The role of Notch signaling in specification of podocyte and proximal tubules within the developing mouse kidney

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The role of Notch signaling in specification of podocyte and proximal tubules within the developing mouse kidney. Notch genes encode transmembrane receptors that mediate intercellular interaction by binding to the ligands on the adjacent cells. Due to early embryonic lethality in mice deficient for some Notch pathway genes, the role of Notch signaling for kidney development has not yet been defined. Using an antibody specific to the N-terminal end of γ-secretase–cleaved Notch 1, we found evidence for Notch 1 activation in the comma-shaped and S-shaped bodies. We therefore cultured embryonic (E) day E12.5 mouse metanephroi in the presence of a γ-secretase inhibitor, N-S-phenyl-glycine-t-butyl ester (DAPT), to block Notch signaling. Fewer renal epithelial structures were observed, with a severe deficiency in proximal tubules and glomerular podocytes. Distal tubules were present but at a reduced number, and this was accompanied by an increase in intervening, nonepithelial cells. By culturing day E14.5 metanephroi, we observed the formation of podocyte clusters after 3 days of DAPT treatment.

These observations suggest that γ-secretase activity, probably through activation of Notch, is not essential for podocyte formation beyond the stage of S-shaped body but is required for the proximal tubule and podocyte fates when S-shaped bodies are forming.

Using an antibody that specifically recognizes the γ-secretase–cleaved intracellular domain of Notch-1, we screened embryonic (E) mouse day E16 embryos and observed activation of Notch-1 in the developing mouse kidney. To define the precise location, we stained metanephroi with this antibody and found the expression was on the comma-shaped and S-shaped bodies. In addition, the cells that experienced Notch-1 activation also express Jagged1 (Fig. 1). No other Jagged1-expressing cells were detected elsewhere except in the blood vessels.

To study the role of Notch signaling in early mammalian kidney development, we treated cultured mouse metanephroi with N-S-phenyl-glycine-t-butyl ester (DAPT) in order to inhibit γ-secretase activity and therefore block all Notch signaling. When day E12.5 metanephroi were cultured for 5 days, we observed a great reduction of renal tubular epithelial cells without the formation of glomeruli [1]. We then attempted to define the nature of the remaining epithelial cells by a series of marker staining. The Wilms tumor-1–expressing podocyte clusters were diminished in the DAPT-treated kidneys (Fig. 2). Proximal tubules, marked with Lotus tetragonolobus lectin (LTL), did not form as well. We used cytokeratin 8 to label ureteric bud derivatives and E-cadherin for distal tubular cells and ureteric bud derivatives. The distal tubules, which expressed E-cadherin but not cytokeratin 8, were still detected and in many instances remained connected to the ureteric bud. The data demonstrated a requirement of γ-secretase activity, probably through Notch signaling, for the formation of podocyte and proximal tubule fate [1]. Importantly, distal tubules formed in the absence of the components proximal to it, and proximal tubules formed after DAPT was removed after 3 days in culture (Fig. 1). These observations imply that formation of each of the three segments (podocyte, proximal tubule, and distal tubule) can occur without input from the structure proximal to it.

Podocyte formation may require γ-secretase activity at two distinctive stages. One may be during S-shaped body formation when podocyte precursors form. The second could be at the capillary-loop stage as podocyte precursors further differentiate into mature podocytes. In additional experiments we asked if γ-secretase activity is required at the second stage by culturing day E14.5 metanephroi and following the differentiation of preformed S-shaped bodies. After 3 days of culture in DAPT, podocyte clusters formed in both treated and control kidneys, indicating that preformed S-shaped bodies could differentiate normally without γ-secretase. However, induced mesenchyme could not produce podocytes in the same metanephroi when treated with DAPT; this defect was visible after 4 days in culture [1].

At the meeting we presented genetic evidence that the DAPT effects (and those seen in mice deficient for presenilin [2]) can be explained by loss of Notch signaling. We
Fig. 2. γ-secretase inhibition causes reduction of podocyte formation while preserves the distal tubules. In the control (A) [dimethyl sulfoxide (DMSO)-treated], the Wilms’ tumor-1–expressing podocytes (red) form clusters throughout the whole metanephros. However, under N-S-phenylglycine-t-butyl ester (DAPT) treatment, the podocyte clusters fail to form normally (B). Nevertheless, the distal tubules, which express E-cadherin (green) by not cytokeratin 8 (red), are still present even when the γ-secretase activity in inhibited (D). Normal formation of distal tubules (green) and extensive ureteric branching morphogenesis (red and green) are observed in control (C). Notice that some the distal tubules are continuous from the ureteric buds in the DAPT-treated metanephros (D, arrow).

are currently pursuing the function of individual Notch genes in kidney development using a variety of genetic approaches and these results will be published elsewhere.

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