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Another niche for Notch

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The Notch signaling pathway patterns the developing nephron along the proximal–distal axis during renal development. In an adult acute tubular necrosis model, Kobayashi *et al*. now show expression of many Notch components and the activation of Notch target genes, suggesting a critical function for Notch in regenerating proximal tubules.

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One of the most striking themes to have emerged from the realm of developmental biology during the past 20 years is the conservation of signaling pathways that control growth, differentiation, pattern formation, and cell death in organisms as disparate as flies and humans. But it is not only the conservation of receptors, ligands, and second messengers across hundreds of millions of years of evolution that is remarkable; it is also the variety of biological outputs that any given pathway can achieve. Indeed it appears that the same pathways are used over and over again often with quite different results. Is there a developing organ system or cell type that does not utilize some aspect of WNT/β-catenin, transforming growth factor-β superfamily, fibroblast growth factor, or hedgehog signaling? Given the diverse biological activities of these signaling molecules and receptors, we should not be surprised that they are also implicated in many disease processes, from cancer to fibrosis. The Notch signaling pathway falls squarely into that category of highly conserved and adaptive mechanisms whose functions in the developing and adult kidney are now being addressed.

The intricacies of Notch signaling are summarized in recent reviews that discuss not only the activation of receptors but the regulation of signaling by receptor–ligand secretion, modification, and degradation.[1,2] A more simplified view of Notch signaling is presented in Figure 1, which also highlights a central theme: that the Notch pathway requires direct cell–cell interactions. Signaling requires cell contact because both receptors and ligands are single-pass transmembrane proteins. In mammals, there are four receptors, called Notch-1 through Notch-4, and at least seven ligands, Delta-1 through Delta-4, Jagged-1 and Jagged-2, and Serrate. Notch proteins are modified by the Fringe glycosyltransferases, which are thought to alter affinity for different ligands. Engagement of the Notch proteins by ligands results in a series of proteolytic processing events that remove first the extracellular domain, via metalloprotease cleavage, and then the intracellular domain, via the γ-secretase enzymatic complex. This liberated Notch intracellular domain (NICD) translocates to the nucleus and regulates gene expression through interactions with the Rel-family transcription factor Csl and the coactivator, Mastermind. Among the best-characterized targets of NICD activation are the Hes and Hey family of helix-loop-helix proteins, which are essential for regenerating stem-cell populations and for complex oscillatory mechanisms of embryonic segmentation.[3] As an aside, I should mention that the γ-secretase enzymatic complex has been well characterized primarily because it is also responsible for cleaving the amyloid-β precursor protein to generate the plaques found in the central nervous system of Alzheimer's disease patients.

The biological effects of activated Notch signaling play out in three conceptually different scenarios. The first is called lateral inhibition, whereby a field of equivalent cells is resolved into different fates. This was originally described in the ommatidia of the fly eye. Once small differences in Notch signaling are detected within a field of cells, a single cell amplifies the signal, is selected for a neuronal fate, and suppresses the neuronal fate of its neighbors.[4] A second effect of Notch signaling is lineage decision making, which may have aspects of lateral inhibition but can also occur independent of the suppressive effects associated with lateral inhibition. A well-studied example is the B- versus T-cell fate decision in the mammalian immune system.[5] Lastly, Notch signaling can form boundaries or establish niches for maintaining specific cell types. It is this ability to establish boundaries between regions fated to make different cell types that may be most relevant to the kidney.

The first direct evidence that Notch signaling was important for boundary formation in the vertebrate kidney was found in the *Xenopus* pronephros, in which Notch activation appears to suppress nephric duct formation and promote pronephric tubule fates.[6] More recently, the Drummond laboratory has shown that Jagged-2 and Notch-3 in the zebrafish embryo promote differentiation of ion transport epithelia while suppressing the multiciliated epithelial fates in a manner more reminiscent of lateral inhibition.[7] In the mammalian kidney, several key papers from Kopan's laboratory demonstrate the effects of Notch signaling on proximal–distal axis specification in the developing nephron. Because of the variety of ligands and multiple Notch receptors expressed in the kidney, one way to completely inhibit signaling is through the use of γ-secretase inhibitors[8] or the specific deletion of presenilin,[9] the enzymatic subunit of the γ-secretase complex. Both of these experimental approaches pointed to a role for Notch in specifying the more proximal elements of the nephron during kidney development. These conclusions were confirmed by the specific deletion of Notch-2 in the kidney,[10] which resulted in the complete absence of glomerular epithelia and proximal convoluted tubules, and is entirely consistent with the appearance of the NICD within the nuclei of proximal and glomerular epithelial precursor cells. Thus, Notch-2 signaling compartmentalizes the

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developing nephron by specifying proximal cell fates and forming a boundary between these proximal and the more distal elements.

