Pax2 in development and renal disease

GREGORY R. DRESSLER1* and ADRIAN S. WOOLF2
1Department of Pathology, The University of Michigan Medical Center, Ann Arbor, Michigan, USA and 2Nephrourology Unit, Institute of Child Health, University College London Medical School, London, United Kingdom

ABSTRACT  Pax genes are associated with a variety of developmental mutations in mouse and man that are gene dosage sensitive, or haploinsufficient. The Pax2 gene encodes a DNA binding, transcription factor whose expression is essential for the development of the renal epithelium. Both gain and loss of function mutants in the mouse demonstrate a requirement for Pax2 in the conversion of metanephric mesenchymal precursor cells to the fully differentiated tubular epithelium of the nephron. However, Pax2 expression is down-regulated as cells leave the mitotic cycle. Humans carrying a single Pax2 mutant allele exhibit renal hypoplasia, vesicoureteric reflux, and optic nerve colobomas. Conversely, persistent expression of Pax2 has been demonstrated in a variety of cystic and dysplastic renal diseases and correlates with continued proliferation of renal epithelial cells. Thus, Pax2 misexpression may be a key determinant in the initiation and progression of renal diseases marked by increased or deregulated cell proliferation.

KEY WORDS: Pax2, fetal kidney obstruction, multicystic dysplastic kidney, renal-coloboma syndrome, renal tumours, vesicoureteric reflux

Introduction

Although many genes which code for transcription factors, growth/survival factors and adhesion molecules have been functionally implicated in the control of murine nephrogenesis, the relevance of most of these molecules to human disease is unclear. In this respect, the PAX2 paradigm is perhaps the best example so far in which results from animal experiments appear relevant to the understanding the pathobiology of a variety of human diseases which are disorders of cell differentiation. Since most of these disorders are congenital anomalies of the kidney and lower urinary tract, we will focus on these malformations. However, we will also briefly allude to the relation between PAX2 and human kidney malignancies.

Pax genes and normal development

The Pax gene family encodes DNA binding, transcription factors whose functions are required for the normal development of a variety of structures and cell types in Drosophila, mouse and man (for review see Stuart and Gruss, 1996). Vertebrate Pax genes were originally identified based on sequence homology to the Drosophila segmentation gene paired. These homologies are maximal within the DNA binding domain, or paired-box, of the proteins but also encompass other conserved domains such as the paired-type homeobox and the octapeptide sequence. In mouse and man, there are 9 known Pax genes, (Fig. 1) whose expression patterns are restricted to developing structures such as the CNS, the vertebral column, the eye, the neural crest, and the kidney. Both naturally occurring mutations in mice and humans, as well as gene targeting in mouse, have revealed essential functions for Pax genes during embryogenesis. For example, the mouse small eye mutant and human aniridia are due to mutations in the Pax6 gene, whereas mouse splotch and human Wardenburg syndrome are caused by Pax3 mutations (Read, 1995). In most of all cases studied, phenotypes are dominant and are due to a gene dosage effect, or haploinsufficiency. Thus, a reduction to one functional Pax gene allele already has deleterious effects on development. This effect has serious implications for human disease, since, unlike many disease genes, loss of heterozygosity is not required for phenotypic manifestations.

Pax2 in kidney development

The expression pattern of the Pax2 gene has been characterised in many vertebrate species, including frog, chick, mouse and human, yet the essential character of this pattern remains constant. Both mRNA and Pax2 protein can be detected in the
pronephric duct, the earliest epithelial structure derived from the intermediate mesoderm, around the level of the 10th to 12th somite. As the duct extends into the mesonephric region, Pax2 expression remains and becomes evident in mesonephric tubules, which are thought to derive from periductal mesenchyme. At the time of ureteric bud outgrowth and invasion into the metanephric mesenchyme, Pax2 expression is activated in mesenchyme cells with particularly high levels in condensing mesenchyme at the ureteric bud tips. Thus, Pax2 is one of the earliest markers for induced mesenchyme and may be activated by the primary inducive signals emanating from the bud. Such Pax2 activating signals have not been identified, although a growing body of evidence points to members of the wnt family of secreted, matrix associated proteins. In the classical transfilter induction assay, the wnt-4 protein can substitute for much of the inducing activity previously associated with the dorsal spinal cord, as can other related wnt-proteins (Herzlinger et al., 1994; Kispert et al., 1998). Although kidneys from wnt-4 mutant mice arrest at the mesenchymal aggregate stage (Stark et al., 1994), they still express Pax2, consistent with the fact that wnt-4 expression follows Pax2 normally.

