ARCHIVES OF DISEASE IN CHILDHOOD

The Journal of the British Paediatric Association

Annotations

Unravelling the pathogenesis of cystic kidney diseases

Human cystic kidney diseases comprise a large group of congenital and acquired disorders, many of which have a defined genetic basis. In none of these diseases do we fully understand the sequence of biological events which generates renal cysts and it is unclear whether there are common mechanisms of cyst formation in these diverse diseases. In this review we will highlight some recent advances in molecular and cell biology that are beginning to shed light on the causes of renal cysts. Based on this information, it is possible to conceive of rational treatments for human cystic kidney diseases and these will be also discussed. It is not our aim to exhaustively catalogue and classify cystic kidney diseases and for this background the reader is referred to recent reviews.[1 2]

The spectrum of cystic kidneys seen by paediatricians

Renal cysts are a prominent feature of many renal malformations.[1] A good example is provided by the multicystic dysplastic kidney which, in addition to large cysts, contains immature ducts surrounded by undifferentiated cells. Here nephrogenesis is abnormal so that mesenchymal precursor cells fail to differentiate into nephrons and the ureteric bud fails to branch serially to form the collecting duct system.[3] These kidneys have no excretory function and bilateral cases often die perinatally from renal failure or associated conditions such as lung hypoplasia and congenital heart disease. Unilateral multicystic dysplastic kidneys, however, may be clinically silent and can 'disappear', thus producing aplastic kidneys.[4] This process can be followed by serial ultrasound scans and it is increasingly apparent that this involution is more common than complications such as sepsis, hypertension, or malignancy.[5] Collectively, bilateral renal aplasias and dysplastic kidneys are the commonest causes of chronic renal failure in infancy, often in conjunction with congenital abnormalities of the urinary tract such as posterior urethral valves or reflux nephropathy.

In the so-called polycystic kidney diseases (PKDs), the major anatomical steps of nephrogenesis are complete but there are more subtle abnormalities which suggest that the epithelial cells lining the cyst lumens are not fully differentiated. These changes are discussed in detail below. Autosomal recessive disease (ARPKD) often causes renal failure in infancy or childhood, while autosomal dominant disease (ADPKD) accounts for 5–10% of end stage kidney failure world wide. While ADPKD usually presents in adulthood, it is increasingly recognised that this disease may present in the paediatric age range. In fact, a recent analysis suggests that some families with ADPKD show the phenomenon of anticipation, with earlier clinical presentations in successive generations.[6]

Multiple cysts may also arise in the kidneys of patients with chronic renal failure of any aetiology and have been reported to regress after successful transplantation.[7] A few cysts may be acquired as the normal kidney ages but these appear to be rare in childhood.

The genetics of dysplastic kidneys

Dysplastic kidneys usually occur sporadically and are often considered to arise as a result of prolonged ureteric or urethral obstruction. Experimental ligation of the lower urinary tract in the prenatal period can sometimes result in a mild form of renal cystic dysplasia.[8] It should, however, be noted that the same genes that direct the growth of nephrons and the collecting ducts are expressed during the development of the lower urinary tract,[3] suggesting that kidney and ureteric malformations could both result from a single genetic aberration. There is now convincing evidence from transgenic animals that 'knock-outs' or null mutations of the WTI[9] and RET[10] genes causes renal and ureteric malformations. WTI codes for a transcription factor expressed in kidney mesenchyme that orchestrates the expression of other nephrogenic genes and is mutated in a minority of Wilms' tumours. RET codes for a cell surface receptor that transduces growth signals between the renal mesenchyme and the ureteric bud. In humans point mutations of RET proto-oncogene are associated with multiple endocrine neoplasia type 2.

Similar genes which control nephrogenesis may eventually be found to be mutated in humans with dysplastic kidneys. This contention is supported by the occurrence of families who inherit dysplastic kidneys in an autosomal dominant manner.[1] In these kindreds the renal malformations may occur in isolation or form one component of a syndrome affecting multiple organs. It is therefore likely that at least some cases of multicystic dysplastic kidneys have a genetic basis and hence feasible that some sporadic cases may be due to new mutations.

