Cell Tissue Res (2006) 326:671–685
DOI 10.1007/s00441-006-0226-0

REVIEW

Molecular and cellular pathophysiology of autosomal recessive polycystic kidney disease (ARPKD)

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Received: 13 March 2006 / Accepted: 20 April 2006 / Published online: 10 June 2006
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Abstract Autosomal recessive polycystic kidney disease (ARPKD) belongs to a group of congenital hepatorenal fibrocystic syndromes characterized by dual renal and hepatic involvement of variable severity. Despite the wide clinical spectrum of ARPKD (MIM 263200), genetic linkage studies indicate that mutations at a single locus, PKHD1 (polycystic kidney and hepatic disease 1), located on human chromosome region 6p21.1–p12, are responsible for all phenotypes of ARPKD. Identification of cystic disease genes and their encoded proteins has provided investigators with critical tools to begin to unravel the molecular and cellular mechanisms of PKD. PKD cystic epithelia share common phenotypic abnormalities despite the different genetic mutations that underlie the disease. Recent studies have shown that many cyst-causing proteins are expressed in multimeric complexes at distinct subcellular locations within epithelia. This co-expression of cystoproteins suggests that cyst formation, regardless of the underlying disease gene, results from perturbations in convergent and/or integrated signal transduction pathways. To date, no specific therapies are in clinical use for ameliorating cyst growth in ARPKD. However, studies noted in this review suggest that therapeutic targeting of the cAMP and epidermal growth factor receptor (EGFR)-axis abnormalities in cystic epithelia may translate into effective therapies for ARPKD and, by analogy, autosomal dominant polycystic kidney disease (ADPKD). A particularly promising approach appears to be the targeting of downstream intermediates of both the cAMP and EGFR axis. This review focuses on ARPKD and presents a concise summary of the current understanding of the molecular genetics and cellular pathophysiology of this disease. It also highlights phenotypic and mechanistic similarities between ARPKD and ADPKD.

Keywords Autosomal recessive polycystic kidney disease · Autosomal dominant polycystic kidney disease · Molecular genetics · Cellular pathophysiology · Phenotype · Disease mechanism

The authors are supported by the National Institutes of Health (grant no. 1-P50-DK57306), the PKD Foundation (grant no. 76a2r), and the Children's Research Institute, Children's Hospital of Wisconsin.

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Introduction

Renal cystic diseases (RCD) include a group of monogenic kidney abnormalities that cause significant morbidity and mortality (Dell et al. 2004). Histopathologically, renal cysts are fluid-filled epithelia-lined dilated saccular lesions that generally arise from tubular segments. Although isolated renal cysts may arise as degenerative lesions in adults, the most common cause of nondysplastic nonsyndromal multiple renal cysts are autosomal dominant (ADPKD; MIM 601313 and MIM 173910; PKD1 and PKD2) or autosomal recessive (ARPKD; MIM 263200) polycystic kidney disease.

ADPKD is the most common renal monogenic disease with an incidence of 1/400–1000. ARPKD comprises 5%–

8% of all individuals requiring renal replacement therapy (dialysis and/or kidney transplantation). Approximately 80%–85% of ADPKD patients have mutations in the *PKD1* gene, located on chromosome 16p13, which encodes polycystin-1 (PC-1). The remaining 10%–15% of ADPKD cases have mutations in the *PKD2* gene, which is located on chromosome 4q21, and which encodes polycystin-2 (PC-2). ADPKD is usually asymptomatic until the middle decades. However, 2% to 5% of ADPKD patients present with a severe neonatal course and significant morbidity and mortality.

ARPKD is a significant cause of renal and liver-related morbidity and mortality in childhood. Estimates of disease prevalence vary widely, but an overall frequency of 1 in 20,000 live births and a carrier level of up to 1:70 have been recently proposed (Zerres et al. 1998).

The majority of ARPKD patients present clinically as newborn or young children. Despite dramatic improvements in neonatal and intensive care over the past decade, neonatal mortality is 25%–35%. The clinical spectrum of surviving patients is considerably more variable. Principal manifestations of the disease involve the fusiform dilation of renal collecting tubules or ducts (CT) and dysgenesis of the hepatic portal triad attributable to ductal plate abnormalities.

The ARPKD disease gene, *PKHD1* (polycystic kidney and hepatic disease 1), is a large gene located on chromosome 6p21.1–p12; it spans 470 kb of genomic DNA and produces a cDNA of 16 kb. A minimum of 86 exons is assembled into a variety of alternatively spliced transcripts. The longest continuous open reading frame (ORF) is predicted to yield a novel 4,074-amino-acid multidomain integral membrane protein of unknown function called fibrocystin (Ward et al. 2002) or polyductin (Onuchic et al. 2002).

Historically, inherited renal diseases were characterized by the description of clinical symptoms, age of presentation, and the history of disease progression. Advances in molecular genetics have led to the identification of a number of cyst-causing genes including the three PKD genes (PKD1, PKD2, and PKHD1). This has allowed previous descriptive cataloging of many renal diseases to be classified and confirmed at the genetic or molecular level. Identification of RCD genes and their encoded cystoproteins has provided investigators with the basic tools to begin to unravel the molecular and cellular mechanisms of PKD. Recent studies have led to the discovery that many of these proteins are expressed in multimeric complexes at distinct subcellular locations within renal epithelia (Wilson 2004b). This co-expression of cystoproteins in complexes suggests that cyst formation in many different diseases may result from similar perturbations in convergent and/or integrated signal transduction pathways (Wilson 2004b). This may provide a molecular basis for the perplexing clinical observation that diseases caused by mutations in many different genes may produce similar, and at times, identical clinical phenotypes.

This review will focus on ARPKD and present a concise summary of the current understanding of the molecular genetics and cellular pathophysiology of this disease. It will also highlight phenotypic and mechanistic similarities between ARPKD and ADPKD.

Clinical spectrum and pathology

ARPKD (MIM 263200) was first recognized as a distinct morphologic form of cystic disease in 1902 (Osathanondh and Potter 1964a). In a landmark study, Blyth and Ockenden (1971) classified ARPKD into four distinct phenotypes (perinatal, neonatal, infantile, and juvenile) on the basis of clinical manifestations and the age at presentation. The classifications focused on the wide spectrum of renal CT and biliary ductal plate abnormalities. These ranged from nephromegaly and the oligohydramnois sequence with minimal biliary abnormalities ("perinatal") to severe portal hypertension with minimal renal cystic disease ("juvenile"). Prior to advances in molecular genetics and the identification of the ARPKD disease gene, the four groups were thought to be distinct disease entities caused by different mutant genes.

