staining is exclusively basolateral and is punctate in nature. Apical desmoplakin staining was noted in ADPKD cells lining the cysts. Basolateral staining was also observed in the ADPKD cells. In order to determine whether the apical desmoplakin was due to a protein sorting defect of a differentiation block, we examined the distribution of desmoplakin in a human embryonic kidney. Apical desmoplakin was observed in immature nephron segments of the embryonic kidney. This suggests that the apical desmoplakin staining observed in ADPKD renal cells is another manifestation of the block in differentiation.

In cultured NK epithelial cells, punctate desmoplakin staining was noted at the sites of cell-cell contact. ADPKD cells had large aggregates of desmoplakin at the cell borders and in a cytoplasmic location. Further experiments are required to conclusively demonstrate that the aggregates are intracellular.

Other investigators have shown that ADPKD renal epithelial cells express a number of additional markers that are also consistent with the idea that the cells are immature or dedifferentiated. Taken together, the data are consistent with the hypothesis that the genetic defect in ADPKD causes a block in the differentiation pathway of renal epithelial cells.

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Regulation of tubular morphogenesis and differentiation

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Certain cystic diseases of the kidney may reflect defects in renal development; some of these may be due to aberrancies in normal renal tubulogenesis. Development of the metanephric kidney begins at day 11 in the mouse when mutual interactions between the metanephric mesenchyme and ureteric bud activate events which ultimately lead to the formation of the nephron and urinary collecting system. When cocultured with MDCK cells seeded in Type I collagen, the embryonic kidney induced branching morphogenesis in MDCK cells, similar to those induced by hepatocyte growth factor (HGF). This branching was inhibited when monospecific antisera to HGF was added to the culture. Thus, at least for MDCK cells, HGF appears to be the primary branching morphogen secreted by the developing kidney. The expression of HGF and c-met during kidney development was examined using PCR. Expression of both messages were highest at day 11.5, when branching morphogenesis of the ureteric bud begins. Immunocytochemical analysis in embryonic kidneys revealed c-met to be present virtually exclusively on the basolateral surfaces of the ureteric bud elements as well as early tubular structures. Addition of anti-HGF serum to day 11.5 kidney organ cultures appeared to inhibit metanephric growth. In these experiments, perturbation of ureteric bud and metanephric development was visible under light microscopy following treatment with the anti-HGF serum. Thus, HGF and its receptor, c-met, appear to be important for normal kidney development, and, in particular, the development of the urinary collecting system. An analysis of factors that affect tubulogenesis and branching morphogenesis in MDCK cells was performed. Beginning with the observation that HGF induces the development of branching tubular structures in MDCK cells cultured in Type I collagen gels, but not in basement membrane Matrigel, we defined individual components in this extract of basement membrane and their effects on HGF-induced morphogenesis. Upon depletion of most of the growth factors from Matrigel, HGF was still unable to induce tubulogenesis, indicating that the remaining extracellular matrix (ECM) proteins or growth factors were exerting an inhibitory effect. Each extracted component was then added back to collagen gels, and we were able to show that: (1) a set of ECM proteins, including laminin, entactin and fibronectin, facilitated the development of branching tubular structures and increased their complexity; (2) certain other ECM proteins, like Type IV collagen, heparin sulfate proteoglycan and vitronectin inhibited HGF-induced morphogenesis; and (3) TGF-β not only inhibited tubulogenesis, but the tubules which did form had very little branching, suggesting that TGF-β inhibits tubulogenesis as well as branching. These results suggest that a tubulogenic morphogen such as HGF and a tubulogenesis-inhibitory morphogen like TGF-β can, in the context of a dynamic matrix known to occur during renal development, modulate the extent of tubulogenesis and the degree of their branching.

Other factors that are involved in tubulogenesis and branching remain to be elucidated. C-met is a transmembrane tyrosine kinase which can be phosphorylated by protein kinase C. Upon activation, c-met is known to be associated with SH2 domain containing effectors of intracellular signaling, including PI-3-kinase, Ras, GAP and src. The specific signaling events involved in HGF-induced morphogenesis are unknown. To gain further insight into these events, MDCK cells seeded in collagen gels were treated with HGF plus agents that modulate protein phosphorylation. Inhibitors of PKC resulted in more complex branching in the presence of HGF. PKA activators and calmodulin antagonists resulted in a marked decline in tubulogenesis. Protein phosphatase inhibition also inhibited tubulogenesis. These results suggest that the induction of branching tubular structures by HGF can be modulated by multiple phosphorylation pathways including those mediated by PKA, PKC and Ca^{++}/calmodulin dependent kinases. These phosphorylation events may play critical roles in modulating the degree of tubule formation and the extent of their branching during renal development.

