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Cellular pathophysiology of cystic kidney disease: insight into future therapies

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ABSTRACT Polycystic kidney disease (PKD) is a developmental kidney disorder which can be inherited as either an autosomal dominant trait, with an incidence of 1:50 to 1:1000, or as an autosomal recessive trait with an incidence of 1:6,000 to 1:40,000. Three different genes have now been cloned that are associated with mutations that cause PKD. Two of these are linked to the most common forms of the dominant disease while the third is associated with the *orpk* mouse model of recessive polycystic kidney disease. Advances in understanding the molecular genetics of PKD have been paralleled by new insights into the cellular pathophysiology of cyst formation and progressive enlargement. Current data suggest that a number of PKD proteins may interact in a complex, which when disrupted by mutations in PKD genes may lead to altered epithelial proliferative activity, secretion, and cell matrix biology. The identification of a unique cystic epithelial phenotype presents new opportunities for targeted therapies. These include targeted gene therapy, gene complementation, and specific immunological or pharmacological interruption of growth factor pathways.

KEY WORDS: *polycystic kidney disease, epidermal growth factor receptor, gene therapy*

Introduction

Polycystic kidney disease (PKD) can be inherited as either an autosomal dominant trait (ADPKD) with an incidence of 1:500 to 1:1,000 individuals or as an autosomal recessive trait (ARPKD), with an incidence of 1:6,000 to 1:40,000 live births (McDonald *et al.,* 1998). Three different genes have now been cloned which are associated with mutations that cause PKD (reviewed by Calvet 1998; Torres, 1998; Murcia *et al.,* 1999). Two of these are linked to the most common forms of the dominant disease: *PKD1*, which encodes polycystin 1; and *PKD2*, which encodes polycystin 2. The third gene *(Tg737)* is associated with the *orpk* mouse model of ARPKD. In parallel with these advances in understanding the molecular genetics of PKD, new insights into the cellular pathophysiology of cyst formation and enlargement have recently emerged (Murcia *et al.,* 1998). These data, combined with powerful new molecular technologies, create a new spectrum of potential therapies for PKD.

This review will: 1) provide an overview of the cellular and molecular pathophysiology of PKD; 2) describe the unique cellular phenotype of PKD epithelium; and 3) demonstrate how targeting the unique PKD epithelial phenotype provides new insights into future therapies for PKD.

Molecular and cellular pathophysiology of PKD

PKD1 is a large gene composed of 46 axons spanning 53 kilobases (kb) of genomic DNA (reviewed by Calvet, 1998; Murcia *et al.*, 1998; Torres, 1998). It produces a 14.5 kb mRNA that encodes a 4,304 amino acid polypeptide, polycystin 1. The complex structure of polycystin 1, which includes many different protein motifs, suggests that it is a large multi-functional molecule that is involved in carbohydrate motif recognition, ligand binding, and calcium regulation. It is also a protein that likely engages in cell-cell and/or cell-matrix interactions which regulate a signal transduction pathway mediated by specific protein-protein interactions at the cytoplasmic surface of the cell membrane (Arnould *et al.*, 1998). Data from *in vitro* protein interactions and heterologous expression systems suggest that polycystin 1 forms heterodimers with the *PKD2* gene product (Tsiokas *et al.*, 1997). The *PKD2* gene expresses a 4.5 kb mRNA which encodes a 968 amino acid polypeptide, polycystin 2 (reviewed by Calvet 1998; Torres, 1998; Murcia *et al.*, 1999). Polycystin 2 is an integral membrane protein which, by structural motif analysis, may be part of a large calcium ion channel family. The *Tg737* gene predominantly expresses a 3.2 kb mRNA which encodes an 824 amino acid polypeptide (reviewed in Murcia *et al.*, 1999). *Tg737* contains ten copies of the

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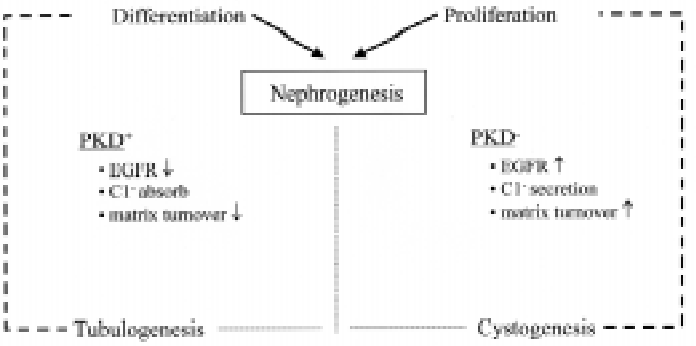