ARPKD belongs to a group of congenital hepatorenal fibrocystic syndromes characterized by dual renal and hepatic involvement of variable severity. Renal manifestations are characterized by both ectasia and cystic dilation of renal CT. The fusiform dilated CT are lined by undifferentiated epithelium and surrounded by abnormal deposition of extracellular matrix. Unlike ADPKD cysts, renal cystic lesions in ARPKD retain both their afferent and efferent connections (Bernstein and Slovis 1992; Dell et al. 2004; Osathanondh and Potter 1964c). The kidney appears spongy, and there is no clear separation of the cortex and medulla. From 10% to 90% of CT are affected, resulting in wide variability of renal dysfunction. Depending on the number of CT involved, the kidneys may be massively enlarged. Histological analysis of the kidney reveals multiple subcapsular cystic lesions corresponding to radially oriented, ectatic CTs (Osathanondh and Potter 1964a, 1964b).

Liver disease is an invariable feature of ARPKD. The manifestations generally vary according to the patient's age at presentation (Blyth and Ockenden 1971; Dell et al. 2004). The liver disease consists of biliary dysgenesis and periportal fibrosis (congenital hepatic fibrosis; CHF). Dilation of both intrahepatic (Caroli's syndrome) and extra-hepatic bile ducts (extra-HBD) and CHF lead to

recurrent ascending cholangitis and portal hypertension with splenomegaly and esophageal varices (Blyth and Ockenden 1971; Davis et al. 2003; Dell and Avner 2003; Desmet 1992; Guay-Woodford and Desmond 2003; Harris and Rossetti 2004; Jorgensen 1977).

Despite advances in neonatal care, the short-term and long-term morbidity and mortality of ARPKD remain substantial. Notwithstanding the variable clinical spectrum of ARPKD, the majority of patients are identified either in utero or at birth (Dell et al. 2004; Guay-Woodford and Desmond 2003). The most severely affected fetuses have enlarged echogenic kidneys and oligohydramnios attributable to poor fetal renal output. The only signs potentially detectable *in utero* are enlargement and increased echogenicity of both kidneys. However such findings are often not present during second trimester fetal sonography (Zerres et al. 1998). Pulmonary hypoplasia, resulting from oligohydramnios, occurs in the majority of affected infants and is a major cause of morbidity and mortality in the newborn period. Improved respiratory treatment leads to increased neonatal survival, but death still occurs in the neonatal period in approximately 25%–30% of affected individuals, primarily because of respiratory insufficiency (Kaariainen et al. 1988; Kaplan et al. 1989; Roy et al. 1997). The prognosis is more optimistic for ARPKD patients surviving the neonatal period (Roy et al. 1997).

Approximately 50% of affected individuals progress to end-stage renal disease (ESRD) within the first decade of life (Cole et al. 1987; Roy et al. 1997). Modern neonatal respiratory support and renal replacement therapies have improved the 10-year survival rate of patients who survive the first year to 82% (Dell and Avner 2003). For infants who survive the perinatal period, a wide range of associated morbidities can evolve, including systemic hypertension, renal failure, portal hypertension, and fibrosis of both the liver and kidneys (Davis et al. 2003; Dell et al. 2004; Guay-Woodford 1996). The 15-year survival rate is estimated to be 67%–79% (Dell and Avner 2003).

A minority of individuals present as older children, usually with hepatosplenomegaly as the presenting feature. Later childhood presentation is usually associated with less renal enlargement and more variability in cyst size (Blyth and Ockenden 1971). As described above, these patients are more likely to develop complications of CHF and Caroli's disease and may require porto-systemic shunting. In rare cases, sequential or simultaneous liver-kidney transplants can be considered a viable therapeutic option (Davis et al. 2003). The clinical spectrum of ARPKD has been expanded by a recent study involving molecular characterization. Almost 1/3 of the patients presenting with classical hepatic phenotype and documented mutations in the *PKHD1* gene are over 20 years old at the time of diagnosis (Adeva et al. 2006).

Hypertension may occur in up to 80% of children with ARPKD, is frequently severe, and is generally correlated with decreased renal function. The mechanisms of hypertension are unknown, although increased intravascular volume secondary to dysregulation of renal sodium transport and activation of the renin-angiotension axis have been implicated (Kaplan et al. 1989). Therapies with angiotension converting enzyme inhibitors or ATII receptor inhibitors are generally effective (Guay-Woodford and Desmond 2003; Jafar et al. 2005; Kaplan et al. 1989). Additional clinical complications include nephrogenic diabetes insipidus, failure to thrive, and hyponatremia (Dell et al. 2004; Guay-Woodford and Desmond 2003; Zerres et al. 1996).

Genetics

Localization, identification, and expression of PKHD1

Despite the variable clinical spectrum of ARPKD (MIM 263200), genetic linkage studies indicate that mutations at a single locus, *PKHD1*, mapped to human chromosome region 6p21.1–p12 are responsible for all phenotypes of ARPKD (Guay-Woodford et al. 1995; Zerres et al. 1998). *PKHD1* has recently been cloned by several independent groups (Onuchic et al. 2002; Ward et al. 2002; Xiong et al. 2002).

PKHD1 is amongst the largest disease genes identified to date in the human genome, with a complicated transcription profile that probably generates multiple variants of the fibrocystin protein. The *PKHD1* gene spans approximately 470 kb of genomic DNA, and an estimated minimum of 86 exons (12,222 bp) are assembled into a variety of alternatively spliced transcripts ranging from 9–16 kb (Losekoot et al. 2005; Onuchic et al. 2002). Both *PKHD1* and its mouse ortholog (*Pkhd1*) encode a complex and extensive array of splice variants, with most abundant transcriptional expression in fetal and adult kidney and weaker expression in other tissues including liver and pancreas (Nagasawa et al. 2002; Onuchic et al. 2002; Ward et al. 2002). Northern and reverse transcription/polymerase chain reaction (RT-PCR) analyses of the murine transcript have also detected low level expression in the lung, testis, vascular smooth muscle, sympathetic ganglia, and trachea, most likely the result of alternative splice variants (Nagasawa et al. 2002). The degree of alternative splicing and differential tissue-specific expression of the various splice forms requires further analysis.

The longest *PKHD1* transcript includes 67 exons with an ORF composed of 66 exons extending from exons 2–67 and encodes a unique 4074-amino-acid protein. The longest ORF of the mouse ortholog (*Pkhd1*) encodes a protein of

4059 amino acids; the mouse and human protein sequences are 73% identical overall and 55% identical in the carboxyl-terminal tail (Nagasawa et al. 2002). Although both the human and mouse proteins have large ORFs, the expressed proteins appear to represent a number of splice variants with tissue-specific patterns of expression (Ward et al. 2003). In addition, a truncated form of PKHD1, missing the final seven exons and a novel terminal exon, has been reported (Xiong et al. 2002).

Characterization of the PKHD1 mutational spectrum

An anticipated benefit from the identification of a human disease gene is the ability to understand the molecular basis of clinical variations in disease presentation, progression, and outcome. Genotype-phenotype correlations should enhance the understanding of the molecular pathogenesis of the disease and provide improved patient care by predicting disease progression. However, the combination of the large size of *PKHD1*, allelic heterogeneity, the high level of missense mutations, and the complicated pattern of splicing poses significant challenges to DNA-based diagnostic testing.

