys, which was paralleled by diminished canonical WNT–β-catenin signalling. DKK3 is secreted into the urine in response to acute or sustained stress acting upon the kidney[153]. Although DKK3 was not detectable in the urine of animals or humans without apparent or occult kidney disease, DKK3 urine levels were substantially increased in different types of kidney injuries[153,162], rendering DKK3 a potential biomarker for ongoing renal stress (FIG. 5). Indeed, we found that urinary DKK3 levels were associated with the degree of tubulointerstitial fibrosis in a cohort of patients undergoing kidney biopsy[163]. Notably, in patients with CKD, increased urinary DKK3 levels were associated with an increased risk of short-term CKD progression (that is, within 1 year). Moreover, in patients with IgA nephropathy included in the randomized, controlled STOP IgAN trial[164], high urinary DKK3 levels were associated with a faster GFR decline within 6 months[163].

A trial from our group has established urinary DKK3 as a preoperative biomarker to identify patients at risk of AKI after cardiac surgery and subsequent loss of kidney function[162,165]. Increased urinary DKK3 levels before surgery were not only associated with an increased risk of postoperative AKI but also with reduced kidney function at discharge and after long-term (that is, 820 days) follow-up. This finding identifies urinary DKK3 as a predictor of AKI and its transition into CKD. These findings were confirmed in the randomized, placebo-controlled RenalRIP trial[166], in which patients before cardiac surgery underwent either remote ischaemic conditioning or sham procedure. Remote ischaemic conditioning was only associated with a reduced risk of postoperative AKI and persistent kidney dysfunction in those patients with preoperatively elevated urinary DKK3 levels. Therefore, urinary DKK3 might aid in the identification of patients in whom therapeutic strategies to prevent AKI might be particularly beneficial[165]. Studies to evaluate the efficacy of DKK3-guided therapies in patients with chronic or acute kidney injury are currently ongoing.