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Hepatocyte growth factor as a renotrophic factor: Significance in kidney disease

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Hepatocyte growth factor (HGF) has been discovered, purified, and molecularly cloned as a potent mitogen for mature hepatocytes. HGF is thought to be a hepatotropic factor which acts as a trigger for liver regeneration after injury. HGF is a heterodimeric molecule composed of a 69 kD α-chain and a 34 kD β-chain which is linked by a disulfide bridge. Four kringle domains are located on the α-chain.

HGF is produced by mesenchymal cells and acts on a wide variety of epithelial cells. It can function as a mitogen (stimulating cell growth), motogen (stimulating cell motility), morphogen (inducing multicellular tissue structure) and tumor suppressor (suppressing cell growth). These pleiotropic functions of HGF are essential biological activities for the construction of normal tissue architecture. Thus, HGF may be one of the long-sought humoral mediators of morphogenic epithelial-mesenchymal interactions which are essential for organogenesis. The HGF-receptor has been identified as the product of the c-met proto-oncogene. This receptor is a 195 kD transmembrane protein that possesses tyrosine kinase activities.

HGF mRNA and HGF activity are rapidly and markedly increased in the liver after various liver injuries. Intravenous injection of recombinant HGF into mouse remarkably enhanced liver regeneration *in vivo*. Moreover, administration of recombinant HGF prevented the onset and progression of hepatic cirrhosis in rats and completely abrogated death caused by severe hepatic cirrhosis. HGF also exerts mitogenic and morphogenic activities for renal epithelial cells. HGF mRNA in injured kidney and blood HGF levels were markedly induced after unilateral nephrectomy and acute renal failure. Exogenously injected HGF remarkably enhanced renal regeneration *in vivo*. Intravenous injection of recombinant HGF into rats with acute renal injury markedly prevented the onset of severe renal dysfunction and enhanced the regeneration of the injured kidney. These findings suggest that HGF, a hepatotrophic factor, also functions as a renotrophic factor required for renal regeneration. This factor may be used for effective 'growth factor therapy' in patients with hepatic and renal dysfunction.

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Role of proto-oncogenes in cell proliferation

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Among renal cystic disorders, polycystic kidney disease (PKD) is considered to be the most prevalent and clinically important inherited disease. The etiology and pathogenesis of PKD still remain to be defined. Three mechanisms of cystogenesis have been proposed to cause tubular epithelial cysts: increased tubular epithelial proliferation, alteration in tubular basement membrane, and mislocation of the Na/K ATPase pump, resulting in abnormalities of ion and fluid secretion.

Recently, several investigators have focused their attention on hereditary models of PKD which resemble the human disorder. These include spontaneously occurring and targeted genetic models of PKD established by transgenic or insertional mutagenesis techniques. Transgenic mice with a PKD phenotype have been produced using oncogenes known to be involved in cell proliferation. Transgenic mice generated with the SV40 early region develop cysts and glomerulosclerosis associated with proteinuria. These mice also display renal tubular epithelial hyperplasia, thymic hyperplasia and lethal choroid plexus tumors. We have produced a transgenic mouse model of PKD known as SBM. The transgene consists of the SV40 enhancer, the adult human β-globin promoter and the coding region of the c-*myc* oncogene. Nineteen transgenic founder mice have been generated.

All SBM founders and mice derived from these founder lines develop a characteristic PKD phenotype with complete penetrance leading to death from renal failure at six weeks to three months of age. Histopathologic studies from gestational age to adulthood have elucidated the evolution of the cystic phenotype. Renal and glomerular cysts are first detectable in the terminal stages of renal organogenesis in SBM fetuses. From a young age (1 to 20 days), transgenic SBM mice develop progressively increasing cyst number and size. In addition, these young SBM mice have severe epithelial hyperplasia primarily in the proximal tubules. Fifty percent of young SBM mice develop one or more microadenomas. Hyperplasia also affected the visceral and parietal epithelium of some glomeruli leading to formation of crescents. Thus, hyperplasia was noted in three related renal epithelium cell types, the proximal tubular, parietal and visceral epithelium, all of which are derived embryologically from the proximal metanephric epithelium. In adult SBM mice, the cystic changes were very severe. Presence of proteinaceous cysts and interstitial fibrosis were consistently associated with the mature phenotype. Segmentally or globally sclerotic glomeruli were frequently observed in the kidneys of adult mice but were not a feature of fetal and young SBM kidneys. Hence, it is likely that glomerulosclerosis occurs secondary to tubular cysts. These results suggests that glomeruloscelerosis may arise from at least two