The mutation detection rate obtained in separate studies has varied substantially, from 42% to 65%, based primarily on the cohort of patients screened within the respective studies (Bergmann et al. 2003, 2004a,b; Onuchic et al. 2002; Ward et al. 2002). These studies have demonstrated that mutations are scattered throughout the gene without evidence of clustering at specific sites, and neutral polymorphisms are common. Most mutations are unique to single families (“private mutations”), and most affected patients represent compound heterozygotes. Approximately 45% of the changes are predicted to truncate the protein. All missense mutations are non-conservative, with the affected amino acid residues found to be conserved in the murine fibrocystin ortholog. One recurrent mutation, thr36 to met (T36M; 606702.0001), is thought to represent a mutation hotspot and has been found in a variety of populations (Bergmann et al. 2003). Two founder mutations, arg496 to thr (R496×; 606702.0007) and val3471 to gly (V3471G; 606702.0008), comprise approximately 60% of PKHD1 mutations in a Finnish population (Bergmann et al. 2003).

However, more recent studies with new algorithms have yielded detection rates of 85% for the entire clinical spectrum of ARPKD patients (Bergmann et al. 2005a,c; Losekoot et al. 2005; Sharp et al. 2005). Strategies based on denaturing high performance liquid chromatography (DHPLC) and PCR have been successfully used for mutation screening. In general, sequence analysis, combined with haplotype analysis in multiply affected families, has proven to provide the most reliable and comprehensive results. To date, however, only micromutations in the 66 exons encoding the longest ORF have been described and account for approximately 85% of mutations (Bergmann et al. 2005b). As of January 22, 2006, 305 different PKHD1 micromutations (point mutations and small deletions/duplications/insertions) on 670 mutated alleles had been listed in the locus-specific database http://www.humgen.rwthaachen.de).

Bergmann et. al. recently described the use of DHPLC for alternatively spliced exons and quantitative real time polymerase chain reaction to detect genomic imbalances (Bergmann et al. 2005b). In 58 patients, three different heterozygous PKHD1 deletions and several single nucleotide changes in alternatively spliced exons were identified, suggesting that gross deletions in PKHD1 account for a detectable proportion of ARPKD cases.

Prenatal diagnosis and genotype-phenotype correlations

Because of the significant morbidity and mortality of ARPKD, many parents of ARPKD children seek prenatal diagnosis to guide future family planning. Prior to the identification of the PKHD1 gene, prenatal diagnosis was only feasible by indirect genotyping (haplotype-based analysis). However, interpretation of haplotype-based analysis is difficult in cases without an unambiguous clinicopathologic diagnosis. If parental renal, hepatic, or pancreatic ultrasound reveals cyst formation, severe, early onset ADPKD must be considered. Thus, in families with diagnostic uncertainties, characterization of PKHD1 mutations by direct sequencing is the only option for accurate genetic counseling and prenatal diagnosis. Prenatal diagnoses of ARPKD based on PKHD1 mutation analysis has been successfully accomplished (Zerres et al. 2004), and clinical gene-based testing will probably become more widely available as the number of diagnostic centers increases. In addition, in vitro fertilization following preimplantation genetic diagnosis will also become available.

Although the improved algorithms provide high detection rates, the ability definitively to assess the likelihood of micromutations being pathogenic is still lacking. In order to predict whether a variant is likely to be disease-causing, several factors are taken into account: evolutionary conservation of the amino acids, class of amino acid, no other mutations in cis, co-segregation with the disease, and/or previously published pathogenic variant.

Obviously, the correct classification of missense variants as pathogenic mutations or harmless polymorphisms is crucial for a correct diagnosis. In addition, the roles of the various splice forms in determining disease severity have yet to be determined. A potential biological function of the alterations identified in alternatively spliced exons must await the confirmation and definition of transcripts con-

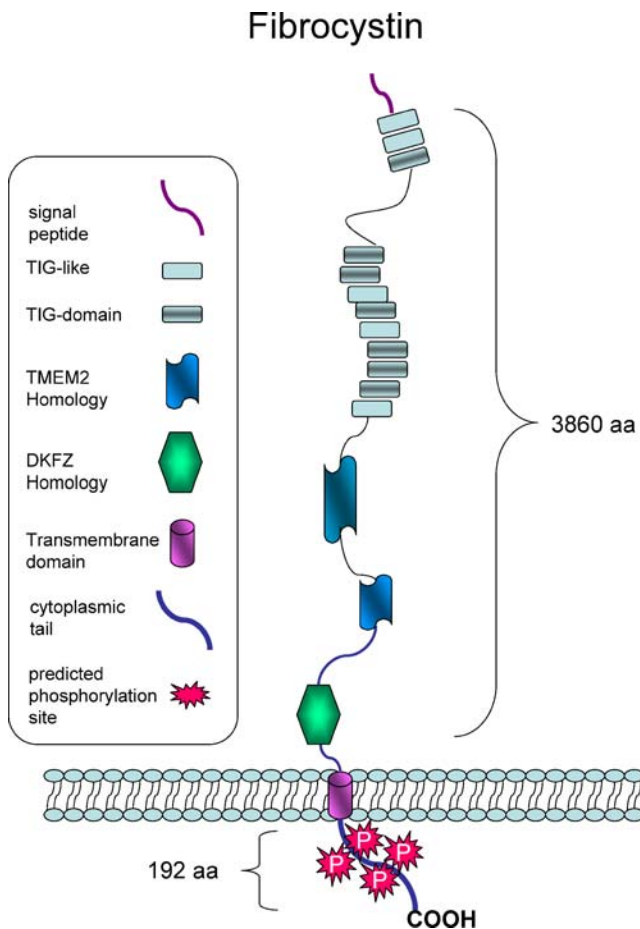

Fig. 1 Predicted structure of fibrocystin, a large integral membrane protein, with the largest known open reading frame, translating a 4074-amino-acid novel peptide (4059 amino acids in mouse). Fibrocystin has homologies to several known proteins, including TIG/IPT (immunoglobulin-like fold shared by plexins and transcription factors) or TIG-like domains in the extracellular region. This domain has 80–100 amino acids and is found in several receptor molecules, including Met, various plexins, and Ron, although the large number of domains seems unique to the fibrocystins. The structure of fibrocystin as an integral membrane protein with a large extracellular portion containing multiple glycosylation sites, and a short intracellular C-terminal domain containing potential protein kinase A and PCK phosphorylation sites suggest that this protein acts as a transducer of extracellular information into the cell by eliciting signal transduction cascades resulting in the modulation of gene transcription (*aa* amino acids)

taining these alternative spliced exons and their predicted reading frames. Continued cataloging of mutations, especially pathogenic missense variants, and the resulting disease phenotypes will be required to define genotype-phenotype correlations fully.

