Kick it up a notch: Notch signaling and kidney fibrosis

Mariya T. Sweetwyne[1], Jianling Tao[1] and Katalin Susztak[1]

[1]*Renal Electrolyte and Hypertension Division, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA*

Notch is a critical regulator of kidney development, but the pathway is mostly silenced once kidney maturation is achieved. Recent reports demonstrated increased expression of Notch receptors and ligands both in acute and chronic kidney injury. *In vivo* studies indicated that Notch activation might contribute to regeneration after acute kidney injury; on the other hand, sustained Notch expression is causally associated with interstitial fibrosis and glomerulosclerosis. This review will summarize the current knowledge on the role of the Notch signaling with special focus on kidney fibrosis.

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Correspondence: *Katalin Susztak, Renal Electrolyte and Hypertension Division, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, 415 Curie Boulevard, Room 405, Philadelphia, Pennsylvania 19104, USA. E-mail: ksusztak@mail.med.upenn.edu*

The terms 'chronic kidney disease' (CKD) and its most severe form, 'end-stage renal disease', describe the progressive functional decline of the kidney.[1] Histological lesions that are associated with CKD are collectively described as fibrosis. Kidney fibrosis is characterized by five distinct changes: glomerulosclerosis, interstitial fibrosis, tubular atrophy, peritubular capillary loss, and inflammation. Different insults can initiate fibrosis, such as epithelial injury, persistent inflammation, or progressive capillary loss. As the functions of different cell types are interrelated in the kidney, these events will feed into each other as the fibrosis progresses. For example, capillary rarefaction will cause tubular atrophy, and loss of tubular epithelial cells will further fuel further capillary loss. These fibrotic changes in the intersitium (termed 'interstitial fibrosis') are currently used as critical pathologic diagnostic criteria in determining the severity of CKD. However, the key process that causes the functional decline of the kidney is unclear. As the primary functions of the kidney (filtration, reabsorption, and secretion) are performed by epithelial cells, we believe that the loss of functional epithelium and tubular atrophy must be the most critical contributors to loss of renal function. Mouse models further support the role of epithelial cells in interstitial fibrosis development. For example, genetic manipulation of the signaling pathway only in epithelial cells has a profound effect on interstitial fibrosis development. Here, we will examine kidney fibrosis and Notch signaling with respect to the renal epithelial cell.

NOTCH SIGNALING: THE BUILDING BLOCKS

In mammals, there are four Notch receptors (Notch 1–4) and two classes of canonical ligands, Jagged (Jag1, 2) and Delta-like ligand (Dll1, 3, and 4).[2] The canonical Notch signaling pathway is initiated when the extracellular domain of a Notch receptor binds to a Notch ligand in a trans-interaction (receptor and ligand on opposite cells).[3] The Notch protein is proteolytically cleaved on the extracellular face by ADAM/TACE proteases and on the intracellular side of the plasma membrane by γ-secretase. The extracellular cleavage product remains bound to the ligand-presenting cell to be endocytosed. The intracellular part, known as the Notch intracellular domain (NICD), translocates to the nucleus where it complexes with transcription cofactors, such as RBPj and Mastermind-like proteins, to alter gene expression. The most commonly recognized Notch target genes are the

helix-loop-helix proteins of the Hey/Hes family. Hey and Hes operate primarily as repressive transcription factors.[4] Although altered expression of the Hey/Hes genes are occasionally reported independently of Notch, their expression patterns are consistent enough to be routinely utilized as proof-of-function for Notch signaling.

Post-translational modification of Notch is equally critical for its function.[5,6] O-fucosylation or *O*-glycosylation via fringe proteins (lunatic, radical, and manic) regulates the specificity of Notch receptor-ligand binding. For example, modification of Notch by manic fringe confers an increased affinity for Dll1 and a decreased affinity for Jag1, essentially allowing Dll1 to outcompete Jag1 for the Notch receptor. Whether or how resultant signaling differs with engagement of one ligand over another is still undergoing evaluation in the field.

When compared with other signaling pathways, several unique features of Notch signaling become apparent. Notch signaling requires the interaction of at least two different cell types. The cell containing the Notch receptor directs neighboring cells to either inhibit Notch ligand/receptor expression (lateral inhibition) or to establish a small population of adjacent Notch signaling cells (inductive signaling). Both types of signaling result in segregated cell populations by establishing boundaries between homogeneous cell types and/or developing heterogeneous cell populations within tissues. These features give the Notch pathway a unique and critical role in distinguishing cell types and deciding cell fates during development, differentiation, and disease pathogenesis.

