

Getting a Notch Closer to Understanding Diabetic Kidney Disease

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D diabetic kidney disease (DKD) is one of the most devastating complications of diabetes. Renal dysfunction develops in about one-third of patients with diabetes (1). Diabetic nephropathy is characterized by albuminuria, glomerulosclerosis, and progressive loss of renal function. Current therapies for DKD, including blood glucose control, angiotensin II receptors blockers, and ACE inhibitors, slow down, but do not halt, the progression to end-stage renal disease after overt nephropathy has been established (2). Recent studies indicate that podocyte injury and depletion play key roles in the pathogenesis of DKD (3,4). Clinical observational studies showed a strong correlation between podocyte density, albuminuria and renal function decline in patients with type 1 and type 2 diabetes (5).

Notch signaling regulates many aspects of metazoan development and tissue renewal (6). Notch is a transmembrane protein that interacts with ligands of the Jagged and Delta family (7). In mammals, there are four Notch receptors (Notch 1–4), two Jaggeds, and three delta-like ligands (8). Each of these proteins show a cell type- and tissue-specific expression. Notch is made in the endoplasmic reticulum as pre-Notch. A furin-like convertase cleaves pre-Notch to intracellular and extracellular domain. The protein is then transported to the plasma membrane. Interaction of the ligand with the Notch receptor triggers a series of proteolytic cleavage, by ADAM, a disintegrin and metalloprotease and by the γ -secretase complex. The final cleavage releases the Notch intracellular domain (NICD), which then moves to the nucleus, where it can regulate gene expressions by binding to the transcription factor CSL. The Notch pathway in the kidney is indispensable for glomerular and proximal tubule development (9). Once the development process is complete, very little Notch activity can be observed in rodent or human kidneys. Using unbiased microarray technologies, several recent studies described the expression of Notch pathway proteins in kidneys of patients with DKD (10,11). Mechanistic studies performed in mouse models and in cultured cells showed that activation of the Notch signaling in podocytes plays a key functional role in the pathogenesis of podocyte injury (11,12). Expression of Notch1 in podocytes caused the development of albuminuria and glomer-

ulosclerosis, whereas genetic deletion or pharmacological inhibition ameliorated DKD in a mouse model of diabetes. In podocytes, Notch signaling interacts with the transforming growth factor (TGF)- β pathway (6,7). This interaction seems to form a positive feed-back loop: TGF- β transcriptionally upregulates notch ligand Jagged1 expression (Fig. 1). On the other hand, Notch activation also increases TGF- β expression. Given the potent profibrotic activity of TGF- β in glomerular disease, this suggests that Notch is an important “master regulator” of glomerulosclerosis (11,13).

Vascular endothelial growth factor (VEGF) plays a key role in vasculogenesis and angiogenesis by stimulating endothelial cell mitosis and migration. Evidence is emerging that VEGF plays a critical role in maintaining renal homeostasis. Altered (increased or decreased) expression of VEGF leads to glomerular dysfunction and proteinuria. In cultured podocytes, VEGF induces the pro-survival phosphoinositol 3-kinase/Akt and suppresses the proapoptotic p38 mitogen-activated protein kinase pathway (14,15). In diabetic kidneys, both decreased and increased expression of VEGF has been reported (16–20). The reason for such discrepancy is not clear, and there might be a stage-specific regulation, i.e., an early increase followed by a decreased expression later on.

In the current issue of *Diabetes*, Lin et al. (21) report that the Notch and VEGF pathways interact in diabetic podocytes and drive the development of DKD. They found that hyperglycemia led to the activation of Notch signaling in cultured human podocytes, in HEK293 cells, and in kidneys from a rat model of diabetes (Fig. 1). *Notch1* activation induced VEGF expression and subsequently caused a decrease in nephrin expression and induced podocyte apoptosis (Fig. 1). Lin et al. also treated diabetic rats with a γ -secretase inhibitor (DAPT), which led to a decrease in albuminuria in a dose-dependent manner. In addition, DAPT treatment also normalized VEGF and nephrin expression in vivo. This is an important translational finding suggesting that blocking Notch activation could provide a new therapeutic strategy for the cure of diabetic nephropathy. Moreover, because hyperglycemia was able to regulate Notch expression in different cell types (including podocytes and HEK293 cells), it also raises the possibility that Notch activation could be a common mechanism leading to the development of diabetes complications.