However, the bulk of mutational data currently available permit broad categorization of missense mutations as severe, moderate, or mild changes. Therefore, genotype-phenotype correlations can be drawn for the type of mutation rather than for the site of individual mutations. All patients carrying two truncating mutations display a severe phenotype with peri- or neonatal demise (Bergmann et al. 2003), whereas patients surviving the neonatal period have, on average, at least one missense mutation (Furu et al. 2003). This indicates that some missense changes may not entirely inactivate the product, but rather generate a hypomorphic allele. Additionally, some missense mutations may only disrupt specific splice forms of PKHD1 without affecting other functional variants (Rossetti et al. 2003).

Regulation of PKHD1 transcription

Insights into the tissue-specific transcriptional regulation of PKHD1 are becoming available with the recent discovery of an evolutionarily conserved transcription factor (TCF)-binding site in the proximal promoter of the mouse *Pkhd1* gene (Hiesberger et al. 2004). TCF-2, or hepatocyte nuclear factor-1β (HNF-1β), is a Pit-1/Oct-1/Unc-86 (POU)/homeodomain-containing transcription factor that regulates tissue-specific gene expression in the kidney, liver, pancreas, and other epithelial organs (Igarashi et al. 2005). Mutations of HNF-1β have been shown to produce maturity-onset diabetes of the young type 5 (MODY5) and are associated with congenital cystic abnormalities of the kidney. Igarashi et. al (2005) have recently demonstrated that kidney-specific deletion of HNF-1β by using Cre/loxP recombination results in renal cyst formation paralleled by a 70% decrease in *Pkhd1* expression. These studies demonstrate that HNF-1β is required for the development of the mammalian kidney, and that HNF-1β directly regulates the *Pkhd1* promoter.

The ARPKD protein, fibrocystin

Fibrocystin is a novel integral membrane protein of 4074 amino acids (4059 amino acids in the mouse) with a signal peptide, an extensive (3860 amino acid) highly glycosylated, N-terminal extracellular region, a single transmembrane (TM) domain, and a short (192 amino acid) cytoplasmic tail containing four putative cAMP/cGMP phosphorylation sites (Nagasawa et al. 2002; Onuchic et al. 2002; Ward et al. 2002; Fig. 1). However, Northern expression analysis suggests the likelihood that splice forms of PKHD1 generate alternative proteins, including secreted products (Nagasawa et al. 2002; Onuchic et al. 2002).

Alternatively spliced transcripts of PKHD1 are predicted to fall into two broad categories. The first is predicted to contain the single TM element but to vary with respect to the insertion of other peptide domains. The second category would include transcripts that lack the transmembrane-spanning domain and may therefore be secreted. This complex pattern of splicing has been found to be highly

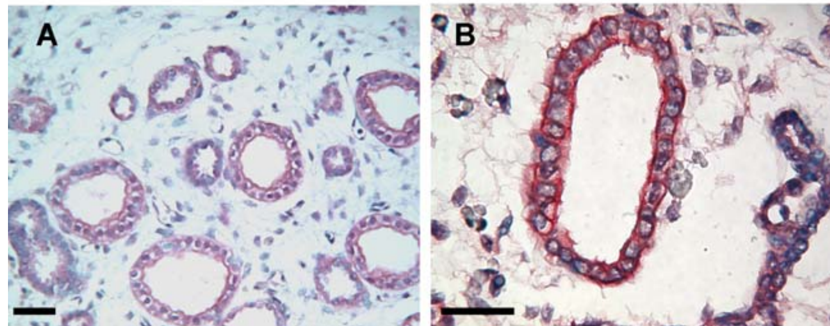

Fig. 2 Immunohistochemical localization of fibrocystin. Sections of human fetal kidneys from severe ARPKD demonstrate robust membrane localization on the apical and basolateral membranes of lectin-identified collecting tubular cysts with two different antibodies to fibrocystin. Cell and tissue lysates subjected to Western analysis demonstrate that both antibodies recognize equivalent MW proteins of >440 kDa and 200 kDa (data not shown). Thus, fibrocystin expression is not limited to cilia, basal bodies, or centrosomes and can be expressed even in severe ARPKD

conserved in the murine ortholog (Nagasawa et al. 2002). Multiple variants of fibrocystin generated from alternatively spliced transcripts of PKHD1 may provide a mechanism for regulating the temporal and spatial functions of fibrocystin isoforms in a tissue-specific manner (Onuchic et al. 2002; Ward et al. 2002).

Fibrocystin has homologies to several known proteins; this provides some clues to the function of the protein. The large amino-terminal extracellular portion contains domains with TIG, TIG-like, TMEM2, and DKFZ homologs (Wang et al. 2004). The predicted extracellular domain contains 10–12 TIG/IPT (IgG-like, plexin, transcription factor) or TIG-like domains that are shared by RON, the hepatocyte growth factor receptor, MET, and the plexin superfamily involved in the regulation of cellular adhesion, repulsion, and proliferation. In addition, the extracellular region of fibrocystin contains TMEM2 and DFKZ homologies, plus multiple PbH1 (parallel beta-helix 1) repeats that may bind to carbohydrate moieties such as glycoproteins on the cell surface or in the basement membrane.

The function of this protein remains to be determined, but the structure and homologies suggest a role as a receptor protein involved in the regulation of cellular adhesion, repulsion, and proliferation and/or the regulation and maintenance of renal CT and HBD (Bergmann et al. 2003; Onuchic et al. 2002; Ward et al. 2002). Delineation of the normal role of fibrocystin in the development, differentiation, and maintenance of renal CT and HBD will provide important insights into the way that mutations in a single gene cause cyst formation with a wide variation in cellular and clinical phenotype.

When fibrocystin was initially identified, there were no known related proteins until the identification of fibrocystin-L (Hogan et al. 2003). This protein is encoded by the *PKHDL1* gene at chromosome region 8q23. Fibrocystin-L (4243 amino acids; 466 kDa) has homology (overall identity of 25.0% and similarity of 41.5%) within the extracellular region to fibrocystin. Despite the similarity of this protein to fibrocystin, mutation analysis indicates that it is not associated with ARPKD or any renal or biliary phenotype. Indeed, expression studies suggest a possible role in cellular immunity with up-regulation in activated T-cells (Hogan et al. 2003).

Fibrocystin expression

During embryogenesis, *PKHD1* is expressed in epithelial derivatives, including ureteric bud branches, intra- and extra-HBD, and pancreatic ducts (Menezes et al. 2004; Ward et al. 2003). This temporal and spatial expression pattern of *PKHD1* parallels the CT and biliary epithelial pathology of ARPKD.

