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Are Pax Proteins Potential Therapeutic Targets in Kidney Disease and Cancer?

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Abstract

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 represent new pharmaceutical targets that merit exploration and further development.

Keywords

Pax2; Pax8; renal cancer; polycystic kidney disease; drug development; renal epithelia

Introduction

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 year¹, 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

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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 settings². 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 podocytes³, to changes in the filtration barrier due to alterations in podocyte specific protein expression⁴, or to defects in the glomerular capillary bed and associated mesangial cells⁵.

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*) which encode developmental regulatory proteins that specify a variety of tissues during embryogenesis (Fig. 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 (OP) sequence, a homeodomain (HD) or a partial homeodomain that divide the Pax family into four subgroups^{6, 7} (Fig. 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 members⁸⁻¹⁴ and can be phosphorylated at multiple serines and threonines¹⁵. The OP is evolutionarily related to the *Drosophila engrailed* family Eh1 repression domain¹⁶ and can bind with the Groucho/Tle (Transducin-like Enhancer of split) family of co-repressors¹⁷.

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 is 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 Lim1¹⁸. The first epithelial derivatives from the intermediate mesoderm are the bilateral nephric ducts, or Wolffian ducts, which extend caudally from the mid thoracic region (Fig. 2). In the mouse, nephric duct development begins at about embryonic day 9 (E9) which is equivalent to approximately 22 days post 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} (Fig. 2). However, as the nephrons mature, Pax2 is down regulated, 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 collecting ducts and the renal papilla²⁰ (Fig. 2, arrows). In the glomerulus of adult human kidneys, Pax2 has also been detected in parietal epithelial cells²¹, some which have been proposed to be a source of renal stem cells^{22, 23}. Just how redundant Pax2 and Pax8 are in the developing and adult kidney is still uncertain, but much of what we do know comes from genetic analyses of *Pax2* and *Pax8* mutations in mice. *Pax2* null mutants form a nephric duct but fail to develop mesonephric tubules or any semblance of a metanephric kidney^{24, 25}. *Pax2* homozygous mutant mice die after birth due to complete renal agenesis. In humans, multiple *Pax2* mutations have been described that are associated with renal-coloboma syndrome, which is characterized by renal hyperplasia, reflux, and optic nerve colobomas^{26, 27}. *Pax8* mutant mice have no discernible kidney defects, suggesting that this gene is entirely redundant with *Pax2*. In humans, no *Pax8* mutations are associated with renal defects. However, *Pax2/Pax8* double mutants are more severe than *Pax2* mutants alone with near complete absence of the intermediate mesoderm and no evidence of nephric ducts²⁸. Compound Pax2 and Pax8 heterozygotes also affect expression of Lim1 and branching morphogenesis of the developing kidney²⁹. Even though Pax2 and Pax8 proteins have identical paired domains and share much sequence identity outside of the paired domain, Pax8 alone is insufficient for kidney development, most likely because its expression cannot be detected in the metanephric mesenchyme prior to invasion by the ureteric bud. Pax8 expression is only observed in mesenchyme derivatives after induction by the ureteric bud and persists in adult renal epithelial cells^{30, 31}. Yet the function of Pax8 in adult kidneys is not known.

Pax Genes in Kidney Cancer

Renal cell carcinoma (RCC) accounts for 90% of kidney cancer cases worldwide with greater than 270,000 new patients diagnosed and 116,000 patients succumbing to their disease each year³². The treatment of choice for RCC was immunotherapy using a combination of interferon alpha and interleukin-2 with high dose interleukin-2 as the only accepted option for patients presenting with metastasis³³. The inactivation of Von Hippel-Lindau (VHL) tumor suppressor gene is associated with the majority of clear cell RCCs³⁴. Inactivation of VHL produces an increase in angiogenic factors including Vascular Endothelial Growth Factor (VEGF). Thus, frontline therapies commonly include the VEGF inhibitors Sunitinib, Pazopanib, or Bevacizumab³⁵. Still, the response rates are limited, patients frequently develop resistance, and the overall long-term survival rates have not dramatically improved. Newly emerging options for second-line therapy include mTOR inhibitors (Temozolimus or Everolimus), multi-tyrosine kinase inhibitors (Sorafenib, Axitinib, or Cabozantinib), and programmed cell death protein 1 (PD-1) inhibition (Nivolumab). Although each has shown benefits in phase III trials, the median overall survival was well below 3 years for all cases³³.