Many gene mutations cause human PKD

Genetic linkage of an ADPKD locus to the α-globin gene on chromosome 16 was reported in 1985. It is only recently, however, that the PKD1 candidate gene has been isolated on 16p.13.3.[11] Mutations of this gene may account for up to 90% of ADPKD cases while another locus, on 4q13-q23, has been implicated in other patients. The normal function of the PKD1 gene is unknown and is difficult to surmise as there is no significant homology of its predicted protein product with known proteins. This year, human ARPKD was mapped to chromosome 6p21-cen, although the specific mutation is not yet defined.[12]

Both tuberous sclerosis and von Hippel-Lindau disease are inherited in an autosomal dominant fashion and may present with PKD. TSC2, a gene mutated in some patients with tuberous sclerosis, is expressed in a wide variety of tissues and its predicted protein product has homology to molecules implicated in the control of cell differentiation and proliferation.[13] Interestingly, it is located very close to PKD1 on chromosome 16. It has been postulated that the normal von Hippel-Lindau gene, located on chromosome 3p25-p26, acts as a tumour suppressor in a similar fashion to the WT1 transcription factor gene.[14]

From the above discussion it is apparent that PKDs, and probably also some multicystic dysplastic kidneys, are caused by mutations of diverse genes. Given that the specific roles of many of these genes, either during or after renal development, remains indeterminate, can we learn anything useful about cyst pathogenesis from the investigation of kidney cells themselves? Here most work has focused on the biology of epithelial cells from patients and animals with polycystic kidneys.

Abnormal proliferation and death in cystic kidneys

Cysts are fluid filled spaces surrounded by a layer of epithelial cells. The mitotic rate of normal adult tubular epithelia is very low but in a cystic kidney epithelial cells must be continually born to provide a lining for enlarging cysts. Epithelial cells derived from renal cysts divide faster than their normal counterparts even when isolated in the test tube. Transgenic mice which overexpress cellular and/or viral oncogenes also develop renal cysts.[15 16] The products of these genes, such as c-myc protein and the SV40 simian virus T antigen, stimulate cell division, directly implicating excessive cell proliferation in renal cystic disease. These transgenic animals sometimes develop small renal epithelial neoplasms, a tendency shared with some patients with cystic kidneys. Further evidence supporting a primary role of proliferation comes from a mouse model that closely resembles human ARPKD. Here the mutated gene has been defined and most likely codes for a protein with a role in cell division.[17]

Paradoxically, recent studies in mice have also directly implicated excessive cell death in the pathogenesis of cystic kidneys. Bcl-2 is a gene that prevents apoptosis (programmed cell death). Genetically engineered mice that do not express a functional Bcl-2 protein have increased renal apoptosis, develop extensive renal cysts and die from kidney failure.[18] Just how excessive death leads to cell proliferation and cyst formation remains a mystery. It is uncertain whether a similar mechanism is important in human diseases, although preliminary observations from our laboratory suggest that apoptosis is widespread in tissue between cysts in both multicystic dysplastic kidneys and in PKD.[19] Based on this evidence, excessive apoptosis would also explain the spontaneous involution seen in some cases of multicystic dysplastic kidneys, and may also contribute to the destruction of normal renal tissue in PKD.

'Upside down' cystic epithelia

Mature kidney epithelial cells are said to be 'polarised'. They have an apical plasma membrane that faces the tubule lumen and a basal plasma membrane facing the interstitium. Each is distinct in its biochemistry and morphology. Subtle aberrations in the polarity of the renal epithelia have been detected in both human and animal PKD.[20 21] Most notably the Na^+-K^+-ATPase ion pump is found on the apical membrane of PKD cells while it is normally situated in the basal membrane. This mislocalisation could contribute to cyst expansion by the active secretion of ions into the lumen. Similarly, other studies have found that ADPKD cysts contain high concentrations of amino acids that are normally pumped out of the tubular lumen.[22]

During nephron formation, the acquisition of polarity by nascent epithelia is directed by matrix proteins that envelop precursor mesenchymal cells.[23] Although biochemical and structural abnormalities have been detected in the basement membranes of cystic epithelia it is unknown whether such abnormalities are the prime movers or just epiphenomena of renal cyst formation.[24]

Renal cysts can be generated in the test tube using immortalised epithelial cell lines.[25] These models have implicated various ion pumps in cyst growth including Na^+-K^+-ATPase, Na^+-dependent H^+ and Ca^{++} transport as well as $Cl^-/HCO3^-$ and Na^+/H^+ exchangers. Additionally, drug treatments such as oubain, amiloride, and cAMP inhibitors slow cyst growth in vitro.