Why should Notch signaling in renal development be of interest to clinical nephrologists? As with most developmental signaling pathways, Notch is reactivated in renal injury. Kobayashi *et al.*[11] (this issue) show quite conclusively that Notch pathway receptors, ligands, and target genes are quickly upregulated in the rat kidney ischemia and reperfusion model of acute tubular necrosis. Furthermore, the ligand Delta-1 was shown to increase survival or proliferation of proximal tubule cells *in vitro*. Given that the proximal tubules are able to regenerate after ischemia or nephrotoxic injury, it is not surprising that many of the same signaling pathways that control differentiation, proliferation, and survival during embryonic renal development are reactivated in the regenerating tubules. Early markers for regenerating renal epithelial cells include the transcription factor Pax2 and the signaling proteins WNT4 and bone morphogenetic protein 7 (BMP7). Indeed, the high levels of expression of BMP7 have prompted its use as an agonist of regeneration after acute injury.[12] Whether the Notch pathway can be accentuated with recombinant proteins or small molecules *in vitro* remains to be determined. Nevertheless, the data provided by Kobayashi *et al.*[11] suggest that this is an important determinant of regenerated proximal tubule cell fate that could be stimulated under appropriate circumstances.

As is often the case, too much of a good thing may be detrimental. Although Notch is clearly essential for development and may play an important role in regeneration, stimulating the Notch pathway in healthy adult kidneys is likely to lead to complications. Evidence that Notch, Jagged, and its transcriptional target Hey-1 are complicit in the transforming growth factor-β-mediated transdifferentiation of epithelial cells to a mesenchymal phenotype is compelling.[13] This process of epithelial–mesenchymal transition is thought to contribute to glomerular and interstitial fibrosis in both chronic and acute renal diseases. Consistent with the potential role for Notch in chronic disease, upregulation of Jagged and Hes-1 has been observed recently in diabetic nephropathy.[14] The problem with these analyses is determining whether activation of Notch is a response to or a primary cause of the diseased state. Clearly, it will require using mutant mice or γ-secretase inhibitors in the various animal models of chronic and acute renal injury to definitively address the role of Notch in disease. If Notch activation is a compensatory or protective measure, it would make sense to develop strategies for activation of the pathway. This would most likely require small-molecule agonists, as the endogenous ligands are large and unwieldy. If Notch activity contributes to chronic disease, then the use of γ-secretase inhibitors may prove protective. Unfortunately, given the activity of Notch signaling in other essential cell types, such as in the immune system, pleiotropic effects of systemic inhibition may be unacceptable.

Whether or not Notch proves to be clinically relevant, it is still remarkable how well the regenerating kidney recapitulates aspects of embryonic development. It is too bad that this capacity for regeneration is limited to proximal tubules. Now if we could only get the glomerulus to behave.

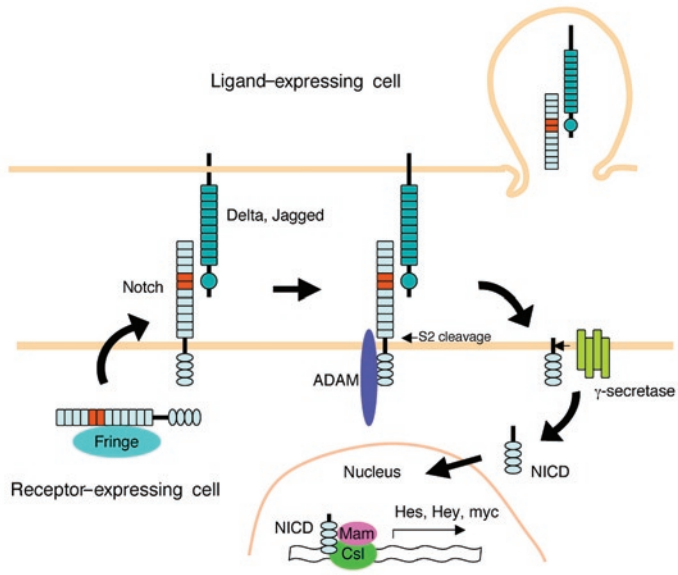

Figure 1 | Basic elements of Notch signaling. A schematic view of two cells is shown, one expressing the receptor, Notch, and an adjacent cell expressing the ligand Delta or Jagged. Maturation of the Notch receptor requires the glycosyltransferase Fringe. Upon binding to Delta or Jagged, Notch is cleaved by the ADAM metalloproteases to release the extracellular domain, which is then endocytosed along with ligands. Further cleavage by γ-secretase releases the Notch intracellular domain (NICD). The NICD translocates to the nucleus and activates gene expression through interactions with CsI and Mastermind (Mam).