The transient nature of Pax2 expression in developing but not adult nephrons led multiple labs to investigate the expression of Pax2 in renal cancer, as reactivation of developmental programs may in part underlie the cancer phenotype. Close to 95% of RCCs have high levels of Pax2 and Pax8 expression and this expression is associated with metastasis^{36–40}. Pax2 expression is also high in embryonic renal cancer such as Wilms' tumor⁸, and in hyperproliferative dysplastic renal syndromes⁴¹. In renal cancer cell lines, inhibition of Pax2 can increase cell death, decrease proliferation, and sensitize cells to other chemotherapeutic agents^{40, 42, 43}. Thus, experiments with cultured cancer cell lines support the idea that targeting Pax2 may be a viable anti-cancer treatment by inhibiting proliferation or pushing cancer progenitor cells into a terminal or benign state.

Pax2 in Polycystic Kidney Disease

Renal epithelia cells, which make up the majority of cell types in the adult kidney, are the primary affected cell type in cystic or dysplastic kidney disease and polycystic kidney disease (PKD). Among the most common single gene disorders⁴⁴ is autosomal dominant polycystic kidney disease (ADPKD), a leading causes of ESRD⁴⁵. ADPKD is characterized by proliferation of tubular epithelial cells, the loss of epithelial cell polarity, and the progressive increase in the number and size of epithelial cysts that ultimately displace normal tubular epithelia⁴⁶. Most cases of PKD are caused by mutations in the *Pkd1* or *Pkd2* genes. However, there are many other juvenile and adult onset diseases that result in renal cysts⁴⁷. *Pkd1/2* encode the Polycystin proteins which localize to the primary cilia and impact signaling by the Hedgehog, Wnt, cAMP, and planar cell polarity pathways. Yet it remains unclear how these pathways contribute to PKD pathogenesis. Conventional PKD treatment regimens reduce disease-associated complications such as hypertension, cyst infection, and chronic pain in order to increase the quality of life and extend lifespan⁴⁴. Unfortunately, such treatments do not slow cyst progression, therefore approximately 70% of patients require renal transplants between 40 and 70 years of age⁴⁸. Only recently have

more targeted therapies been tested. The most promising is the vasopressin V2 receptor antagonist Tolvaptan which slows the increase in total kidney volume and the decline in glomerular filtration rate⁴⁹. Tolvaptan has since been approved for ADPKD treatment in Japan, although the United States Food and Drug Administration raised concerns and declined approval⁵⁰. However, more recent phase III clinical trial data suggest that FDA approval for Tolvaptan could be forthcoming⁵¹. While these data are encouraging, there remains a desperate need for better treatment options.

As with cancer, high Pax2 expression has been observed in the proliferating cystic epithelial cell in PKD. This is consistent the re-entry of cystic epithelial cells into the mitotic cycle. All Pax genes exhibit haploinsufficiency, meaning that heterozygous individuals, both mice and humans, already show phenotypes due to a loss of gene dosage. Thus, one can use mouse genetics to address the role of Pax2 in PKD by crossing the PKD mutations into a Pax2 heterozygous background. A reduction of *Pax2* gene dosage slowed the progression of PKD in two different mouse models of PKD^{52, 53}, consistent with the hypothesis that continued Pax2 expression is necessary for continued renal epithelial cell proliferation and cyst expansion.

Given the reactivation of Pax2 gene expression in specific disease of the renal epithelia, developing therapies that modulate Pax protein function requires intimate knowledge of the biochemistry and mechanisms of Pax target gene regulation.