Immunohistohemical analysis demonstrates that fibrocystin is expressed along the apical and upper lateral cell surface and within the cytoplasm in cortical and medullary CT epithelia (Menezes et al. 2004; Nagano et al. 2005; Wang et al. 2004), biliary and pancreatic ductal epithelia, and thick ascending limbs of Henle (Menezes et al. 2004; Zhang et al. 2004). Immunofluorescence studies have revealed that, like other cystoproteins including the ADPKD proteins PC-1 and PC-2, fibrocystin co-localizes in centrosomes, basal bodies or primary apical cilia in renal epithelial cells (Menezes et al. 2004; Wang et al. 2004; Ward et al. 2003; Zhang et al. 2004) and in the cilia of cholangiocytes of intra-HBD (Masyuk et al. 2003).

The precise pattern of staining appears to vary with the stage of cellular differentiation (Menezes et al. 2004;

Nagano et al. 2005; Zhang et al. 2004). This variation may be attributable solely to differences in antibody specificity and technical aspects of the fixation and/or staining procedures employed. Alternatively, differences in antibody specificity combined with the temporal and spatial expression of alternatively spliced isoforms of fibrocystin may represent a regulatory mechanism inherent to the biological function of fibrocystin in renal and biliary epithelia.

The general genotype-phenotype correlations outlined above suggest that severe manifestations of ARPKD are generally attributable to the presence of two truncating mutations in *PKHD1* resulting in the absence of fibrocystin expression. This appears to be supported by study reports that ARPKD tissue samples lack fibrocystin expression (Ward et al. 2002; Zhang et al. 2004). Evidence from our laboratory does not support this generalization. Immunohistochemical staining of freshly processed renal samples from severely affected ARPKD patients with well characterized monoclonal antibodies generously provided by Dr. Christopher Ward (Ward et al. 2003) and affinity-purified polyclonal antibodies generated in our laboratory has demonstrated equivalent robust fibrocystin expression in CT cystic lesions (Fig. 2). These results suggest that not all severely affected patients lack fibrocystin expression and that collection, processing, and fixation are critical variables that may hamper the detection of fibrocystin. Additional immunohistological and Western analysis, especially from severely affected patients, in conjunction with mutational analysis, may provide important insights into the complexity of temporal and spatial expression of fibrocystin splicing variants.

Cilia and multimeric protein complexes in PKD

Observations initially made in animal models of RCD showing that protein products of cyst-causing genes, such as polaris (*Tg737*), cystin (*cpk*), and inversin (*Nphp2*), were in part localized to the primary cilia of renal epithelia led to the “primary cilia” hypothesis (Guay-Woodford 2003). Simply stated, the hypothesis is that structural or functional abnormalities in the primary apical cilia of tubular epithelia play a role in renal cyst development (Pazour and Rosenbaum 2002). This suggests that the cilia serve as an organizing center for the early steps of signal transduction pathways that are responsible for monitoring the integrity of the nephron and bile ducts and participate in regulating epithelial proliferation and differentiation.

Structurally, cilia consist of a microtubule-based axoneme covered by a specialized plasma membrane. The ciliary axoneme is built from one of two basal bodies that form the core of the centrosome and extend from the cell surface into the extracellular space (Praetorius and Spring 2005). Cilia are defined as motile, primary, or nodal (for a comprehensive review, see Praetorius and Spring 2005).

Primary cilia are non-motile hair-like structures that emerge as single projections from one of the two basal bodies or centrioles (Pazour 2004). Researchers have recognized for decades that individual cilia appear, at least temporarily, on most cells in the body, but most cell biologists have dismissed them as evolutionary remnants. This assertion has recently been challenged by the observations that the three proteins associated with human PKD, including the ADPKD proteins, PC-1 and PC-2, and the ARPKD protein fibrocystin, are expressed in cilia and in other apical, lateral, and basal locations.

Additional cystoproteins responsible for non-PKD forms of human RCD, such as nephronophthisis (nephrocystin-1, nephrocystin-2, and nephrocystin-4) and Bardet-Biedl syndrome (BBS 1–8), are also partially expressed in the basal bodies and/or the primary cilia (Hildebrandt and Otto 2005). These findings indicate a striking association between proteins that are involved in RCD (cystoproteins) and cilia (Igarashi and Somlo 2002).

Additional compelling data linking cilia structure and/or function in renal cyst formation cyst development have been reported in studies of mice with mutations in *Kif3a*. Lack of *Kif3a* in mice produces embryonic lethality; however, conditional disruption of the floxed allele of *Kif3a* in the kidney gives viable offspring with cystic lesions in the distal nephron and CTs, specifically where cre recombinase is expressed (Lin et al. 2003). This study has demonstrated that tissue-specific inactivation of *Kif3A* in renal tubular epithelial cells results in viable offspring that develop rapidly progressive CT cystic lesions leading to ESRD and death by postnatal day 21. The epithelial cells lining the cystic lesions exhibit the distinctive abnormal cellular phenotype associated with PKD, including increased proliferation, apical mislocalization of the epidermal growth factor receptor, and increased expression of β-catenin and c-Myc, and inhibition of $p21^{CIP1}$ (Lin et al. 2003).

The pathogenic link between cystoprotein expression in cilia, basal bodies, and centrosomes, on the one hand, and the renal cystic phenotype, on the other hand, remains unknown. However, recent studies have demonstrated that physical manipulation of the primary cilium, including bending or removal, elicits changes in Ca^{2+} flux (Praetorius et al. 2003, 2004; Praetorius and Spring 2001, 2003a,b) demonstrating that cilia can function as mechanosensors or chemosensors to sense fluid movement or ionic composition in the kidney. The physiological consequence of alterations of calcium concentrations in the renal CT epithelium remains to be clearly defined, particularly in ARPKD. Of note, the only binding partner for fibrocystin identified to date is calcium-modulating

