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INVITED CONTRIBUTION

Editor's Note:

The International Society of Nephrology sponsors, under the Editorship of Gerhard Giebisch, a series entitled Forefronts in Nephrology. *A recent meeting was held May 13–15, 1994 in Ontario, Canada, on* "The Molecular Basis of Renal Cystic Disease," *chaired by Jared J. Grantham and John H. Dirks, with the assistance of Clarissa Davidow and Ivy Foo.*

These four individuals have edited the key summaries of the presentations at that meeting, and these appear in following Invited Contribution.

THE EDITORS

Forefronts in Nephrology: The molecular basis of renal cystic disease

In few fields of nephrology is the pace of scientific discovery as swift as in the study of renal cystic disorders. In less than a decade the number of scientists working on this common clinical problem has risen from a handful to several hundred, worldwide. The pathophysiology of the hereditary and acquired cystic disorders has been found to involve the abnormal proliferation of renal epithelial cells, the abnormal accumulation of fluid within the cyst cavities by transepithelial secretion and the abnormal remodeling of extracellular matrix.

To enhance dissemination of the latest information about the molecular basis of renal cystic disease pathogenesis the International Society of Nephrology sponsored a symposium led by established researchers in renal cystic disease and related fields, and attended by trainees and other interested scientists. Summaries of the formal presentations are included here.

Within two months after the meeting concluded a 15 kb transcript of the ADPKD-1 gene had been described and the location of the ARPKD gene had been assigned to chromosome 6.

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Queen's Landing Inn
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I. ABNORMAL REGULATION OF EPITHELIAL CELL GROWTH

Abnormal epithelial cell proliferation in renal cyst formation and growth: The maturation arrest hypothesis

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It is proposed in the maturation-arrest hypothesis that a single abnormal genetic event triggers a cascade of abnormal gene expression events that ultimately locks tubular epithelial cells in a less-than-terminally differentiated state. The central feature of this is that cysts form because of their unique differentiated state. Cysts are thought to arise through a process that involves epithelial proliferation, and this proliferation is believed to be associated with this unique state of differentiation. If the proliferating cells dedifferentiate too far, they would lose their epithelial phenotype, and result in the formation of a solid tumor. Cystic cells, while not terminally differentiated, are not completely dedifferentiated. As such, they retain their epithelial phenotype, forming tight monolayers, and secreting fluid. This secretion keeps the cysts filled as they expand.

According to this hypothesis, cysts are envisioned to arise by either of two pathways: one that would occur during the process of tubular differentiation; the other following dedifferentiation of mature tubules. In the first, which may be more typical of rapidly progressive PKD, the cyst would arise as a consequence of a block in tubular development, causing arrested differentiation and cyst formation. In the second, which may be more typical of slowly progressive PKD or acquired PKD, tubules would undergo partial dedifferentiation to the point at which cyst growth would occur. With either pathway to cyst formation, the epithelial cells destined to form cysts would undergo continued cell proliferation and aberrant morphogenesis, and the cells would carry out secretion as opposed to absorption. This might be caused by underlying abnormalities in epithelial polarization, the cytoskeleton, the basement membrane or extracellular matrix, or in growth factor responsiveness.

Cyst formation is the result of the action of a single abnormal gene. However, to change the differentiated state of the cystic epithelium, it is likely that the action of this gene triggers the abnormal expression of other genes, initiating an abnormal cascade of gene expression events that ultimately results in cells that function abnormally. Among the genes expected to be abnormally expressed are transcription factors. If so, there would be an abnormal regulatory cascade involving the aberrant expression of a number of transcription factors and the genes under their control.

Differential cDNA library screening was carried out to identify genes abnormally expressed in polycystic kidneys. Results for 115 cDNA clones indicated that there is a wide variety of genes that

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are abnormally expressed, but that there is also a substantial number of genes that are not abnormally expressed. The large number of abnormally expressed genes in cystic kidneys suggests that there might be an abnormal transcription factor cascade that would have an effect on a variety of genes. To investigate this, the expression of several transcription factor families was examined in cystic kidneys. These families included the Hepatocyte Nuclear Factor (HNF)-1 and HNF-4 proteins. Both are expressed in liver, intestine, and kidney. They are thought to be involved in switching the developmental program, controlling the acquisition of the epithelial phenotype, and regulating terminal differentiation. While a great deal is known about the functions of these transcription factors in the liver and intestine, very little is known about their functions in the kidney.