As the induced mesenchyme undergoes epithelial conversion, forming first the renal vesicle and then the S-shaped body, Pax2 expression is down regulated. This is first apparent in the podocyte precursors at the proximal end of the S-shaped body where Pax2 repression may be mediated by increasing levels of the Wilms' tumour suppressor gene, WT1 (Ryan et al., 1995). In mature proximal and distal tubule cells, Pax2 expression is undetectable but still persists in the major collecting ducts. How Pax2 expression is suppressed in proximal and distal tubules remains unclear, but most probably works through a WT1 independent mechanism.

Both in vitro loss of function experiments (Rothenpieler and Dressler, 1993) and gene targeting (Torres et al., 1995) demonstrate a clear requirement for Pax2 to generate tubules from the mesenchyme. Homozygous Pax2 null mice initiate pronephric duct development and express at least some of the known markers, such as the receptor tyrosine kinase c-ret, in the ductal epithelium as it extends caudally. However, at the level of the mesonephros, there is no evidence of tubule formation in the Pax2null mice. Furthermore, the mesonephric duct fails to extend completely caudal to the level of the metanephric mesenchyme and is unable to produce a ureteric bud. In vitro tissue recombination experiments suggest that Pax2 mutant mesenchyme is unable to respond to wild-type inductive signals (G. Dressler, personal observation), just as the mesonephric mesenchyme is unable to form tubules despite the apparently normal nephric duct more anterior. The phenotype of the Pax2 null mice is supported by previous organ culture experiments which indicated that Pax2 was required for the early aggregation stage of the induced mesenchyme. It remains to be determined whether the block in nephric duct extension and maintenance is due to lack of signals emanating from mesonephric tubules or is a cell autonomous effect due to lack of Pax2 expression in the ductal epithelium.

**Biochemistry of Pax proteins**

As outlined in Figure 1, Pax genes fall into three main subgroups based on conserved domains and expression patterns. The subdivision of the Pax gene family was based on the presence or absence of the paired-type homeodomain and the octapeptide sequence, as well as common elements of the expression patterns. Pax proteins interact with DNA through the characteristic 128 amino acid paired-domain at the amino-terminal end of the protein and/or the homeodomain located at the amino-terminus. Experiments in the fly demonstrated that the paired-domain and the homeo-domain could bind DNA independently (Treisman et al., 1991). Although most Pax genes were cloned by sequence homology, the Pax5 gene was identified because it encodes the B-cell specific transcription factor BSAP which binds to an immunoglobulin enhancer sequence (Adams et al., 1992). Thus, Pax5 is probably the best model for paired domain/DNA interactions because of the known recognition sequences. Much of the available evidence now indicates that the 128 amino acid paired-domain binds to a bipartite DNA recognition sequence, allowing for a high degree of sequence divergence (Czerny et al., 1993; Epstein et al., 1994b; Phelps and Dressler, 1996). The bipartite nature of the paired-domain was confirmed by the crystal structure of the Drosophila paired protein bound to its recognition oligonucleotide (Xu et al., 1995). The paired-domain consists of three amino-terminal α-helices that resemble a homeo-domain, followed by a carboxyl terminal region with three smaller α-helices. The amino terminal α-helices contact the 3' part of the bipartite target sequence, whereas the carboxyl terminal tail recognizes the 5' end of the DNA sequence. Furthermore, interactions between the paired-domain and the DNA recognition sequences can change the conformation of the Pax protein (Epstein et al., 1994a) and the target DNAs (Chalapsakis et al., 1994). The importance of DNA binding is underscored by specific mutation in the DNA binding domains of Pax2 (Sanyanusin et al., 1995a) and Pax6 (Hanson and van Heyningen, 1995) in human disease.

The Pax2, 5, and 8 constitute a subfamily that was probably derived from a single ancestral gene. Each gene contains a paired domain and an octapeptide, but only the first helix of the homeo domain. Although DNA binding activities map to the amino terminal paired domain, transcription activation and repression is mediated by more C-terminal domains of the Pax2 family of proteins (Lechner and Dressler, 1996). For example, amino acid residues (aa) 197-415 of murine Pax2 are required for maximal transcription transactivation in cultured mammalian cells (Lechner and Dressler, 1996). The activation domain is generally rich in proline (P), serine (S), threonine (T) and tyrosine (Y) and is phosphorylated at serine residues. Missense mutations have rarely been identified in the C-terminal coding region of Pax genes, most likely because multiple regions can contribute to full activity. Surprisingly, Pax2 also contains a repressor motif, the octapeptide sequence, that is similar to the engrailed and goosecoid repression domains (Smith and Jaynes, 1996; Mailhos et al., 1998). In all these proteins, the octapeptide is found N-terminal to the HD suggesting a potential interaction which has survived evolution. The presence of both activation and repression domains in Pax proteins suggests multiple functions that may ultimately depend on the context of target sequences within a given locus.

