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Wnt6 - another player in the Yin and Yang of renal Wnt signalling

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Running title: Role of Wnt6 in diabetic renal fibrosis

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Diabetic nephropathy (DN) remains the single most common cause of end-stage kidney disease, necessitating dialysis or transplantation, in the Western world. Hence, novel therapies beyond tight blood pressure and glycaemic control are required to slow or reverse progression of nephropathy in patients with diabetes. Whilst significant efforts have been made to understand the molecular basis of DN, further delineation of the final common pathway of renal fibrosis, where the functioning nephrons are replaced by scar tissue, may identify novel therapeutic targets.

The WNT pathway is a highly conserved signalling pathway that is essential during development in several organs including the kidney. There are 19 mammalian Wnt ligands and these are spatially regulated during development. During nephrogenesis the secreted Wnt ligands, Wnt9b and Wnt4 are indispensable and stimulate mesenchymal cells to differentiate into epithelial cells that subsequently generate the nephron (9). WNT signalling is carefully regulated by endogenous suppressors of WNT signalling such as Dickkopf-1 (Dkk1) and Axin. Crosstalk between renal stromal cells and the nephron epithelia are required to regulate nephron elongation and differentiation including suppression of Wnt signalling by DKK-1 to allow branching morphogenesis to occur (7)

The classical model of Wnt signalling is that Wnt ligands interact with heterodimeric receptor complexes consisting of a Frizzled (Fz) receptor and low-density lipoprotein-related receptor 5 or 6 (LRP5/6). Recruitment of axin promotes phosphorylation of the cytoplasmic tail of the LRP5/6 receptor, which ultimately leads to cessation of B-catenin phosphorylation followed by its translocation to the nucleus where it binds and activates TCF/LEF family transcription factors to induce target genes (2).

In the normal kidney the Wnt pathway is active in cells in the papilla, however after injury Wnt pathways become activated throughout the kidney. This activation of Wnt signalling can be protective or deleterious depending on the cell type. The Wnt pathway has been implicated in human diabetic renal disease by high throughput transcriptomic analysis and in preclinical models of diabetic nephropathy and renal injury. Within injured podocytes there are increased levels of Wnt1, Wnt2b, Wnt4, Wnt6 and Wnt16 (3). In contrast in mesangial cells, high glucose culture down regulated Wnt4 and Wnt5a expression and induced apoptosis which was also observed in diabetic rats (4).

In this issue, Beaton et al (1) have provided functional insight regarding the role in diabetic nephropathy of the hitherto poorly characterised Wnt6. As expected Wnt/β-catenin signalling was increased in the diabetic kidney, however Wnt6 expression was decreased in the tubulointerstitium of patients with DN. Using preclinical models of DN and renal fibrosis they found a progressive reduction in Wnt6 expression. They demonstrated for the first time that during development Wnt6 expression was detectable in the mesonephric duct and urogenital membrane at E9.5. Wnt6 co-localised with Frizzled 7 (FzD7) expression and coincided with canonical Wnt signalling in a TCF/Lef reporter mouse. Therefore they suggest that FzD7 is a putative receptor of Wnt6, for which they provide further evidence by demonstrating that siRNA knockdown of FzD7 blocked phosphorylation of GSK3β by Wnt6 in renal tubular cells. This led to their hypothesis that Wnt6 may play a role in epithelial cell fate. Transfection of renal tubular cells grown in 3D culture with Wnt6 led to new tube-like protrusions indicating that Wnt6 can drive *de novo* tubulogenesis. In addition, transfection of renal epithelial cells with Wnt6 prior to or after TGFβ stimulation prevented epithelial to mesenchymal trans-differentation by inhibiting expression of vimentin although this had no effect on the loss of E-cadherin. Analysis of the promoter revealed that vimentin has a NF-

Kβ binding site so the authors explored if non-canonical TGFβ signalling through NF-Kβ was involved in the regulation of vimentin. Using TGFβ stimulation of p65 -/- and IKK-/- fibroblasts they observed that vimentin expression was undetectable compared to wild-type fibroblasts. This interesting study reveals differential expression patterns of the Wnt ligands following injury. Loss of Wnt6 is permissive for loss of epithelial integrity and function, while restoration of Wnt6 may increase repair of the tubular cell population by inducing tubulogenesis.

How do the current findings compare with previous studies examining other Wnt ligands? During the repair phase following ischemia reperfusion (I/R) injury Wnt2, Wnt2b, Wnt4, Wnt7b and Wnt10a expression is upregulated (5). Consistent with this, genetic ablation of β-catenin in the renal epithelia has been found to aggravate acute kidney injury (10). Macrophages may be a major source of Wnt ligands during the repair phase following I/R injury, with macrophage-derived Wnt7b ligand binding to FzD4:LRP5/6 on tubular epithelial cells being critical for the repair phase (5). Wnt7b signalling crosstalk between macrophages and tubular cells promotes tubular membrane repair and drives epithelial cells through the G2 arrest as they repopulate the tubules (5). Thus Wnt signalling is critical for kidney repair following acute kidney injury and inhibition of signalling may be deleterious in this context.

Myofibroblasts exhibit increased Wnt/β-catenin signalling following kidney injury. Blockade of Wnt signalling through systemic administration of DKK-1 inhibits myofibroblast expansion and renal fibrosis (8). Recent studies by the Humphreys' group have revealed that paracrine Wnt signalling by the Wnt1 ligand is sufficient to drive fibrosis in the absence of inflammation (6). Induction of Wnt1 expression specifically in cortical proximal tubular cells in a transgenic mouse resulted in renal fibrosis by 12 weeks. Although the fibrosis observed

was mild there was a significant increase in the number of platelet-derived growth factor-β^+ and α-smooth muscle actin$^+$ proliferating myofibroblasts in the interstitium. Interestingly, no epithelial cell injury was noted, nor was there evidence of an inflammatory cell infiltrate. There was, however, a small but significant increase in TGFβ and Smad3 expression in the kidneys which indicates cooperative and potentially synergistic convergence of the Wnt and TGFβ signalling pathways.