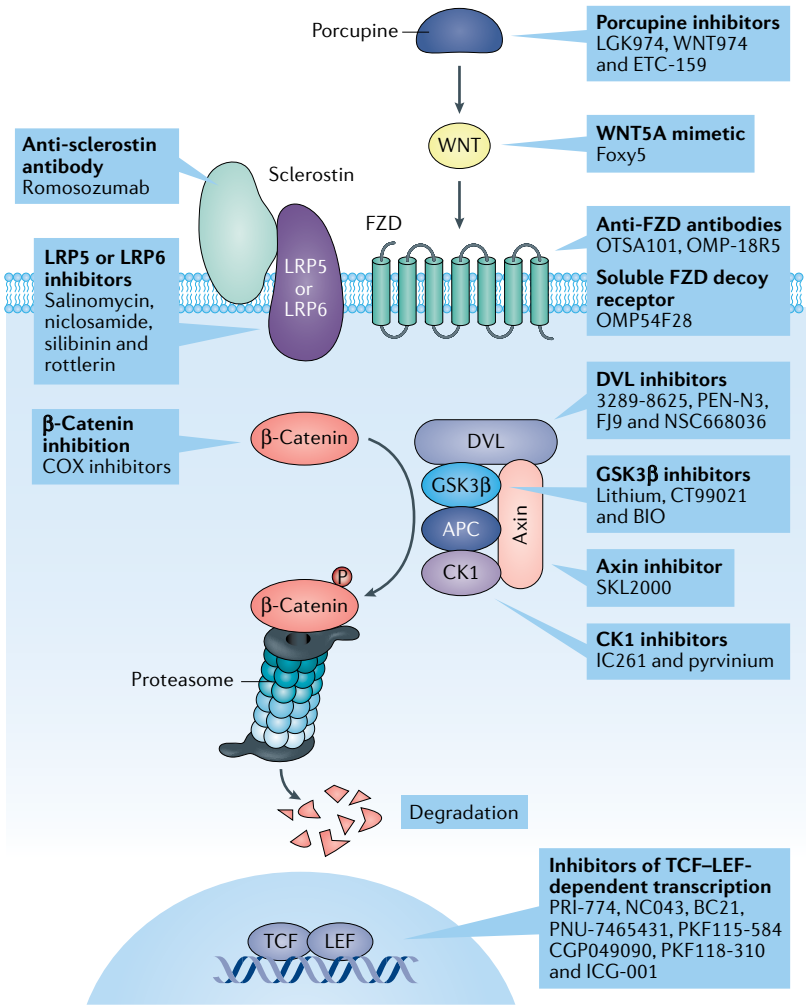

Fig. 6 | **Potential therapeutic targets in the WNT–β-catenin signalling pathway.** The WNT–β-catenin signalling pathway can be therapeutically targeted at several steps. Porcupine inhibitors prevent the release of WNT ligands. The anti-sclerostin antibody, romosozumab, the only agent available for therapeutic use in clinical practice, prevents the WNT–β-catenin-inhibiting effect of sclerostin on the bone. LRP5 and LRP6 inhibitors and anti-FZD antibodies prevent the receptor actions of WNT ligands. Inhibitors of DVL, GSK3β, axin and CK1 interfere with WNT–β-catenin downstream signalling. By contrast, Foxy5 is a WNT5A mimetic that promotes WNT–β-catenin signalling. Cyclooxygenase inhibitors (COX) serve as β-catenin inhibitors. Finally, inhibitors of TCF–LEF prevent WNT–β-catenin-dependent gene transcription.

WNT–β-catenin as a therapeutic target

Therapeutically targeting the WNT–β-catenin system is emerging as a treatment strategy in patients with acute and chronic kidney injury and associated extra-renal complications. Several small molecules modulating WNT–β-catenin signalling at different steps of the pathway are in development, as summarized in FIG. 6. To date, clinical studies focusing on WNT–β-catenin-targeted therapies are mainly confined to cancer[2]. Owing to the diverse actions of the WNT–β-catenin pathway, molecules targeting specific components of this signalling cascade might be more promising than pan-WNT signalling inhibitors in order to limit potential adverse effects. Currently, only inhibition of sclerostin by the humanized monoclonal anti-sclerostin antibody romosozumab has entered clinical practice. A phase II clinical trial demonstrated increased mineral density and bone formation, and reduced bone resorption, in postmenopausal women with low bone-mineral density treated with romosozumab[167]. A large clinical trial documented the beneficial effects of romosozumab compared with denosumab (a RANKL inhibitor) for reducing the risk of vertebral

fractures in postmenopausal women with osteoporosis[168]. Moreover, in postmenopausal women on bisphosphonate treatment, romosozumab significantly increased hip bone-mineral density compared with teriparatide (a form of PTH)[169] or bisphosphonates alone[170].

Besides the WNT–β-catenin pathway, Notch and Hedgehog (HH) represent two other developmental signalling pathways that are activated in kidney injury (reviewed elsewhere in detail[3]). The HH pathway is thought to act upstream of WNT–β-catenin signalling, and WNT–β-catenin and Notch signalling might synergize in a positive feedback loop[171]. Improved understanding of the interaction of these pathways could help in the identification of key molecules that regulate their interaction as potential therapeutic targets.

Conclusions

WNT–β-catenin signalling is an important regulator of embryogenesis and physiological growth. Upon kidney injury, WNT–β-catenin can be re-expressed and contribute to abnormal tissue responses. Transient activation promotes repair and regeneration after AKI. If sustained, however, it contributes to kidney fibrosis, podocyte damage, cystic kidney diseases and CKD-associated complications such as vascular calcification and CKD-MBD. Although a clear role of WNT–β-catenin in kidney injury has been established, the precise signalling events leading to activation of the WNT–β-catenin pathway in kidney diseases remain largely elusive. Cellular effects of WNT–β-catenin signalling are highly diverse across different cell types and tissues. Therefore, cell-specific or tissue-specific WNT–β-catenin-targeting approaches are desirable for a successful WNT–β-catenin-modulating therapy. Inhibition of the WNT–β-catenin inhibitor sclerostin via romosozumab increases bone formation and bone mass without adverse effects on other WNT–β-catenin-dependent processes. Targeting abnormal WNT–β-catenin activation in kidney diseases has the potential to provide a novel and promising treatment approach, which requires the identification of overarching patterns of WNT–β-catenin-dependent effects and their activating upstream events.

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