Fig. 1. Nephrogenesis represents a balance between cellular differentiation which leads to tubular heterogeneity and segment specific development, and proliferation which is necessary for overall kidney growth. *Cystogenesis, as opposed to normal tubulogenesis, can be considered an abnormal pattern of nephron formation in which proliferation associated with a specific epithelial phenotype (see text) is accentuated relative to differentiation.*

tetratricopeptide repeat, suggesting a role in cell cycle control as well as protein-protein interactions. Preliminary yeast 2-hybrid screens with *Tg737* have identified several potential protein partners which include polycystin 1, catenin, P120 catenin, Snx1, and HNF4 (Yoder *et al.*, 1997b).

Molecular genetic evidence suggests that the protein products of *PKD* genes participate in a common disease pathway and provides a working hypothesis for critical analysis (Murcia *et al.*, 1998). These data suggest that polycystin 1, polycystin 2, and Tg737 form a macromolecular regulatory/signaling structure at the cytoplasmic membrane which may regulate fundamental aspects of renal epithelial cell biology. Mutations in *PKD1*, *PKD2*, or *Tg737* (as well as other *PKD* genes) may disrupt this macromolecular protein complex and lead to abnormal epithelial cell proliferation, secretory activity, and altered cell-matrix interactions. Over the past decade these three features – proliferation, secretion and abnormal matrix – have emerged as the key pathophysiological features mediating epithelial cyst formation and enlargement in PKD (Orellana and Avner, 1995; Murcia *et al.*, 1998).

The cellular phenotype of PKD epithelium

As noted above, cystic epithelial cells are characterized by altered proliferative activity, secretion rather than absorption, and an abnormal matrix microenvironment. The identification of a unique cystic epithelial cell phenotype provides insight into disease pathophysiology and suggests possible direction for new therapies. The cystic epithelial cell has both quantitative (too much) and qualitative (apical cell surface localization) abnormalities of epidermal growth factor receptor (EGFR) expression which mediate enhanced proliferation; 2) secretes chloride which obligates net fluid secretion in what is a normally absorptive epithelium; and 3) exists in an abnormal matrix microenvironment (Orellana and Avner, 1995; Murcia *et al.*, 1998). Some of these features resemble an early developmental epithelial cell phenotype. From a developmental perspective, cystogenesis can be considered as a state of abnormal tubulogenesis in which proliferative influences predominate over differentiation factors (Fig. 1).

Proliferation is the single most common characteristic of all PKD epithelia reported to date and appears to be necessary for cyst formation and progressive enlargement. We have therefore focused on the role of abnormal EGFR expression in mediating cystic tubular hyperplasia. A number of studies to date have demonstrated a potential role for the transforming growth factor (TGFα)/epidermal growth factor (EGF)/EGFR axis in cystic epithelial hyperplasia: TGFα and EGF are cystogenic in a number of epithelial systems *in vitro*; TGFα overexpression in transgenic mice is cystogenic *in vitro*; renal cyst fluid contains EGF/EGF-like peptides in mitogenic concentrations and cystic renal tissue has increased TGFα and EGFR expression (reviewed in Orellana and Avner, 1995; Murcia *et al.,* 1998). EGFR protein is overexpressed and mislocalized to the apical and basolateral surfaces of cystic tubular epithelium in human ADPKD (Du and Wilson, 1995). We have recently demonstrated that in human and 3 murine models of ARPKD the EGFR is mislocated to the apical surface of cystic collecting tubule cells (Sweeney and Avner, 1998). Since the apical cell surface is in direct contact with cystic fluid, apical localization of the receptor places it in direct contact with a pool of biologically active stimulatory polypeptides (Fig. 2). We have demonstrated that cells from cystic and control collecting tubules can be isolated and that such cells maintain their *in vivo* EGFR phenotype *in vitro* (Sweeney and Avner, 1998). Domain-specific high affinity ligand binding was assessed by standard Scatchard analysis in control and cystic cells. High affinity binding was detected in the basolateral domain of control and cystic cells from human ARPKD and all murine ARPKD models. However, apical domain high affinity ligand binding was detected only in cystic cells from human and murine ARPKD (Fig. 3). To further assess the functional activity of abnormally