Pax protein function must be mediated by interactions with other nuclear proteins that complex at the DNA binding site and mediate activation or repression. For example, Pax5 can recruit members of the Ets family of proteins to a B-cell specific promoter sequence (Fitzsimmons et al., 1996). This recruitment implies Pax5 binds first
and requires both Pax5 binding sites and an Ets consensus sequence. However, if the Pax5 binding site is sub-optimal, the Ets protein binds first and can recruit, or stabilise, Pax5 binding, although it is not clear if Ets proteins interact with unbound Pax5. A recent Pax3 binding protein was identified mapping to the DiGeorge/velocardiofacial syndrome locus (DGCR) (Magnaghi et al., 1998). This protein, called HIRA, shares homology with yeast transcriptional co-repressors. However, it remains to be determined how these protein interactions affect Pax mediated function.

PAX2 in human kidney disease

The human metanephros appears at five weeks of gestation and the first glomeruli form by nine weeks (Risdon and Woolf, 1998). Nephrons are generated until 34 weeks with tubule maturation continuing postnatally. Malformations of the kidney and urinary tract are often diagnosed by fetal ultrasound examination, a routine procedure performed in many countries from midgestation. Such malformations contribute to 30% of all prenatally diagnosed anomalies and the most severe cases are associated with a significant decrease in fetal urine formation and hence reduced amounts of amniotic fluid: this is often associated with an intact DNA-binding paired box, an ‘unperturbed’ Potter sequence’ (Noia et al., 1996). The human fetus can survive without kidney function since the placenta removes circulating waste but, after birth, severely affected infants may require dialysis and subsequent renal transplantation. In fact, kidney malformations are the commonest causes of chronic renal failure in young children (McEnery et al., 1992). Renal malformations can occur in isolation or as part of a multiorgan syndrome and, additionally, they can be sporadic or inherited (Woolf and Winyard, 1998). The renal-coloboma syndrome is a rare collection of anomalies including blindness due to an optic nerve malformation called a ‘coloboma’ associated with congenitally small kidneys and vesicoureteric reflux (Rieger, 1977; Schimmenti et al., 1995; OMIM #120330). The renal lesion has sometimes been called ‘hypoplasia’, although the absolute nephron numbers have not been formally counted. The syndrome has a striking phenotypic similarity to Pax2 heterozygous mutant mice (Keller et al., 1994; Torres et al., 1995; Favor et al., 1996) and, following this clinical observation, Sanyanusin and colleagues (1995b) defined a heterozygous mutation of PAX2 in a human kindred where the mutation arose de novo and was transmitted from father to sons in an autosomal dominant manner consistent with the gene location on 10q24.3-q25.1. The mutated gene was predicted to code for a protein with an intact DNA-binding paired box, an interrupted octapeptide domain and a novel, truncated, carboxyterminal: it was postulated that this would perturb the transactivation of target genes. Subsequent reports of further patients confirmed other PAX2 mutations including a de novo t(10;13) translocation (Narahara et al., 1997) and a mutation in the paired box which would affect DNA binding.
The phenotype of this disease would be consistent with a haploinsufficiency state causing a lack of growth of both the kidney and the ureter. The ocular coloboma, which can cause severe visual impairment, is explained by PAX2 expression during eye development and the high frequency hearing loss, which is a more variable feature of the syndrome (Schimmenti et al., 1995, 1997) is consistent with gene expression in the inner ear. Homozygous PAX2 mutations have yet to be described in humans, although kindreds with kidney and Mullerian malformations (Battin et al., 1993) superficially resemble mouse null-mutants (Torres et al., 1995).

The renal coloboma syndrome is a rare entity but the question arises whether PAX2 mutations might account for some individuals with non-syndromic malformations of the kidney and ureter. Primary, non-syndromic vesicoureteric reflux occurs in 1% of children and can be associated with renal dysplasia and hypoplasia (Feather et al., 1996). Inheritance in some kindreds appears dominant with variable penetrance and expression. So far, families with this disorder have failed to show linkage to the PAX2 locus or mutations of the gene (Feather et al., 1997; Cho et al., 1998). Very recently, there is preliminary evidence that a subset of patients who have non-syndromic renal hypoplasia have PAX2 mutations (Salomon et al., 1998). Finally, although the closely-related PAX8 gene is expressed during human kidney development (Poleev et al., 1992; Eccles et al., 1995), mutations have yet to be associated with renal malformations.