These studies demonstrate that there are cell-specific responses to Wnt signalling with activation being either protective or detrimental to the injured kidney depending on the context (Figure). While targeting the Wnt signalling pathway represents an attractive novel anti-fibrotic strategy, further studies will be required to further define the role of specific Wnt ligands and their receptors to ensure successful translation to the clinic.

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Figure legend

Figure 1: Dual role of Wnt signalling in kidney injury and repair.

a) High glucose results in a decrease in Wnt6, which facilitates increased expression of vimentin , a marker of tubular de-differentation. **b**) Macrophage derived Wnt7b induces basement membrane repair and tubular epithelial repopulation during the repair phase following ischaemia-reperfusion (I/R) injury. **c**) Over-expression of Wnt1 in cortical epithelial cells is sufficient to drive myofibroblast activation and proliferation in the absence of inflammation.

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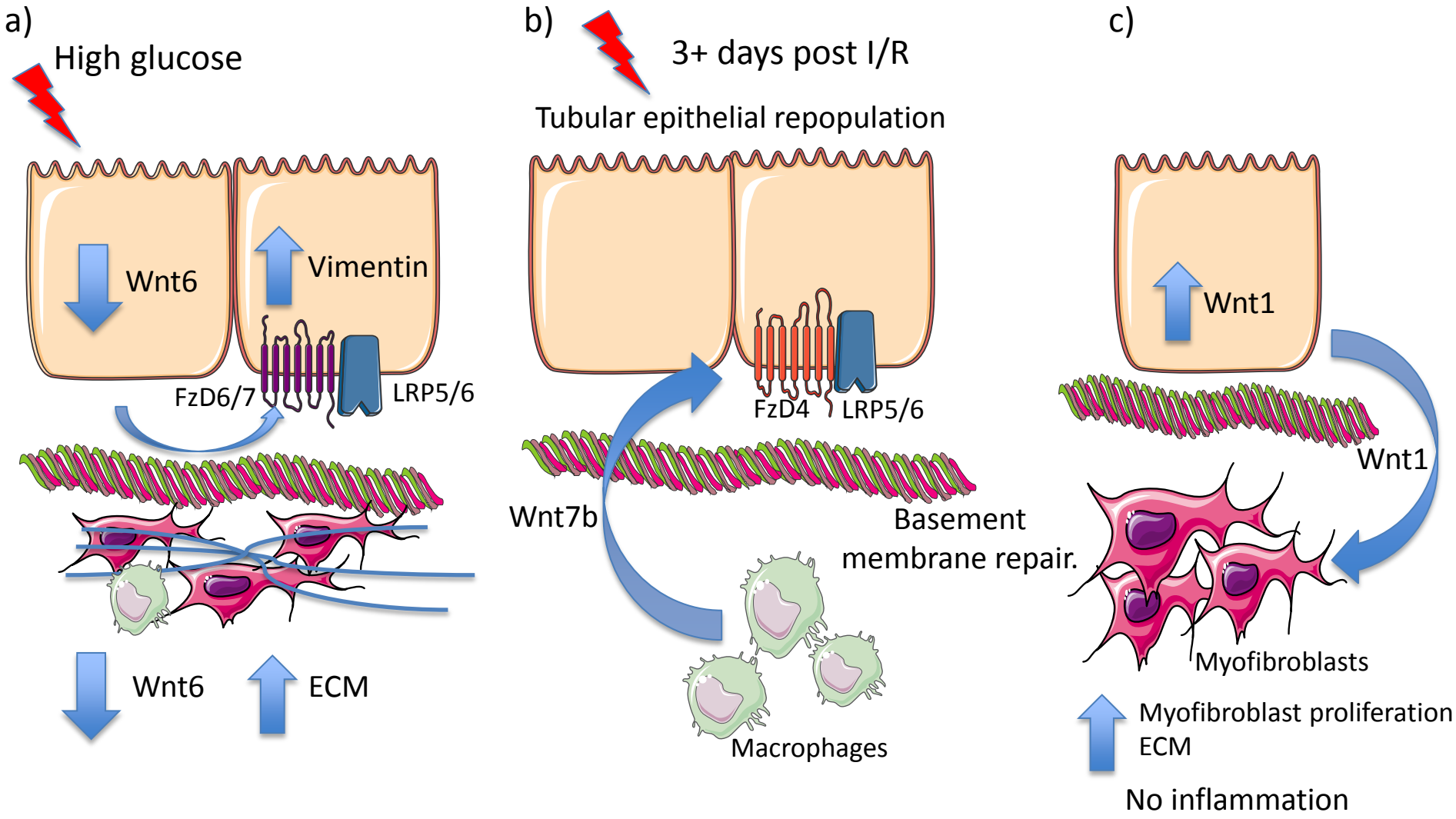
a)
High glucose
Wnt6
Vimentin
FzD6/7
LRP5/6
Wnt6
ECM
b)
3+ days post I/R
Tubular epithelial repopulation
FzD4
LRP5/6
Wnt7b
Basement
membrane repair.
Macrophages
c)
Wnt1
Wnt1
Myofibroblasts
Myofibroblast proliferation
ECM
No inflammation