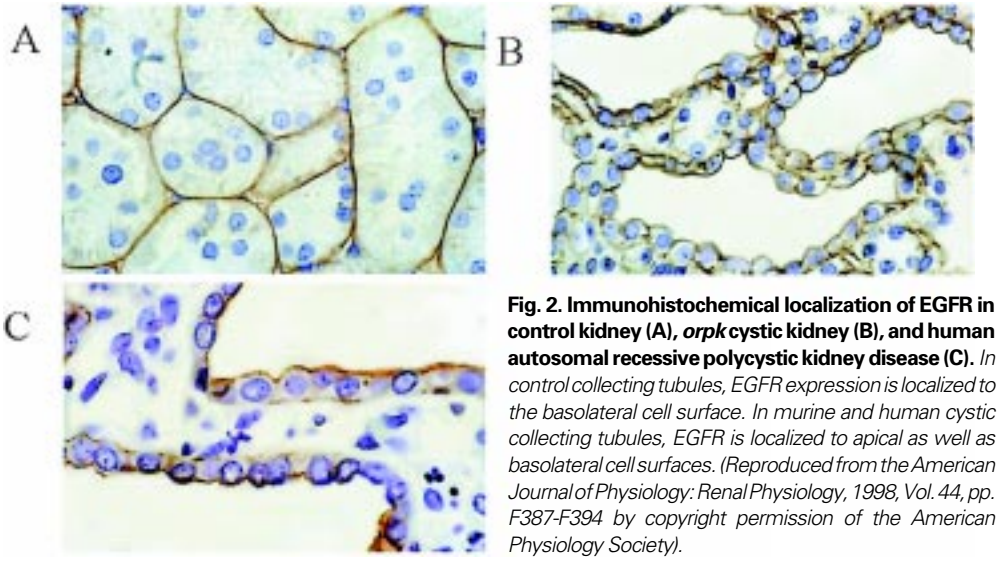

Fig. 2. Immunohistochemical localization of EGFR in control kidney (A), *orpk* cystic kidney (B), and human autosomal recessive polycystic kidney disease (C). *In control collecting tubules, EGFR expression is localized to the basolateral cell surface. In murine and human cystic collecting tubules, EGFR is localized to apical as well as basolateral cell surfaces. (Reproduced from the American Journal of Physiology: Renal Physiology, 1998, Vol. 44, pp. F387-F394 by copyright permission of the American Physiology Society).*

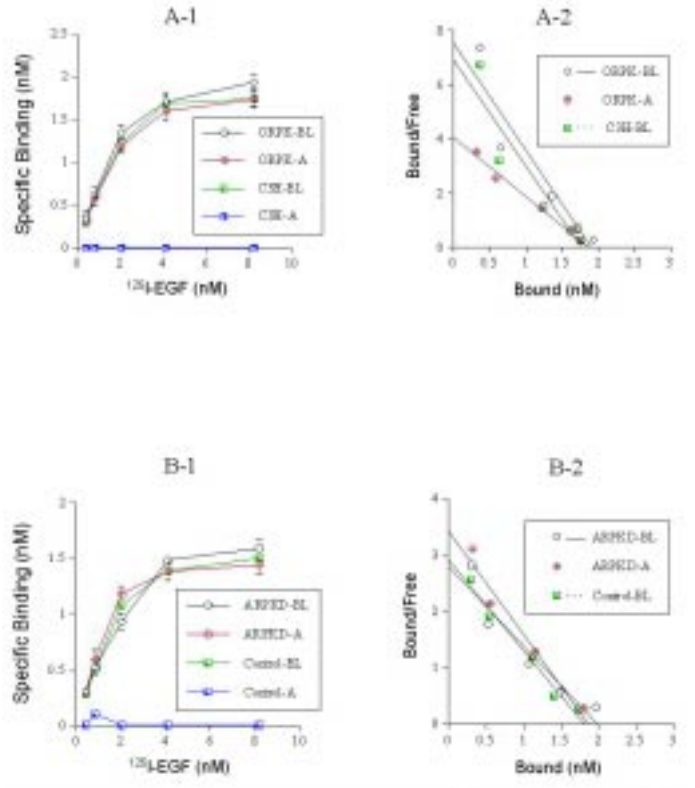

Fig. 3. Domain specific binding (BL, basolateral binding; A, apical binding) curve of ^{125}I-EGF stimulated isolated collecting tubule cells. *Specific binding (nM) versus label added is plotted for orpk model* **(A)** *and human ARPKD* **(B)**. *Curves generated from Scatchard analysis are shown on the right of each curve. (Reproduced from the American Journal of Physiology: Renal Physiology, 1998, Vol. 44, pp. F387-F394 by copyright permission of the American Physiology Society).*