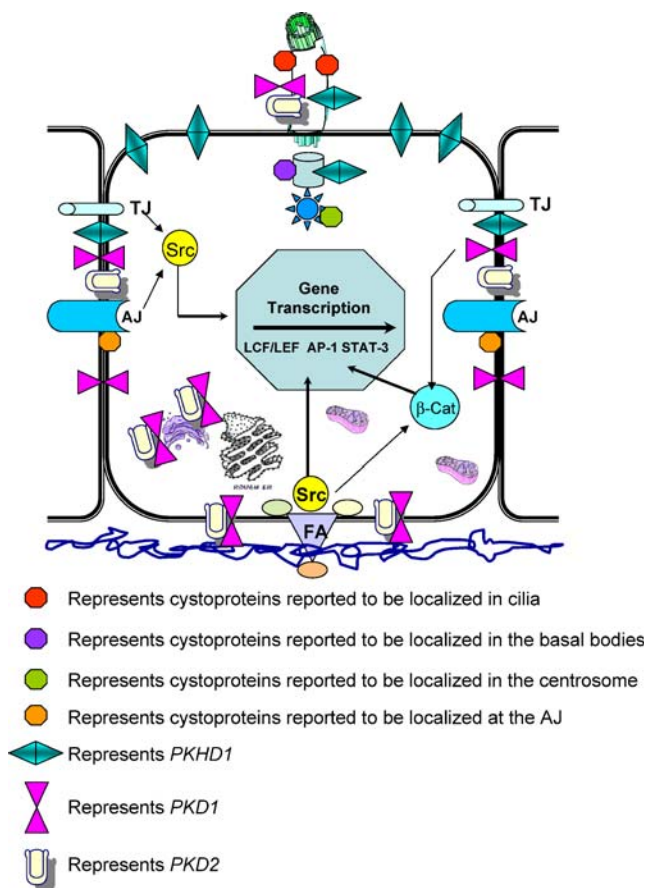

Fig. 3 PKD proteins in multimeric complexes. Summary of recent advances suggesting that cystoproteins exist in multimeric protein complexes and function at distinct sites within epithelial cells that include: basal cell-matrix focal adhesion sites to regulate epithelial cell adhesion and migration; apical-lateral cell-cell adherens sites to regulate differentiated cell shape; within Golgi; apical-central ciliary sites to sense flow and regulate tubule diameter. Expression and signaling from multiple sites establishes signaling platforms and feedback loops that regulate cell growth, proliferation, differentiation, and dedifferentiation. Elucidation of the dynamic regulated signaling from these multiple sites are a current focus of investigation (*AJ* adherens junction, *TJ* tight junction)

cyclophilin ligand, a protein involved in Ca^{2+} signaling (Nagano et al. 2005).

Cyst development and growth is a complex multi-mechanistic process. Immunohistological and immunofluorescent analyses of fibrocystin expression demonstrate that fibrocystin is only partially localized to the basal body and primary ciliary of renal epithelia and that, like ADPKD proteins PC1 and PC2, fibrocystin is localized at additional sites. The expression of PKD proteins in cilia, basal bodies, and intercellular junctions and at focal adhesions suggests that there may be common signaling pathways for cyst formation through the abnormal integration of signal transduction pathways, as shown in Fig. 3 (Nauli et al. 2003; Wilson 2004a).

Cellular pathophysiology of ARPKD, with comments on ADPKD

Despite the identification of the genes responsible for ADPKD and ARPKD, the precise function of these genes and their protein products remains incompletely characterized, because of the novel attributes of the genes (including the complexity of their structure), the large size of *PKD1* and *PKHD1*, and the multiple transcripts produced by these genes. Despite these difficulties, the development of antibodies to PKD proteins coupled with the study of non-orthologous animal models of PKD have provided important insights regarding cystogenic pathways and cellular pathophysiology common to many cystic diseases (Guay-Woodford 2003; Nauli and Zhou 2004; Pazour et al. 2000, 2002; Phillips et al. 2004; Praetorius et al. 2003; Sun et al. 2004; Witzgall 2005; Yoder et al. 2002).

PKD cystic renal epithelia share common phenotypic abnormalities despite the different genetic mutations that underlie the disease. Numerous animal models and in vitro cell culture systems utilizing human and animal CT cells have established that the development of PKD is characterized by a switch from a well-differentiated non-proliferative reabsorptive epithelia to a partially dedifferentiated, secretory epithelia characterized by polarization defects and high rates of proliferation and apoptosis (Dell et al. 2004; Harris and Rossetti 2004; Murcia et al. 1999; Wilson 2004a,b). This is a critical functional change, since mathematical modeling of epithelia demonstrates that proliferation and secretion are necessary and sufficient to account for cyst growth in PKD (Welling and Grantham 1991). The fluid secreted from these abnormal cells is rich in cytokines, lipid factors, and epithelial growth factors, which further stimulate epithelial proliferation resulting in significant ductal ectasia (Grantham et al. 1995; MacRae Dell et al. 2004). Cultured epithelial cells from patients or animal models of PKD have consistently demonstrated an increased intrinsic capacity for proliferation and survival (Gabow 1993; Grantham 1996, 1987; Wilson 2004a).

cAMP-mediated proliferation

Mounting evidence suggests that the adenylyl cyclase-adenosine 3′,5′-cyclic monophosphate (cAMP) pathway promotes both fluid secretion and cell proliferation in both ADPKD and ARPKD renal epithelia. Mutated PKD proteins are thought to disrupt intracellular Ca^{2+} homeostasis or Ca^{2+} signaling leading to cellular dedifferentiation and hyperproliferation through an abnormal cAMP-mediated proliferative pathway.

In normal human and mouse renal epithelial cells, cAMP has been shown to inhibit the Ras/Raf-1/MEK/ERK pathway at the level of Raf-1 and to decrease cell proliferation. In

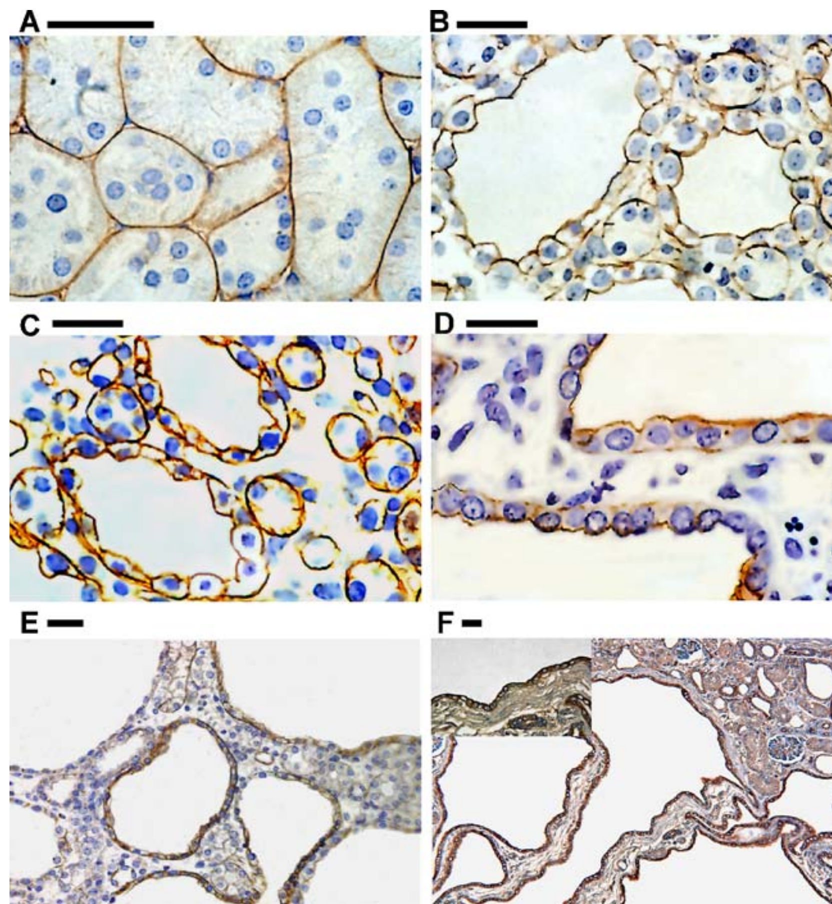

Fig. 4 Immunohistochemical localization of ErbB family receptors. **a** Basolateral staining of EGFR (ErbB1) in a normal murine kidney section. **b–d** Cystin (CPK) deficiency, B-cell progenitor kinase (BPK) deficiency, and human ARPKD, respectively, demonstrate increased expression and apical mislocalization of EGFR (ErbB1) in lectin-identified collecting tubules. Note normal basolateral localization in non-cystic tubules within sections in **b**, **c**. **e**, **f** PKD and human ARPKD, respectively, demonstrate high expression and apical localization of ErbB2. *Bars* 0.1 mM

contrast, cAMP has been shown to stimulate B-Raf and activate the MEK/ERK pathway in ARPKD and ADPKD cells leading to increased cell proliferation (Grantham 1997a,b; Sullivan and Grantham 1996; Wallace et al. 2002; Yamaguchi et al. 2000, 2003, 2004, 2006). Therefore, cAMP can be either mitogenic or anti-mitogenic.