Biochemistry and Function of Pax Proteins

In eukaryotes, nuclear DNA is maintained in a highly-structured state, known as chromatin. The primary unit of chromatin is the nucleosome, which consists DNA wrapped around a core histone octamer^{54, 55} that contains two each of the histone proteins H2A, H2B, H3, and H4. The amino-terminal tails of the core histone proteins extend out of the nucleosome and can be post-translationally modified to dramatically impact chromatin structure and function. Well over 100 different post-translational modifications can be observed across all four core histone tails⁵⁶ and include acetylation, methylation, phosphorylation, ubiquitination, and sumoylation^{57, 58}. Altering the electrostatic charge of the nucleosome through the addition of a charged functional group, as with histone acetylation, can relax chromatin and enable transcription⁵⁹. Alternatively, post-translational modification of histone tails creates binding sites for epigenetic readers that interpret histone modifications⁵⁹. Multiple histone binding domains have been identified, many of which are capable of distinguishing between post-translationally modified and unmodified histones. Among the most stable and instructive marks on histone tails are methylation of H3 at specific lysine residues. These methylation marks are thought to imprint epigenetic information that can be inherited from mother cells to daughter cells and can maintain the phenotype or transcriptome in a stable manner. Histone methyl marks allows reader proteins to be recruited to target genes depending on the specific lysine methylation status to control gene expression and chromatin accessibility⁶⁰.

Despite the absolute need for Pax2 in kidney development, how a single gene and protein exerts such profound effects in specifying entire regions or tissues remained puzzling. In

order to begin to understand the biochemical activity of Pax proteins it is important to place them within a larger context of protein-protein interactions. Multiple Pax proteins have been linked to epigenetic remodeling complexes that regulate both activation and repression of gene expression⁶¹ (Fig. 3). The ubiquitous nuclear protein PTIP binds to the carboxy-terminal domains of Pax2, 5, and 8^{15, 62}, which can be highly phosphorylated at serines and threonines⁶³ (Fig. 3A). PTIP contains 3 pairs of BRCT domains that are known to bind P-serine and thus their interactions with Pax proteins may be regulated by specific kinases such as the c-Jun N-terminal Kinase^{63, 64}. PTIP is part of the MLL3/4 (KMT2C/D) histone H3K4 methyltransferase complex and thus acts as an adaptor linking Pax proteins to the H3K4me machinery^{62, 65}. Methylation of H3K4 is generally associated with poised genes or active gene expression. Upon binding of Pax2 to DNA, PTIP and the MLL3/4 complexes are recruited to chromatin. In the absence of PTIP, Pax proteins still bind but MLLs are not recruited and no increase in H3K4me is observed. In kidney organ cultures, deletion of PTIP abrogates the de novo activation of Pax2 target genes but does not affect the expression of genes already activated prior to PTIP deletion⁶⁶, suggesting the establishment of true epigenetic memory. In B cells, which depend on Pax5 for differentiation, deletion of PTIP also impacts the ability of Pax5 to recruit the MLL3/4 complex to new transcription start sites but has little impact on pre-existing sites of high H3K4 methylation^{67, 68}. These results suggest that Pax proteins can provide the tissue and locus specificity for histone methylation complexes, which by themselves do not have DNA binding activities.

The association of repressor complexes with Pax proteins has also been described in a variety of biological contexts. Among the best characterized is the interaction of Pax2/5/8 with the co-repressor proteins of the Groucho/Tle family (Fig. 3B). The Grg4 protein binds to the octapeptide domain of Pax5 and thereby inhibiting the activation of Pax target genes^{64, 69}. In kidney development, Grg4 expression is tightly regulated, with low levels in metanephric mesenchyme and early epithelial derivatives but high levels in the proximal parts of the s-shaped body that generates the podocyte progenitors. Grg4 continues to be highly expressed in adult podocytes. In cell culture, Grg4 expression displaces PTIP and the MLL3/4 complex at sites of Pax2 binding⁷⁰. This displacement is thought to be due to the recruitment of the PPM1B phosphatase that dephosphorylates Pax2, eliminating the P-serine PTIP binding residues⁷¹. Grg4 then recruits epigenetic repressive complexes consisting of the histone arginine methyltransferase PRMT5 and the Polycomb group proteins Ezh2 and Suz12, which result in H3K27 methylation and gene silencing^{70, 72}. Together these findings show that Pax proteins exhibit versatility in regulating gene expression and that they can provide the locus specificity for both activating and repressive epigenetic complexes. Such epigenetic complexes imprint chromatin with specific histone methylation marks that can determine chromatin structure and accessibility for gene expression. This epigenomic imprinting occurs early in development and likely underlies the loss of pluripotency and maintains the restriction of cell fates to ultimately determine specific cell types. Thus, Pax proteins can be modeled as DNA binding factors that help imprint epigenetic information in a locus and tissue specific manner.