Interaction between VEGF and Notch signaling has been reported in the vascular endothelium, and it plays a key role in angiogenesis. VEGF, by inducing AKT phosphorylation, is a potent pro-survival cytokine (15). However, in the report by Lin et al., Notch and VEGF signaling appears to have a pro-apoptotic effect (21). Further studies are needed to define these key differences. In addition, in *Drosophila*, Notch is a strong inducer of the nephrin homologue *hibris* (22); however—according to Lin et al.—in

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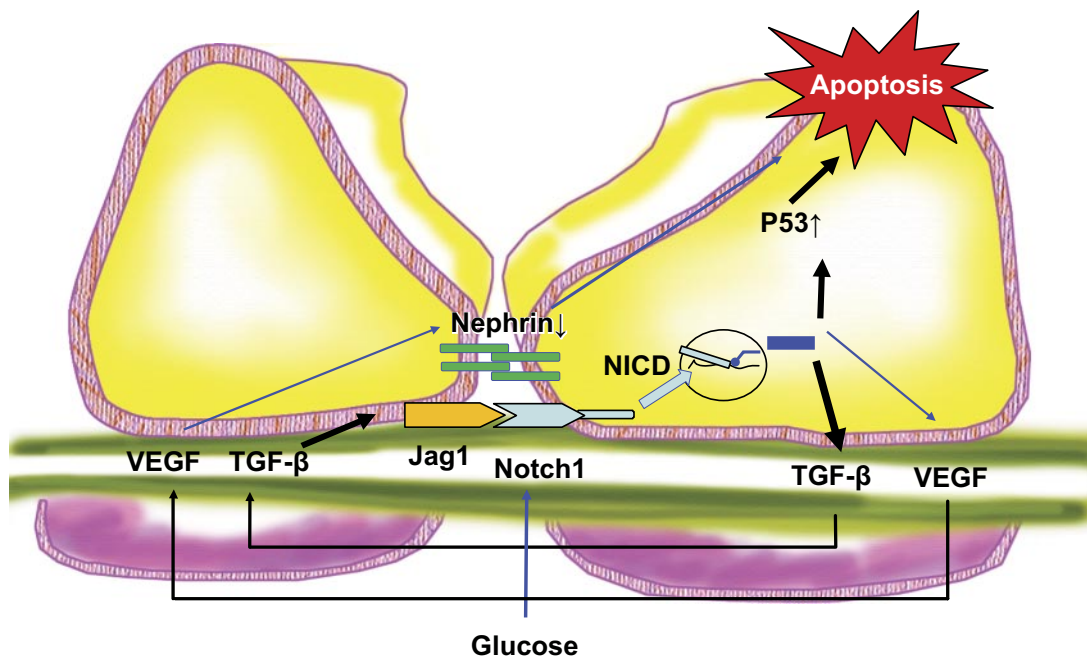


FIG. 1. The mechanism and consequence of Notch activation in podocytes. The proposed model for Notch activation in podocytes based on data from Lin et al. (indicated by blue lines) and prior published work (11–13) (black lines). In podocytes, hyperglycemia or TGF- β leads to increased Notch activity and an increase in NICD. Increased Notch activity in podocytes causes VEGF release, decrease in nephrin expression, and apoptosis. In addition, prior experiments also showed that Notch activation leads to increased TGF- β expression and p53 mediated apoptosis. (A high-quality digital representation of this figure is available in the online issue.)

podocytes, Notch appears to downregulate nephrin expression. A key confounding factor is that many of the experiments were performed in a cultured human embryonic kidney cell line (HEK293), which appears to express a key podocyte-specific protein, nephrin. Whether the expression and regulation of nephrin in this “heterologous system” are the same as in podocytes remains to be established.

Nevertheless, the study by Lin et al. (21) strongly supports the view that Notch signaling in podocytes plays a critical role in the development of albuminuria. There are several key questions to be answered in this area. First, in the Notch signaling pathway, there are differences in ligand-expressing and signal-receiving cells, and often the ligand and the receptor are expressed in different cell types. It is not clear how the Notch signaling works in the kidney. Therefore, it would be important to better characterize the expression and localization of Notch ligands and receptors in the glomerulus. Are they both expressed on the podocytes? Further studies will be needed to analyze the complex interaction between the VEGF, Notch, and the TGF- β pathways. Is Notch a common downstream regulator? Another key question will be to determine the targets of Notch activation in podocytes to understand how and why this important developmental pathway causes damage to the kidney.

Taken as a whole, the study by Lin et al. suggests that there is a critical interaction between HIF/VEGF and Notch signaling in diabetic podocytes, thus expanding our understanding of diabetic nephropathy.

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