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Biotransformation enzymes in development of renal injury and urothelial cancer caused by aristolochic acid

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Ingestion of aristolochic acid (AA) is associated with the development of AA-nephropathy and Balkan endemic nephropathy, which are characterized by chronic renal failure, tubulointerstitial fibrosis, and urothelial cancer. Understanding which enzymes are involved in AA activation and/or detoxification is important in assessing susceptibility to AA. Xiao *et al.* demonstrate that hepatic cytochrome P450s in mice detoxicate AA and thereby protect kidney from injury. The relative contribution of enzymes activating AA to induce urothelial cancer in humans remains to be resolved.

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The paper by Xiao *et al.*[1] (this issue) addresses the interesting and still unsettled question of whether the metabolism of aristolochic acid (AA) determines its pathophysiological effects, and, if so, which enzymes participating in this process are responsible. AA, a naturally occurring nephrotoxin and carcinogen, is associated with urothelial cancer development in patients suffering from Chinese herb nephropathy, now termed aristolochic acid nephropathy (AAN), and may also be a cause of the development of a similar type of kidney fibrosis with malignant transformation of the urothelium, Balkan endemic nephropathy (BEN).[2,3] The molecular mechanisms for AA-mediated renal injury, and whether it is an early stage of the urothelial-specific tumor development, are still matters of debate and need further investigations. In this context, it is noteworthy that a case of AA-induced tumor development without renal injury[4] suggests dissociation between AA-mediated nephrotoxicity and carcinogenicity. AA seems to directly cause renal injury by activating mitochondrial permeability transition, which was found recently in human renal tubular epithelial cells,[5] and metabolic activation of AA to species forming DNA adducts is an important step for AA-induced malignant transformation.[3,6] Indeed, the molecular mechanism of AA-induced carcinogenesis demonstrates a strong association between DNA adduct formation, mutation pattern, and tumor development.[3] The predominant AA-DNA adduct, 7-(deoxyadenosin-N^6-yl) aristolactam I (dA-AAI), which is the most persistent of the adducts in the target tissue, is a mutagenic lesion leading to A→T transversions in the *p53* gene in DNA from urothelial tumors of AAN and BEN patients.[3,7]

One of the common features of AAN and BEN is that not all individuals exposed to AA suffer from nephropathy and tumor development. We have suggested earlier that one cause of these different responses may be individual differences in the activities of the enzymes catalyzing the biotransformation (detoxication and/or activation) of AA (for a summary, see Stiborová *et al.*[6]). Many genes of enzymes metabolizing toxicants and carcinogens are known to exist in variant forms or show polymorphisms resulting in differing activities of the gene products. These genetic variations appear to be important determinants of cancer risk or other toxic effects of xenobiotics.[6]

The proposed activation and detoxication pathways for the major component of AA, aristolochic acid I (AAI), are shown in Figure 1. AAI is activated by simple nitroreduction to *N*-hydroxyaristolactam I, which forms a cyclic *N*-acylnitrenium ion as the ultimate carcinogenic species binding to DNA. The most important human and rat enzyme activating AAI *in vitro* is hepatic and renal cytosolic NAD(P)H:quinone oxidoreductase (NQO1), followed by hepatic microsomal cytochrome P450 (CYP) 1A1/2 and renal microsomal NADPH:CYP reductase (CPR), besides prostaglandin H synthase (cyclooxygenase (COX)), which is highly expressed in urothelial tissue.[6] It is of note that NQO1 polymorphism (the genotype *NQO1*2/*2*) was found to predispose patients suffering from BEN to the development of urothelial malignancy of the upper urinary tract (odds ratio 13.75, 95% confidence interval 1.17–166.21).[8] This finding, together with the demonstration of the importance of NQO1 in AAI activation, could be an explanation for cancer induction by AAI in only some of the AAN and BEN patients.

The competing conversion of *N*-hydroxyaristolactam I to the corresponding 7-hydroxyaristolactam or its further reduction to aristolactam I should be considered a detoxication pathway;

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