Are Pax proteins potential therapeutic targets in kidney disease and cancer?

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Pax genes encode developmental regulators that are expressed in a variety of tissues and control critical events in morphogenesis. In the kidney, Pax2 and Pax8 are expressed in embryonic development and in specific renal diseases associated with aberrant epithelial cell proliferation. Prior genetic and cell biological studies suggest that reducing the activity of Pax proteins in renal cancer or in polycystic kidney disease can slow the progression of these conditions. The Pax proteins may be critical for providing tissue and locus specificity to recruit epigenetic modifiers that control gene expression and chromatin structure. Although they are nuclear, targeting Pax proteins to inhibit function may be feasible with small molecules. Such inhibition of Pax protein function may provide novel therapies for subsets of renal disorders that are tissue- and cell type–specific and avoid systemic effects on non-Pax–expressing cells and tissues. Given the paucity of effective treatments for renal cancer and cystic disease, the Pax family of proteins represents new pharmaceutical targets that merit exploration and further development.

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Adult and pediatric renal disease place a significant clinical and financial burden on public health. With the prevalence of obesity and diabetes, the overall incidence of end-stage renal disease (ESRD) is increasing at an alarming rate and was recently estimated at 336 per million per year,[1] such that the number of ESRD patients may approach 2.24 million by the year 2030. Yet, the biochemical and genetic bases of chronic and acute renal disease are still poorly characterized, and effective therapies are sorely lacking. In simple terms, kidney disease can be divided into chronic, slowly progressing loss of renal function and more acute disease that develops rapidly and is often unrepairable. However, for the purpose of understanding disease mechanisms, it is also advantageous to distinguish renal diseases based on the affected cell types.

Chronic kidney disease (CKD) is attributable to known environmental factors, which include obesity, hypertension, and diabetes but can also be immune complex–mediated or genetic as in Alport's syndrome or polycystic kidney disease (PKD). Acute kidney disease or acute kidney injury (AKI) is the result of renal ischemia or nephrotoxic cell death, and despite hemodialysis still results greater than 50% mortality in clinical settings.[2] On a cellular level, most chronic renal diseases involve an expansion of fibroblasts within the interstitium or the expansion of mesangial cells within the glomerulus. Disease of the glomerulus can also be attributed directly to the death of podocytes,[3] to changes in the filtration barrier due to alterations in podocyte specific protein expression,[4] or to defects in the glomerular capillary bed and associated mesangial cells.[5]

For kidney disease that originates within the epithelial cell population, it would be advantageous to develop drugs that specifically affect cellular pathways unique to renal epithelia cells. In order to realize these goals, it is prudent to understand the molecular and genetic bases for renal epithelial cell development, differentiation, and proliferation. Through the study of embryonic kidney development, multiple genes and pathways have been described that could prove to be specific targets for the development of new therapeutics. Among these are the Pax genes and proteins, which are essential for renal epithelial cell development and function. This review will discuss the evidence that Pax proteins function in renal disease, discuss how Pax proteins may function within the context of chromatin, and whether Pax genes and proteins are viable targets for the development of new therapies for specific renal diseases.

Pax genes and kidney development

The mammalian Pax (Paired-box) gene family includes 9 genes (*Pax1–9*) that encode developmental regulatory proteins that specify a variety of tissues during embryogenesis (Figure 1). Pax genes encode an N-terminal paired domain (PD), a DNA-binding domain first found in the *Drosophila* segmentation gene *paired*. Other conserved domains found in some but not all Pax genes include the octapeptide sequence, a homeodomain, or a partial homeodomain that divides the Pax family into 4 subgroups[6,7] (Figure 1). These domains impart positive or negative transcription regulation depending on cellular context and the availability of cofactors. The carboxy-terminal domain confers transcription activation potential to most of the Pax family members[8–14] and can be phosphorylated at multiple serines and threonines.[15] The octapeptide is evolutionarily related to the *Drosophila engrailed* family Eh1 repression domain[16] and can bind with the Groucho/Tle (Transducin-like enhancer of split) family of corepressors.[17]