expressed EGFR in ARPKD, selective ligand stimulation of apical versus basolateral EGFR in human and murine ARPKD cells was followed by measurement of receptor autophosphorylation and determination of cell proliferation. Stimulation of the basolateral domain of all control and human and murine ARPKD cells resulted in a detectable level of phosphorylated EGFR. Stimulation of the apical domain with EGF resulted in detectible phosphorylated receptor only in human and murine ARPKD cells (Fig. 4). These studies demonstrate that apically expressed EGFRs in PKD exhibit high affinity binding for EGF, autophosphorylate in response to EGF, and transmit a mitogenic signal when stimulated by the appropriate ligand (Sweeney and Avner, 1998). Developing strategies to target abnormally expressed, functional EGFRs in ADPKD and ARPKD represents an attractive new approach for future pharmacological and/or gene therapy in these disorders.

Targeting PKD epithelium: new therapies

The identification of a unique cellular phenotype for PKD epithelium, as noted above, presents new opportunities for therapy. It will be difficult to define specific therapies targeting matrix or secretory alterations in PKD epithelium until the pathophysiology of these processes is more clearly defined. However, recent data suggest that specific targeting of chloride transporters and/or channels (particularly the CFTR) may have potential in this regard (Murcia *et al.*, 1998; Sullivan *et al.*, 1998). Targeting abnormal proliferation in PKD epithelium, particularly specific alterations in EGFR expression, has particular appeal. New therapies might include targeted gene replacement, gene complementation or specific immunological or pharmacological blockage of altered EGF receptor activity.

The ability to target a foreign gene into the appropriate cells of the mammalian kidney is the goal of renal gene therapy. Selective targeting could be enhanced by taking advantage of apical EGFR expression in the collecting tubules of cystic epithelium. It has been reported that targeted delivery and persistent expression of a foreign gene is possible by utilizing polycations such as poly-L-lysine which can promote DNA compaction (Wu *et al.*, 1989). Using this approach, which depends on receptor mediated endocytosis, ligands such as EGF or TGF- could be covalently coupled to polycations to target wild type (or other) genes to PKD epithelium. The potential of this approach is demonstrated by studies in which the PKD phenotype has been rescued in the orpk model of ARPKD through expression of the cloned wild type cDNA (Yoder *et al.*, 1996, 1997a). In these studies of full length *Tg737* cDNA under the control of either a ubiquitous or gene specific promoter was introduced into *orpk* mutants. This resulted in renal epithelial

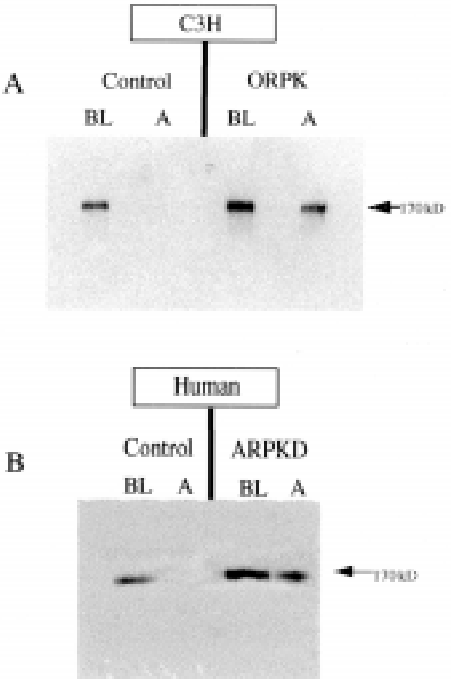

Fig. 4. Western analysis of phosphorylated EGFR after domain specific stimulation with EGF. *After stimulation EGFR was immunoprecipitated, resolved by SDS-PAGE, transferred to nitrocellulose, probed with antiphosphotyrosine antibody and visualized with enhanced chemiluminescence. Phosphorylated receptor was detected after basolateral (BL) stimulation in both cystic and control cells. There was no detectible phosphorylated receptor after apical (A) stimulation of control cells. Apical stimulation produced detectible phosphorylated receptor only in cystic cells derived from orpk* **(A)** *and human ARPKD kidney* **(B)**. *(Reproduced from the American Journal of Physiology: Renal Physiology, 1998, Vol. 44, pp. F387-F394 by copyright permission of the American Physiology Society).*

