Cystic kidney diseases: learning from animal models

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Introduction

Renal tubular cysts arise in several inherited human disorders which include autosomal dominant polycystic kidney disease (ADPKD), as well as rarer disorders such as autosomal recessive polycystic kidney disease (ARPKD) nephronophthisis and medullary cystic kidney. Despite their genetic, clinical and histopathological heterogeneity, all these diseases involve a dilation of tubules leading to cyst formation. This suggests that the gene defects underlying these cystic disorders might disrupt common molecular pathways.

Numerous mouse and rat polycystic kidney disease (PKD) models have been described over the last few years. Some of these models are the result of spontaneous mutations; others were generated through random mutagenesis or gene targeting of mouse PKD orthologue genes. Despite not strictly reproducing the human disease with respect to disease progression and cyst localization, these animal models have provided new insights into the mechanisms underlying cyst formation.

Rodent models of cystic diseases: not strict phenocopies of human diseases

Most of the spontaneous rat and mouse cystic disease models are transmitted as autosomal recessive, monogenic disorders. With respect to cyst localization and disease progression, many of them reproduce phenotypic abnormalities that resemble human ARPKD, with massive cyst formation and rapid progression towards renal insufficiency. Surprisingly, in some cases, the phenotypes differ significantly between rodents and humans. Some gene mutations that are responsible for ARPKD or nephronophthisis in humans give rise to a rodent phenotype that corresponds to ADPKD. For example, mice carrying homozygous pcy (polycystic) mutations were initially proposed as an ADPKD model due to slow disease progression, cyst localization and the occurrence of cerebral vascular aneurysms [1]. However, analysis of the pcy locus showed that the mutated gene is the mouse orthologue of the human \textit{NPHP3} gene, which is responsible for the adolescent form of nephronophthisis [2]. Similarly, in the rat strain Crj:CD/SD, homozygous pck (polycystic kidney) mutants develop renal cysts 1 week after birth and biliary dilation with marked hepatomegaly [3]. The phenotypic alterations of the pck rat model were proposed initially as a model of human ADPKD. Nonetheless, genetic studies have shown that \textit{Pkhd1}, the rat orthologue of the \textit{PKHD1} gene that is responsible for ARPKD in humans, is disrupted in this model [4]. One explanation for this phenotypic discrepancy is that the nature of the mutation is different between humans and rodents. Indeed, genetic studies have demonstrated that different mutations within the same gene can be responsible for different phenotypes. For example, the \textit{Bicc1} gene encoding Bicaudal C is disrupted in two mouse strains. In \textit{bpk} (BALB/c polycystic kidney) mice, homozygous mutants develop renal cysts and biliary dysgenesis and die within 4 weeks after birth [5]. In \textit{jcpk} (juvenile congenital polycystic kidney) homozygous mice, the phenotype is more severe, with cysts in all nephron segments and enlargement of biliary and pancreatic ducts [6]. Genetic analysis showed that the \textit{jcpk} mutation results in a truncated transcript, whereas the \textit{bpk} mutation affects the 3' region of the \textit{Bicc1} mRNA [7]. Despite not strictly reproducing human disease with respect to disease progression and cyst localization, these models provide new clues about cyst formation.

Identification of new genes

Several new genes involved in cyst formation have been identified by means of rodent models. For example,
a mutation in *Nek8*, a gene encoding the NIMA (never in mitosis-A)-related kinase 8 and located in the *jck* (juvenile cystic kidney) locus, is responsible for slowly progressing cyst development in early postnatal life [8]. A mutation in the gene encoding *Nek1*, another kinase of the same family, was recently identified in ‘kidney, anaemia, testes’ (*kat*) mice [9]. These mutants exhibit a pleiotropic phenotype, which includes renal cystic lesions, facial dysmorphism, dwarfism, male sterility, anaemia and choroid plexus cysts. In the *cpk* (‘congenital polycystic kidney’) model, a mutation in the *Cys1* gene, encoding cystin, is responsible for massive renal cystic disease and rapid progression to renal insufficiency [10]. Thus, mouse models can provide clues to genes involved in cystic diseases. These genes probably represent new candidates for cystic kidney diseases in patients that lack mutations in known disease genes.

**Phenotypic variability**

One characteristic of human ADPKD is the broad phenotypic variability of the disease, even between members of the same family. Predisposing genetic factors (modifier genes) may influence the expression of the mutated disease gene, and consequently alter disease severity. However, the nature of these modifiers is not as yet completely characterized. Similarly, in several murine models, the genetic background can affect the phenotype. For example, biliary disease is not penetrant in *cpk/cpk* mice in the C57BL/6J genetic background, but is observed in BALB/c, DBA/2J or CD1 backgrounds. Moreover, heterozygous *cpk* mice in a C57BL/6J background do not suffer from any disease, whereas some aged heterozygous mice of other strains develop biliary cysts [11]. Thus, careful analysis of mutant animals in different genetic backgrounds should help identify modifier genes that could be considered as candidates for human cystic kidney diseases.