Once the mammalian Pax genes were cloned and their expression patterns analyzed, it became clear that all were expressed in unique cell types and tissues during embryonic development, often marking a region of progenitor cells for specific structures, such as the case with *Pax2* and *Pax8* in the developing kidney. The adult kidney or metanephros develops from a region of mesoderm called intermediate mesoderm that can be distinguished from paraxial, or somitic mesoderm, and lateral plate mesoderm by the expression of Pax2, Pax8, and Lim1.[18] The first epithelial derivatives from the intermediate mesoderm are the bilateral nephric ducts, or Wolffian ducts, which extend caudally from the mid-thoracic region (Figure 2). In the mouse, nephric duct development begins at about embryonic day 9, which is equivalent to approximately 22 days after fertilization in humans. As the nephric duct extends caudally, a series of transient nephron-like structures are formed in a linear array along the anterior-posterior axis. These mesonephric tubules are similar to the functioning mesonephric nephrons of aquatic vertebrates. The adult kidney forms at the caudal end of the nephric duct when an epithelial outgrowth, called the ureteric bud, extends into the surrounding metanephric mesenchyme and initiates a series of reciprocal inductive interactions such that the ureteric bud is induced to undergo branching morphogenesis, whereas the metanephric mesenchyme is induced to condense around the tips of the branching ureteric bud and undergo a mesenchymal-to-epithelial conversion to generate nephrons. With each successive branching of the ureteric bud tips, new nephrons are induced such that the youngest nephrons are located at the periphery and the oldest near the medulla.

The Pax2 protein is expressed in the nephric duct, the mesonephric tubules, and in the metanephric mesenchyme and all epithelial derivatives from the mesenchyme[8,19] (Figure 2). However, as the nephrons mature, Pax2 is downregulated, first in the podocyte progenitor cells of the developing glomerulus and subsequently in all proximal and distal tubules of the nephron. However, expression of the related protein Pax8 remains strong in all the epithelial cells of the mature nephron, whereas Pax2 remains high in the

Pax family subgroup	Pax family member	Pax protein structural elements (PAI RED Paired domain; Octapeptide; HD Homeodomain)	Genomic location	Protein size	Expression domain
I	Pax1		20p11	446aa	Sclerotome, thymus, skeleton
	Pax9		14q12-13	342aa	Sclerotome, thymus, skeleton, teeth, craniofacial
II	Pax2		10q25	414aa	CNS, kidney, eye, ear
	Pax5		9p13	391aa	CNS, B cells, testis
	Pax8		2q12-14	457aa	CNS, kidney, thyroid
III	Pax3		2q35	479aa	CNS, neural crest, muscle
	Pax7		1p36.2	505aa	CNS, neural crest, muscle
IV	Pax4		7q32	349aa	CNS, pancreas
	Pax6		11p13	422aa	CNS, eye, nose, pancreas, pituitary

Figure 1 | The mammalian Pax family of transcription factors. Schematic drawings of the 9 Pax family members showing the conserved structural domains. These structural domains include the highly conserved paired DNA domain (blue), which endows these proteins with DNA binding capability and is the defining domain for this family of transcription factors. The paired domain is composed of 2 globular helix-turn-helix subdomains that are connected by a disordered linker. The subdomain (PAI) and the C-terminal subdomain (RED) make contacts with nucleotide bases in adjacent major grooves of the DNA double helix, whereas the extended linker makes substantial contact with bases in the intervening minor groove. The Pax family of transcription factors are further divided into 4 subgroups based on the presence of additional conserved structural elements. These elements include the octapeptide sequence (gold), which is found in all members except Pax4 and Pax6. Pax3, Pax4, Pax6, and Pax7 also contain a full paired-type homeodomain (green), whereas Pax2, Pax5, and Pax8 contain only a truncated version of the paired type homeodomain. CNS, central nervous system.