**PAX2 expression in human cystic dysplastic kidneys**

Human multicystic dysplastic kidneys represent a model of perturbed epithelial/mesenchymal interaction (Woolf and Winyard, 1998). Microdissection studies had revealed that dysplastic epithelia were malformed branching tubules terminating in cysts surrounded by poorly differentiated cells resembling mesenchymal cells. We found that human renal dysplastic epithelia had a high level of cell division and expressed an array of fetal proteins including PAX2, the cell survival molecule BCL2 (Winyard et al., 1996b) and galectin-3, a ureteric bud lineage cell signal molecule (Winyard et al., 1997). Strikingly, dysplastic organs harvested postnatally show persistent patterns of fetal gene expression whereas normal organs downregulated proliferation as well as these genes in mature epithelia (Winyard et al., 1996a). Since transgenic Pax2 expression causes renal cysts (Dressler et al., 1993), persistent expression in human fetal kidney epithelia may drive proliferation and cause cyst growth. We are now aware that the biology of multicystic kidneys is complex, with cell death upregulated in undifferentiated cells located around proliferating epithelia (Winyard et al., 1996b). Recently, Groenen et al. (1996, 1998) described a patient with bilateral multicystic renal dysplasia with a t(6;19) (p21;q13.1) translocation which interrupted the upstream stimulator factor 2 transcription factor gene on chromosome 19 and the CDC5L cell cycle gene on chromosome 6. However, the significance, if any, of these mutations to the abnormal biology the multicystic dysplastic kidney is currently unclear.

Multicystic dysplastic kidneys are usually associated with an atretic ureter, and obstruction early in gestation has been invoked as a cause of the malformation. Therefore, in order to follow up our observations on cell turnover and gene expression in the human disease, we decided to follow the biological changes in a reproducible animal model of fetal kidney obstruction (Attar et al., 1998). The sheep metanephrors appears at 27 days of gestation and this species constitutes a good experimental model because the urinary tract can be obstructed in vivo relatively early in gestation to generate pathologies resembling human malformations. We caused urinary flow impairment in fetal sheep by surgical unilateral ureteric anatomical obstruction at 90 days of gestation when a few layers of glomeruli had formed (Attar et al., 1998). After ten days, the nephrogenic cortex was replaced by disorganised cells separated by oedema and prominent...
vascular spaces. Cortical histology was dominated by cysts which expressed PAX2 (Fig. 2) and PCNA. In addition, there was an increase in apoptosis in tissues around obstructed nephrons. Hence proliferation, PAX2 expression and programmed cell death were upregulated, as described in human dysplastic organs (Winyard et al., 1996a,b). The mechanisms by which urinary flow impairment might initiate these profound effects remain to be explained.

PAX2 and human renal tumours

Renal tumours are disorders of cell growth and differentiation and some variations may even originate in the nephrogenic period (Grignon and Eble, 1998). Therefore, it was logical to examine these lesions for PAX2 expression. The gene was found to be expressed in the condensed blastema and dysplastic epithelium of human Wilms’ tumours (Dressler and Douglas, 1992; Eccles et al., 1995), a relatively common childhood neoplasm, and in renal carcinoma (Gnarra and Dressler, 1995), which affects adults. Furthermore, the reduction of PAX2 expression by antisense oligonucleotides in human renal carcinoma cell lines was associated with reduced growth (Gnarra and Dressler, 1995). Therefore, it can be speculated that the continued, postnatal expression of PAX2 may drive kidney tumour growth in vivo. In support of this contention are other experiments demonstrating that the gene can transform murine cells (Maulbecker and Guss, 1993) and inhibit the promoter of p53, a tumour suppressor (Stuart et al., 1995). Germline and somatic mutations of diverse genes have been documented in some Wilms’ tumours (e.g. WT1) and adult kidney cancers (e.g. VHL and MET), but further work is necessary to unravel the relation of these genetic lesions to deregulated PAX2 expression. Finally, it is notable that a variety of tumours occasionally arise in multicystic dysplastic kidneys and these organs may contain nephrogenic blastema and peripelvic rests, the latter being considered a Wilms’ tumour precursor (Barrett and Winel, 1980; Cromie et al., 1980): as discussed above, there is evidence for PAX2 overexpression in these organs.

Conclusion

Normal renal development requires two copies of the PAX2 gene, which is expressed in the early mesenchymal derived epithelium and in the branching ureteric bud. In both mice and humans, PAX2 deficiency causes defective growth of the fetal kidney and ureter, while overexpression is associated with epithelial overgrowth with cyst or tumour formation.

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References