In renal epithelia, the switch in cAMP from an anti-mitogen to a mitogenic stimulus has been shown to be directly correlated with intracellular calcium levels $[Ca^{2+}]i$ (Yamaguchi et al. 2004). The PI 3-K/Akt pathway has been shown to regulate cAMP-dependent activation of B-Raf/MEK/ERK and cell proliferation in CT principal cells (M-1; Yamaguchi et al. 2003). Phosphorylation of B-Raf by Akt has been shown to regulate B-Raf activity negatively (Guan et al. 2000; Zhang and Guan 2000). Yamaguchi et. al (2004) have demonstrated that reducing intracellular $[Ca^{2+}]$ i in immortalized mouse M-1 CT cells or primary human kidney epithelial (NHK) cells for 3–5 h converts both cell types from a normal cAMP growth-inhibited phenotype to an abnormal cAMP growth-stimulated phenotype, characteristic of PKD renal epithelia. Reduction of $[Ca^{2+}]i$ decreases Akt activity, allows cAMP-dependent B-Raf activation, and stimulates cell proliferation. Direct inhibition of either PI 3-K or Akt causes cAMP-dependent ERK activation and cell proliferation, thus inducing a phenotypic switch that imitates Ca^{2+} restriction (Yamaguchi et al. 2004). Pharmacological elevation of Ca^{2+} increases P-Akt levels, whereas Ca^{2+} channel blockers decrease P-Akt (Yamaguchi et al. 2006). This observation that the reduction of intracellular $[Ca^{2+}]$ in M-1 or NHK cells with calcium channel blockers replicates the abnormal proliferative response of PKD cells to cAMP establishes a link between the PKD proliferative phenotype, reduction in $[Ca^{2+}]i$, and cAMP activity. Evidence for the role of $[Ca^{2+}]i$-mediated proliferation has been further strengthen by studies showning that the elevation of $[Ca^{2+}]i$ levels in ADPKD and ARPKD cultured cells increases Akt activity and blocks cAMP-dependent B-Raf and ERK activation (Yamaguchi et al. 2006). The study has also demonstrated that increases in $[Ca^{2+}]i$ restore the normal anti-mitogenic response to cAMP

in renal cells derived from either human ADPKD or ARPKD kidneys. These data suggest that the PKD proteins (PC1, PC2, and fibrocystin) play a role in maintaining Ca^{2+} homeostasis. Mutations in any of the PKD proteins may lead to reduce $[Ca^{2+}]$i and activate the cAMP mitogenic pathway.

Epidermal growth factor receptor axis-mediated proliferation

Evidence from a number of laboratories has demonstrated a significant role for the epideraml growth factor receptor (EGFR) axis in promoting epithelial hyperplasia in cystic epithelia, with resultant renal cyst formation and progressive enlargement in both murine and human ADPKD and ARPKD (Du and Wilson 1995; Gattone et al. 1996; Lowden et al. 1994; Lu et al. 1999; Nauta et al. 1995; Neufield et al. 1992; Orellana et al. 1995; Pugh et al. 1995; Richards et al. 1998; Sweeney and Avner 1998; Sweeney et al. 2003; Torres 2004). Renal cystic epithelia demonstrate both quantitative (overexpression) and qualitative (mislocalization) expression of one or more members of the ErbB family of receptors as shown in Fig. 4. In addition, evidence from rodent models (including the orthologous polycystic kidney rat; PCK rat) suggests that similar abnormalities of the EGFR axis may mediate biliary epithelial hyperplasia and biliary ductal ectasia (Nauta et al. 1995; Sato et al. 2005; Sweeney et al. 2000, 2003). EGF has an important role in the expansion of renal cysts. Cystic epithelial cells from patients with ARPKD or ADPKD are unusually susceptible to the proliferative stimulus of EGF. Moreover, cyst fluid of patients contains mitogenic concentrations of EGFR ligands, which are secreted into the lumen of cysts in amounts that can induce cellular proliferation (Klingel et al. 1992; Lakshmanan and Fisher 1993; Neufield et al. 1992; Rohatgi et al. 2004; Sullivan et al. 1998; Sweeney and Avner 1996; Ye et al. 1992). In all animal models studied to date, abnormalities in the expression and localization of members of the EGFR axis have been reported. Cyst-causing genes on different chromosomes, irrespective of whether they are orthologous with human PKD genes, result in cystic epithelia with EGFR-axis abnormalities. This includes the previously noted *Kif3A* cystic model that has a tissue-specific inactivation of *Kif3A* in renal tubular epithelial cells. As previously noted, *Kif3A* inactivation results in cystic lesions lined by epithelia that demonstrate pronounced apical expression of EGFR.

A recently developed murine model of ADPKD that produces reduced levels of full-length *Pkd1*-encoded protein, PC-1, develops renal cystic lesions suggesting that reduced *Pkd1* expression is sufficient for renal cyst formation in ADPKD. This model demonstrates up-regulation of heparin-binding epidermal growth factor-like growth factor accompanied by increased protein levels and activity (phosphorylation) of EGFR (Jiang et al. 2006).

These data indicate that abnormalities in the EGFR axis are a common cellular phenotype downstream from a number of different primary gene mutations. Despite interesting speculations, the precise mechanisms of the striking relationship remain unknown (Dell et al. 2004; Du and Wilson 1995; Grantham 2000; Lu et al. 1999; Sweeney et al. 2003; Wilson 2004b).

The overexpression and abnormally located EGFRs on the apical (luminal) surface of cyst-lining epithelia in human ARPKD and murine models have been shown specifically to bind EGF/TGF-α with high affinity, to autophosphorylate, and to generate mitogenic signals in response to EGF and/or cyst fluid (Sweeney and Avner 1996, 1998). This creates a sustained cycle of autocrine-paracrine stimulation of proliferation in cysts similar to that seen in many forms of cancer.