Northern blot analysis revealed that HNF-1α and HNF-1β mRNAs are reduced in *cpk* cystic kidneys at 2- and 3-weeks of age. HNF-4 mRNA was also reduced in the cystic kidneys of both the *cpk* mouse and the *cy* rat, at 2- and 3-weeks and at 3-weeks, respectively. Among the genes regulated by HNF-1 and HNF-4 in the liver are PEPCK and aldolase B, which were both isolated by differential screening from cystic kidneys. Very little is known about the regulation of these genes in the kidney, but if they are under the control of HNF-1 and HNF-4 as they are in the liver it is possible that their underexpression in cystic kidneys is due to the underexpression of HNF-1 and HNF-4. If so, it appears that an abnormal regulatory cascade which involves HNF-1 and HNF-4 and the genes under their control is beginning to be defined. It is also possible that c-*fos* and c-*jun*, both overexpressed in the cystic kidneys, are involved in this abnormal cascade, either directly as the AP-1 transcription factor or indirectly through the glucocorticoid receptor. It is known in the liver that AP-1 regulates HNF-1; it is also known that Fos and Jun proteins down-regulate glucocorticoid receptor function and that a number of the genes under HNF-1 and HNF-4 control are regulated by glucocorticoids. Thus, a number of regulatory networks may be misfunctioning, all leading to decreased expression of genes important for kidney function.

The maturation-arrest hypothesis supposes that cysts develop because of their unique differentiated state. This is supported by accumulating morphological and biochemical evidence which has suggested that the cystic epithelium is immature. If the state of epithelial differentiation has been affected in these cysts, it is likely that abnormal transcription factor cascades are responsible. It would also follow, therefore, that the expression of a large number of genes usually expressed in the terminally differentiated kidney would be abnormal, and that the kidney would not be able to properly carry out its terminally differentiated functions.

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Epithelial differentiation in ADPKD

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It has been proposed that the gene defect in ADPKD causes a block in the differentiation of renal epithelial cells. One group of well characterized markers of differentiation are the intermediate filament proteins. The expression of the intermediate filament proteins, vimentin and cytokeratin have been used to characterize the differentiation state of malignancies and to identify the cell lineage of carcinomas. During nephrogenesis in the mouse embryo it has been shown that metanephric mesenchyme expresses vimentin. Upon induction, the metanephric mesenchyme begins the differentiation process that leads to the formation of a polarized epithelium. Cytokeratin replaces vimentin as the intermediate filament expressed in these epithelial cells. We reasoned that if a block in differentiation occurs in ADPKD then one should find evidence for this block in the pattern of intermediate filaments expression in ADPKD kidneys.

ADPKD and normal kidney sections were fixed and stained using antibodies to vimentin and cytokeratin. Sections from four separate normal and ADPKD kidneys were obtained from patients with end stage renal failure, one kidney was obtained from a patient without renal failure. We also examined the expression of cytokeratin and vimentin in cells grown in culture. Cells were grown from both normal kidney and microdissected cells lining the cysts found in ADPKD kidneys. Cytokeratin was noted in both normal kidney and ADPKD epithelial cells. Vimentin staining was observed exclusively in the interstitium in normal kidney cells. However, vimentin was also expressed in the epithelial cells lining the cysts in ADPKD kidneys. The observation was made in both the end stage ADPKD and in the kidney obtained from a patient without renal failure. Seventy percent of the cysts examined stained positive for vimentin.

We next examined the expression of vimentin and cytokeratin in primary culture of renal epithelial cells. The cells were grown on collagen coated coverslips and grown up to seven days in culture. An initial plating density of 50,000 cell/cm^2 was used for both ADPKD and NK cells. In both cell types, confluence was achieved by day 3 after plating. Second passage cells were used for all the experiments. Cytokeratin staining was observed in 98% of the NK and ADPKD cells one day after plating. This percentage remained relatively constant for both cell lines even after seven days in culture. One day after plating, 98% of the NK and ADPKD cells had vimentin staining. On the seventh day, the percentage of NK cells that were vimentin positive decreased to less than 10%. In contrast, 45% of the ADPKD cells were vimentin positive. The ADPKD cells were more likely to maintain vimentin expression even after prolonged culture.

Intermediate filaments are cytoskeletal proteins whose function is still unknown. These filaments do form stable attachments with specialized cellular junctions called desmosomes and hemidesmosomes. The junctions help mediate cell-cell and cell-matrix interactions. Given the alteration in intermediate filament expression in ADPKD, we examined the distribution of desmosome proteins. Kidney sections from ADPKD and NK were stained with antibodies that recognize desmoplakin I/II. In NK tubules, the