NOTCH ACTIVITY IS CRITICAL FOR KIDNEY DEVELOPMENT

Notch has an important role in kidney development. The variable severity of developmental defects resulting from loss of different Notch receptors or ligands further illustrates that, despite utilization of the same signaling architecture, these are not redundant genes. Global Notch3 and Notch4 knockout animals are viable with minimal, if any, phenotypic changes. Mice with a global Notch1 deletion are embryonic lethal; however, deletion of Notch1 from renal epithelial precursors has no effect on kidney development. On the other hand, ablation of only Notch2 from epithelial renal precursors severely compromises renal development with loss of proximal epithelium including podocytes and proximal tubules.[7] With respect to Notch ligands, both Dll1 and Jagged1 are expressed in the developing kidneys. Gene-deletion studies indicate that Jagged1 is the dominant ligand. The primary role of Notch in kidney development appears to be in deciding proximal epithelial fate, as genetic overexpression of Notch is sufficient to direct cells to proximal tubule and glomerular epithelial fates.

Human genetic studies support animal model observations. Autosomal dominant mutations of JAGGED1 were identified as a cause of Alagille Syndrome.[8] Alagille syndrome was first described as a collection of disorders including craniofacial/skeletal abnormalities, cardiac malformation, and hepatic ductal hyperplasia.[9] Recent studies indicate that 40–60% of Alagille patients have some type of renal involvement as well.[10] NOTCH2 mutations have been associated with an Alagille-like phenotype in patients, who also present with renal abnormalities.[11] In summary, although many Notch ligands and receptors are expressed during development, it seems that Jagged1/Notch2 is the dominant axis for proximal epithelial specification.

PUTATIVE ROLE OF NOTCH IN ACUTE KIDNEY INJURY

The renal epithelium can fully regenerate after an episode of an acute insult. Using a rat model of ischemia-reperfusion injury, increased expression of Dll1 and Hes1 mRNA and proteins along with processed Notch2 was noted by Kobayashi *et al.*[12] Gupta *et al.*[13] also described the increased expression of Notch ligand Dll4 during regeneration after acute renal failure. Treatment of rats with recombinant Dll4 improved the recovery after the kidney injury. Incubation of renal tubule cells with Dll1 *in vitro* stimulated epithelial cell proliferation, indicating a potential beneficial role for Dll1/Notch signaling in epithelial cell recovery.[14] *In vivo* studies using the gamma secretase complex inhibitor (GSI) to reduce Notch signaling in the setting of acute kidney injury showed mixed outcome. Huang *et al.*[15] found that GSI treatment ameliorated the severity of tubular damage after renal ischemia-reperfusion injury in rats, whereas Chen *et al.*[16] described that GSI treatment delayed functional renal recovery in mice.

The mechanism of renal regeneration after an acute injury is a hotly debated issue. The specific question is whether it occurs from a specialized stem/progenitor compartment or via dedifferentiation and redifferentiation of existing epithelial cells. As previously noted, Notch has a role in directing cells to a proximal tubule fate during development, making Notch an attractive candidate for regulating proximal tubule regeneration. In addition, Notch has a critical role in maintaining the stem cell compartment in various organs. To further dissect Notch's potential role in maintaining a renal stem cell reserve, the Romagnani group isolated CD133/CD24 double-positive cells from human kidneys. They showed that under the right conditions *in vitro*, these putative renal progenitor cells exhibit high Notch activity[17,18] and differentiate into podocytes and tubule cells. According to their recent studies, these progenitor cells can replace podocytes after podocyte loss, and this differentiation into podocytes depends on the Jagged1/Notch2 axis.[17]

Parietal epithelial cells (PEC) have also emerged as important putative podocyte progenitors. In a recent study that used a transgenic podocyte-depletion system, increased Notch signaling was described in PECs after podocyte ablation.[19] In this model, parietal cells proliferated and became hyperplastic after severe podocyte loss. Increased Notch1 and Jagged1 were also detected by immunohistochemistry in hyperplastic PEC. *In vitro* studies indicated that Notch is responsible for transforming quiescent PECs into activated parietal cells. Treating these mice with GSI to block