Targeting Pax Genes with Small Molecules

Given the limited cell specific expression of Pax2 and Pax8, targeting these proteins in diseases of the renal epithelial cells could prove beneficial for slowing progression of renal cysts or inhibiting proliferation of renal cancer with less risk of adverse side effects. However, targeting nuclear proteins presents its own unique set of challenges and requires intimate knowledge about the biochemistry of Pax protein/DNA and Pax protein/protein interactions. In fact, transcription factors have classically been considered “undrugable”⁷³ because of their nuclear localization, the charged nature of their interaction with DNA, and their lack of classical binding pockets. With the exception of the nuclear receptor class of transcription factors (retinoic acid receptor, androgen receptor, estrogen receptor, etc.), most others do not have endogenous ligands and therefore lack a well-defined pocket for small-molecule binding. This makes them much less amenable to traditional approaches of inhibition with small-molecules.

One strategy may be to inhibit Pax-DNA interactions by targeting the PD, the structure of which has been solved for several Pax proteins^{74 75 76}. Using these structures and known point mutations that disrupt PD-DNA interactions, a homology model for the Pax2 PD was generated (Fig. 4). The PD is composed of two globular subdomains that are connected by a disordered linker. Both of the globular subdomains adopt a helix-turn-helix (HTH) motif that binds DNA. Additionally, the co-crystal structures indicate that the N-terminal HTH subdomain (PAI subdomain) and the C-terminal HTH subdomain (RED subdomain) make contacts with nucleotide bases in adjacent major grooves of the DNA double helix while the extended linker makes substantial contact with bases in the intervening minor groove. While each of the HTH subdomains are capable of independently binding DNA⁷⁷, both are required for comprehensive regulation of target genes⁷⁸. Given the high degree of concordance within these structural determinations, it is likely that this structure is representative of the entire Pax family.

One approach to inhibiting Pax protein activity is to prevent binding to DNA by competition with small molecules that interact with the DNA binding pocket (Fig. 4A, B). Such an approach was recently utilized to identify a small molecule inhibitor of Pax2 activity⁷⁹. This molecule, called EG1, was able to phenocopy a Pax2 mutation in developing kidney organ cultures (Fig. 4C) and also slowed the growth of Pax2 positive renal and ovarian cancer cells. Whether such molecule can slow renal cancer growth in vivo remains to be determined and would require the development of reproducible genetic models for renal cancer in mice. Fortunately, such a renal cancer model was recently described by creating mutations in the genes Vhl, Trp53, and Rb1⁸⁰.

Given the high conservation of the PD among Pax genes, it is likely that small molecules that compete for the binding pocket may also inhibit multiple Pax proteins. That would certainly be the case for the Pax2/5/8 subfamily as their PDs are virtually identical. For the kidney, inhibition of both Pax2 and Pax8 might be beneficial for treating PKD or renal cancer, whereas inhibition of Pax5 might have some transient immunological effects. Other strategies might identify small molecules that prevent protein-protein interactions necessary for transducing Pax function. Thus, inhibiting the Pax2-PTIP interaction might add a degree

of specificity unattainable with a Pax2-DNA inhibitor. Additional concerns include the potential need for Pax2/8 in renal regeneration after acute injury. Acute kidney injury due to ischemia or nephrotoxins results in cell death within the S2 and S3 segments of the proximal tubules. If the injury is sub-lethal, cells within these regions can reenter the mitotic cycle, proliferate, and repopulate areas denuded of epithelia. These regenerating epithelial cells transiently reactivate the Pax2 gene expression but suppress expression once the site of injury is repaired^{81, 82}. As with the disease models, expression of Pax2 correlates with proliferation, whereas the expression of Pax8 remains on in non-dividing epithelial cells. Thus, the effects on kidney injury and regeneration should be considered when using any potential Pax2 inhibitors.