Secretion

ADPKD cysts have a fundamental structural difference from ARPKD cysts. ADPKD cysts rapidly close off from urinary flow and continue to expand by transepithelial secretion. As stated above, ARPKD cysts remain open with respect to the entire nephron, maintaining both afferent and efferent tubular connections. However, secretion is still a necessary element of cyst formation in ARPKD, and the difference in cyst structure suggests a different secretory mechanism.

In ADPKD, the weight of the evidence indicates that Cl^- is secreted via a cAMP-mediated co-transport mechanism in the basolateral membrane and the cystic fibrosis transmembrane conductance regulator (CFTR) in the apical membrane, leading to expansion of cysts, especially after they have detached from the nephron of origin (Belibi et al. 2004; Sullivan and Grantham 1996). This does not appear to be the case in ARPKD. By using a genetic complementation approach, the BPK mouse (deficient in B-cell progenitor kinase) has been crossed with a CFTR knockout mouse (Clarke et al. 1992). The results demonstrate that the absence of CFTR does not alter the course of renal or biliary cyst development or growth (Nakanishi et al. 2001).

ARPKD cystic epithelia have been shown to exhibit net fluid secretion through a decrease in principal cell sodium absorption. This is attributable to an EGF-mediated decrease in the alpha-subunit of the epithelial Na channel (Veizis et al. 2004; Veizis and Cotton 2005). However, conflicting data has been reported (Rohatgi et al. 2003).

Biliary epithelia in ARPKD have been shown to be hyperproliferative in response to EGF (Nauta et al. 1995; Sato et al. 2005). However, the effect of EGF hypersentivity in biliary epithelia on secretory mechanisms is unclear.

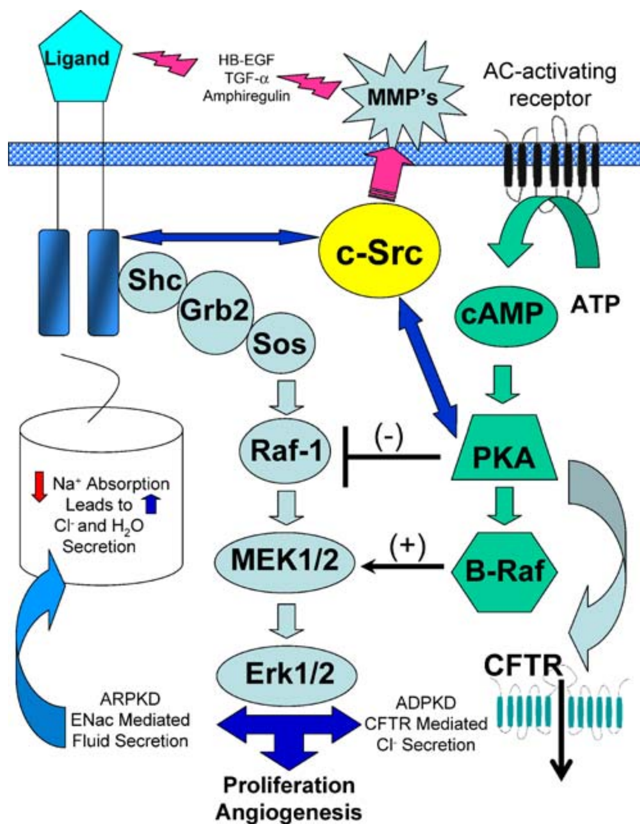

Fig. 5 Signaling pathways involved in PKD pathogenesis. Both cAMP- and EGFR-axis-mediated pathways have been shown to be involved in the development and progression of PKD. Representation of the way that these pathways may intersect, and the manner in which c-Src (pp66Src) may act as a downstream intermediate of both the cAMP- and EGFR-axis-mediated pathways. Proteins such as c-Src that interact with critical pathophysiological pathways and exist at multiple locations in proximity to multermeric polycystic protein complexes may provide attractive therapeutic targets (*ENac* epithelial Na channel, *CFTR* cystic fibrosis transmembrane conductance regulator, *PKA* protein kinase A, *MMP's* matrix metalloproteinases, *HB-EGF* heparin-binding epidermal growth factor-like growth factor, *AC* adenylate cyclase, *MEK* MAP/ERK kinase)

Translational implications

A critical test of the veracity of proposed pathophysiology is the effectiveness of targeted therapy on modulating disease. To date, no specific therapies that limit renal cyst development and progressive enlargement or biliary ductal ectasia in ARPKD are in clinical use (Davis et al. 2001). However, the studies noted above suggest that therapeutic targeting of the cAMP and EGFR-axis abnormalities documented in cystic epithelia may translate into effective therapies for the proliferation and secretory abnormalities of ARPKD (and, by analogy, ADPKD).

To date, decreasing cAMP through vasopressin V2 receptor (VPV2R) antagonism in renal CTs has demonstrated efficacy in animal models of PKD orthologous to human diseases (ARPKD → PCK rat: adolescent nephronophthisis → pcy mouse; ADPKD → pkd2$^{ws25/-}$ mouse; Gattone et al. 2003; Torres 2005; Torres et al. 2004). However, because of the restricted expression of the VPV2R on renal principal cells, such approaches are limited to the renal lesions of nephronophthisis and may provide only partial benefit in such multisystem diseases as ADPKD, and particularly ARPKD.

Therapeutic targeting of the EGFR axis has promise in ADPKD, particularly in the dual renal and biliary cell abnormalities in ARPKD. To date, genetic complementation to decrease EGFR (Richards et al. 1998), the inhibition of EGFR, c-ErbB2, and c-ErbB4 through small molecule covalent inhibitors (Dell et al. 2001; Sweeney et al. 2000, 2003), and the inhibition of EGFR ligand availability, alone and in combination with EGFR tyrosine kinase activity inhibitors (Dell et al. 2001; Sweeney et al. 2003) have demonstrated dramatic efficacy in ameliorating renal CT abnormalities, biliary ductal ectasia, and periportal fibrosis in a variety of rodent ARPKD models. Similarly, therapeutic targeting of substrates downstream from the EGFR, such as MAPK/MEK5/ERK5/MEK1/2 are effective in ameliorating biliary dysgenesis of the PCK rat, in vitro (Sato et al. 2005).

A particularly promising approach appears to be the specific targeting of downstream intermediates of both the cAMP- and EGFR-axis-mediated pathways. As shown in Fig. 5, cSrc (pp66Src) is one such component that interacts with critical pathophysiological pathways and exists at multiple locations in proximity to multermeric polycystic protein complexes. Preliminary studies targeting cSrc with a specific inhibitor (WY-606; Boschelli et al. 2005a,b) have been particularly effective in ameliorating both renal and biliary epithelial abnormalities in ARPKD models (Gunay-Aygun et al. 2006).

Concluding remarks

The delineation of the molecular and cellular pathophysiology of ARPKD has led to a new era of therapeutic innovation through targeting specific abnormalities of cystic epithelia. Such innovations bring great hope to patients with RCD, and particularly children devastated by the dual organ pathophysiology of ARPKD.

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