**Primary cilia and cystogenesis**

Until recently, the importance of cilia in the pathogenesis of cyst formation was largely ignored. Analysis of mouse models has highlighted the pathogenetic importance of this organelle. Primary cilia are long, thin structures that are present on the surface of most cells. They emerge from the basal body, an intracellular organelle corresponding to one of the centrioles. The basic structure of the primary cilium consists of a central core (axoneme) composed of nine peripheral microtubule bundles and a cilium membrane continuous with the cell membrane. Renal tubular epithelial cells contain one primary cilium, in rare cases two, located at the apical pole of every cell type, except for intercalated cells (reviewed in [12]).

Studies in *orpk* (*Oak Ridge polycystic kidney*) mice suggested for the first time a role for the cilium in PKD. These mice carry a hypomorphic mutation of the *Tg737* gene encoding polaris, a protein localized to the ciliary axoneme and basal body [13,14]. Subsequent studies have shown that polaris is part of the intraflagellar transport machinery, which is critical for cilia assembly and maintenance. Homozygous *orpk* mice develop a heterogeneous phenotype that includes renal cysts, hepatic ductal plate malformations and pancreatic ductal hypoplasia. In these mice, the primary cilia in renal, biliary and pancreatic epithelia are severely stunted. Moreover, many of the proteins mutated in animal PKD models, such as cystin, KIF3A (a kinesin II subunit) [15] and Bicaudal C, are also localized to the primary cilium (reviewed in [16]). Notably, all the proteins involved in ADPKD, ARP KD and nephropathies were shown to be part of this organelle (reviewed in [17]).

Numerous studies have suggested that primary cilia act as a mechano-sensor of urinary flow in renal tubular cells. Fluid flow perception through the primary cilium affects tubular cell proliferation. In this way, anatomical and/or functional abnormalities of the cilia could contribute to aberrant tubular growth and cyst formation (reviewed in [18]). Primary cilia also play a role in left–right axis determination during embryonic development. Interestingly, random left–right asymmetry was observed in mice carrying a homozygous null allele of *Tg737*. In these mice, the defect was attributed to the absence of primary cilia in the ventral nodal cells [19].

**Transcriptional defects and cystogenesis**

Human diseases have demonstrated that disruption of a single gene can give rise to a cystic phenotype. One could hypothesize that this cystic phenotype might also be reproduced by mutation of a gene which regulates the expression of these cystogenes. Relatively little is known about the transcriptional networks in cystic kidney diseases that are controlled by these regulatory genes. A candidate for such a factor is Hepatocyte Nuclear Factor 1β (*HNF1β*), a homeodomain-containing transcription factor (reviewed in [20]) that is expressed in tubular epithelial cells of the kidney [21]. Heterozygous mutations in the human *HNF1β* gene are associated with a particular form of type II diabetes (maturity onset of diabetes of the young type 5/MODY5) and with diverse forms of non-diabetic nephropathies (for a review see [22] and associated Editorial). The most penetrant clinical feature of MODY5 patients is the presence of renal cysts. To circumvent the early embryonic lethality of *HNF1β-null* mice [23,24], a Cre-LoxP strategy was used to assess the roles of HNF1β during renal development [25]. The absence of HNF1β expression in medullar tubules led to a polycystic phenotype. Renal cyst formation was accompanied by severe defects in the transcriptional activation of *Pkhd1*, *Pkd2* and *Umod* (encoding uromodulin or Tamm Horsfall protein, mutated in MCKD type 2 [26]) genes.
Strikingly, a single defect in any one of these genes in humans is sufficient to elicit renal cyst formation. The combined defects in expression of these genes could be the origin of the massive cyst formation observed in this homozygous \( HNF1\beta \)-deficient mouse model. These results could also explain why carriers of autosomal dominant mutations in \( HNF1\beta \) develop renal cysts. Interestingly, \textit{de novo} mutations occur frequently in MODY5 patients, suggesting a genetic instability of the locus. To understand further dominant inheritance in humans, it would be interesting to test whether renal cysts in MODY5 patients develop upon focal loss of the residual wild-type \( HNF1\beta \) allele.

**Conclusion**

Rodent models of cystic diseases share several common pathogenic features with human diseases, without being strict phenocopies. These models are nevertheless essential tools for identifying the molecular pathways underlying cystogenesis. These pathways could provide specific therapeutic targets for the prevention of cyst formation.

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[See related article by Bingham and Hattersley (this issue, pp. 2703–2708)]

**References**