Conclusions

Despite the clinical and financial burden renal disease places on the public health, few effective treatments are currently available for slowing the progression of chronic renal disease, for minimizing the effects of acute kidney injury, or for inhibiting proliferation of renal cancer cells. Through the study of kidney development, kidney cancer, and regeneration, some common genes and proteins may lend themselves to targeting by small molecules or other inhibitory strategies. Given their cell-type specificity, limited expression in healthy adults, and high level of expression in disease, certain Pax proteins may be viable targets for inhibition, despite their nuclear localization. A reduction of Pax activity is often sufficient to generate a biological effect, suggesting that Pax inhibition need not approach 100% efficacy. Furthermore, combining Pax inhibitors with other chemotherapeutic drugs may improve their efficacy while reducing the toxic side effects on other healthy tissues.

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Pax family subgroup	Pax family member	Pax protein structural elements			Genomic location	Protein size	Expression domain
		<div><div>PAI</div><div>RED</div></div>	Paired domain	<div><div>Octapeptide</div><div>HD</div></div>			
I	Pax1				20p11	446aa	Sclerotome, thymus, skeleton
	Pax9				14q12-13	342aa	Sclerotome, thymus, skeleton, teeth, cranio-facial
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

Fig. 1. The Mammalian Pax Family of Transcription Factors

Schematic drawings of the nine 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 PD is composed of two 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 while the extended linker makes substantial contact with bases in the intervening minor groove. The Pax family of transcription factors are further divided into four 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) while Pax2, Pax5, and Pax8 contain only a truncated version of the paired type homeodomain.

Fig. 2. Pax Expression in the Developing Kidney

By embryonic day 9.0 (E9.0) in the mouse, Pax2 is expressed in the nephric duct which forms within the intermediate mesoderm. Pax2 expression is visualized in a heterozygous embryo carrying an EGFP insertional mutant allele²⁴. At E10.0, the anterior mesonephric tubules are clearly evident and a mass of Pax2 positive cells, the metanephric mesenchyme, sits adjacent to the posterior nephric duct. By E10.5, the ureteric bud branches off of the nephric duct and invades the mesenchyme. Inductive interactions cause the ureteric bud to bifurcate, as Pax2 positive mesenchyme condenses around the tips of the buds (E11.5). Repeated branching and condensed mesenchyme is evident by E12.5. In newborn kidneys, the nephrogenesis is still ongoing in the peripheral zone (z) where ureteric bud epithelial cells (E-cadherin+ in green) are inducing new nephrons. Pax2 (red, top panel) expression is high in the nephrogenic zone but is down regulated in more mature epithelial derivatives from the mesenchyme. The ureteric bud derived collecting ducts remain Pax2 positive (arrows), whereas Pax8 (red, bottom panel) expression persists in maturing proximal and distal tubules. E-cadherin marks the more differentiated epithelia in green. Scale bars are in μm as indicated. The Pax2 antibodies used have been described⁸; the Pax8 antibody was from Proteintech (Cat. No. 100336-1-AP).

Fig. 3. An Epigenetic Model of Pax2 Mediated Gene Regulation

A) The Pax2 protein binds DNA and recruits the adaptor protein PTIP, which interacts with an Mll3/4 histone H3K4 methyltransferase complex. High levels of H3K4me2/3 are associated with active or poised chromatin, which remains more open and accessible. When Grg4 is highly expressed, interactions with Pax2 result in the recruitment of a phosphatase PPM1B, which can dephosphorylate Pax2, displace PTIP and recruit additional repressors such as the arginine methyltransferase PRMT5 and the polycomb repressor 2 complex. The placement of histone H3K27 and H4R3 methyl marks act to silence gene expression and may promote chromatin compaction.

Fig. 4. Targeting Pax2 with Small Molecules

A) The PAI subdomain of Pax2 paired domain is shown with a DNA binding pocket and key amino acid residues marked. The red square outlines the pocket used for a computational screen to identify the small molecule DNA binding inhibitor EG1. B) Point mutations in critical glutamine (Q) and cysteine (C) residues abrogate DNA binding and transactivation of a reporter gene, even upon stimulation with a JNK activator MEKK1. C) In kidney organ cultures, the small molecule EG1 is able to prevent Pax2 positive cells (red) from condensing at the tips of the ureteric buds (green), thus failing to stimulate further branching of the bud. The expression of the Pax2 target gene and nephrogenic stem cell marker Cited1

is greatly reduced upon Pax2 inhibition by EG1. All cultures were done for 48 hours and controls had DMSO only added. Scale bars are in μm as indicated and detailed methods are as described⁷⁹.

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