Treatments for cystic kidney diseases

The studies described above suggest specific aberrant biological processes that might be targeted using anticyst treatments. Instead, treatment of human PKD has been based on more general strategies used for the amelioration of progressive renal failure. The large multicentre Modification of Diet in Renal Disease Study found no benefit on the progression of renal cystic disease in ADPKD with either low protein diets or blood pressure reduction.[26] Cyst decompression surgery has also been tried but has no significant effect in slowing long term tissue destruction in ADPKD.[27]

Clinical trials in patients are hampered by the heterogeneity in presentation and the slowly progressive nature of many cystic kidney diseases. The ideal animal model with which to test potential anticyst treatments would be engineered to have the same gene defect as the human disease. Such a strategy has been used to produce a 'cystic fibrosis mouse'[28] and could be developed for PKD1. In the meantime the nearest animal models are phenotypic, rather than genotypic, although some recent studies look promising.

Homozygous cpk/cpk mice develop cystic kidneys and die from uraemia at 1 month of age in a disease that resembles early onset human ARPKD. Treatment with Taxol (paclitaxel) prevents cyst formation in vitro and preserves renal function in vivo, prolonging life span beyond 6 months of age.[29] Taxol stabilises microtubules in the cell cytoskeleton; these structures have been implicated in the transport of newly synthesised proteins to the apical plasma membrane. Therefore, in the cpk/cpk cystic mice, Taxol may prevent the incorporation of Na^+-K^+-ATPase into the apical epithelial membrane and hence the abnormal movement of water and solutes into the lumen of the cysts. Taxol also inhibits cell proliferation by disrupting the function of mitotic spindles and it is possible that this action also contributes to the inhibition of the growth of renal cysts.

Other drugs have been reported to be effective in slowing renal cystic diseases in animals, including corticosteroids[30] and epidermal growth factor (EGF).[31] The basis

for the action of corticosteroids is currently unknown whereas EGF is thought to drive the potentially cystic renal tubular epithelia into a more mature, less proliferative state. It is of note that EGF also prevents apoptosis in the embryonic kidney thus providing an alternative mechanism of action.[32]

Therefore, at least for certain strains of mice with cystic kidneys, medications appear promising. It now needs to be determined whether these treatments are effective in other animal models before these powerful drugs with potentially toxic side effects can be recommended for use in patients with cystic kidneys. It should also be remembered that human cystic kidneys often occur in the context of complex syndromes that affect multiple organs and we do not know whether the novel treatments which prevent the progression of renal cysts will also affect the natural history of associated conditions such as the intracranial aneurysms in ADPKD and hepatic fibrosis in ARPKD.

The future – gene therapy for cystic kidneys

The alternative long term approach that must now be given serious consideration is renal gene therapy. The success of this strategy depends on defining all the genes that cause cystic renal disease and then refining the technology for gene transfer into the kidney. It is currently feasible to insert novel genes directly into the tubular epithelia of postnatal kidneys,[33 34] but there are problems in achieving long term gene expression in a biologically significant number of kidney cells. In addition, because many cystic kidney diseases progress in utero, the therapeutic window may only occur before birth, making the technology for successful gene transfer even more of a challenge.[33] However, it is tantalising to speculate that gene transfer could correct specific genetic defects leading to normal, cyst free kidneys.

ADRIAN S WOOLF
PAUL J D WINYARD

Units of Medicine and Developmental Biology,
Institute of Child Health,
30 Guilford Street,
London WC1N 1EH

ASW and PJDW are supported by Action Research, the National Kidney Research Fund and the Kidney Research Aid Fund.

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