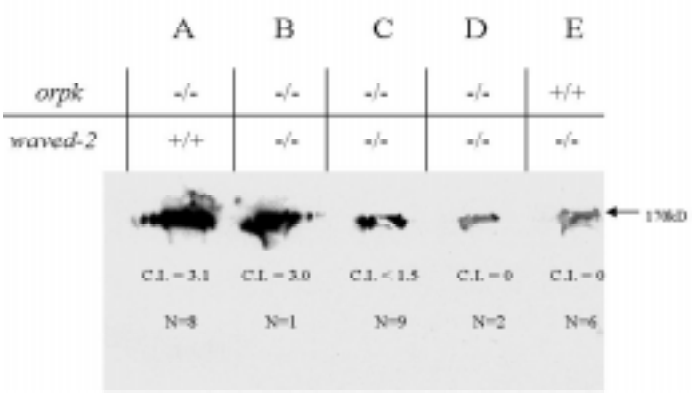

Fig. 5. Whole kidney EGFR tyrosine kinase activity. *Composite Western depicts the range of EGFR tyrosine kinase activity in orpk; wa-2 double mutants. Equal amounts of proteins were immunoprecipitated with an anit-EGFR antibody, and the immunoprecipitate was Western blotted and probed with antiphosphotyrosine antibody. The genotypes of the animals are shown above each lane. The cystic index (CI) of the kidneys are shown below the blots, and the number of animals that demonstrate these levels is also shown. Orpk; wa-2 mutants (three middle lanes) demonstrate a range of tyrosine kinase activity levels which correlate directly with the CI of the kidney. (Reproduced from The Journal of Clinical Investigation, 1998, Vol. 101, pp. 935-939 by copyright permission of The American Society for Clinical Investigation).*

expression of the wild-type gene, correction of renal histopathological cystic abnormalities, and functional correction of renal tubular defects. Though not immediately applicable to humans at this time, these reports demonstrate for the first time that renal cystic disease can be corrected by gene replacement therapy.

Another approach to targeting specific phenotypic abnormalities of PKD epithelium is to use genetic technology to correct a pathophysiological process downstream from the primary gene defect rather than employing wild-type gene replacement. This strategy is particularly appealing in diseases such as ADPKD1, where the complexity and size of the wild type gene are significant barriers to successful primary gene replacement therapy. In recent studies the potential of this approach in targeting altered EGFR activity in cystic epithelium was demonstrated by utilizing the orpk and *waved-2 (wa-2)* mutations (Richards *et al.*, 1998). The *wa-2* allele encodes a protein with a single amino acid change in the EGFR, which dramatically decreases EGFR tyrosine kinase activity. *Wa-2*; *orpk* double homozygotes were generated to genetically test whether reducing EGFR activity would alter genetically-determined cyst formation and progressive enlargement. *Wa-2*; *orpk* double homozygotes exhibited dramatically reduced collecting tubule cyst formation and enlargement, which correlated directly with reduced levels of kidney EGFR tyrosine kinase activity (Fig. 5). These double mutants had improved ability to concentrate urine compared to orpk cystic homozygous animals (Richards *et al.*, 1998). These data unequivocally establish a direct role for the EGFR in the pathophysiology of collecting cystogenesis, and demonstrate a potential role for gene complementation therapy in PKD.

In addition to genetic complementation, future therapies for PKD could immunologically or pharmacologically target altered EGFR expression in PKD epithelium. Recent reports demonstrate that EGFR inhibition with specific blocking antibody, or pharmacological inhibition of EGFR tyrosine kinase activity with tyrphostins markedly block *in vitro* tubular cystogenesis in organ culture systems (Avner *et al.*, 1995; Pugh *et al.*, 1995). Protein tyrosine kinases like the EGFR are members of a growing family of oncoproteins and proto-oncoproteins that play a pivotal role in normal and abnormal proliferative processes. Protein tyrosine kinase inhibitors, particularly the tyrphostins, have particular therapeutic potential as antiproliferative agents in cancers, leukemias, cirrhosis, and restenosis following vascular injury in addition to PKD (Levitzki, 1992). The potential for successful *in vivo* therapy of PKD with newly developed, specific EGFR inhibitors has been demonstrated in preliminary animal studies (O'Neill, 1998).

Conclusion

The analysis of the cellular and molecular basis of PKD in humans and mice has generated new data which demonstrate how mutations in a number of primary cystic disease genes lead to abnormalities in renal epithelial cell proliferation, secretion, and matrix biology. The identification of a unique cystic cellular phenotype permits the targeting of specific pathophysiological processes to interrupt the complex cystic disease pathway at a number of different levels. Increased understanding of the molecular pathophysiology of PKD will lead to further innovations in therapy for human cystic diseases.

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