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Functional Renal Proximal Tubulogenesis in a Reconstituted Basement Membrane

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Background: Epithelial branching morphogenesis has been observed to occur in vitro, in reconstituted basement membranes, as well as in vivo during early development. Notably, the tubulogenesis observed in the kidney mesenchyme has been attributed to the inductive signals of growth factors. In this report we address the question: are the kidney tubules which form in matrigel are functional?

Methods: Primary rabbit kidney tubules were observed to form in matrigel supplemented with either EGF or HGF. Confocal microscopy was employed to examine the capacity of the tubules for transepithelial transport of organic anions, a distinctive property of renal proximal tubules.

Results: we observed that fluorescent substrates of the basolateral p-Aminohippurate (PAH) transport system (lucifer yellow and 5,6-carboxyfluorescein (FAM)) were concentrated into the luminal space of the tubules. Lucifer yellow accumulated into tubules with partially formed lumens, as well as into complete lumens. However BODIPY FL verapamil (a substrate of the P-glycoprotein mediated drug efflux system) was only concentrated intracellularly. Consistent with the renal tubules being "proximal" in origin, the apical membranes of the tubules became stained with TRITC-labeled Wheat Germ Agglutinin (WGA), as well as Tetragonolobus Purpureas lectin (rabbit renal proximal tubule markers), unlike peanut lectin. The process of tubulogenesis was inhibited by PD98059, a MEK inhibitor, rather than by Wortmannin, an inhibitor of PI3 Kinase. However, PD98059 and Wortmannin individually did not inhibit renal proximal tubule cell growth. In contrast, 8-bromocyclic AMP stimulated the formation of multicellular cysts, as well as the activity of the Na,K-ATPase.

Conclusion: In this report we present evidence indicating that primary rabbit renal cells not only possess the capacity to form proximal tubules in matrigel, but in addition, that these tubules are capable of performing physiologic transport functions.