Overview of the Pathway

The core kinases in mammals are the Hpo ortholog Mst1/2 in a complex with its regulatory protein SAV1, acting upstream of Lats1/2 also in a complex with regulatory Mob1 (Fig. 3). MST is activated in a phosphorylation-dependent manner by upstream tumor suppressors NF2/Merlin and KIBRA (1, 16, 22, 40, 72, 76). MST phosphorylation in turn induces phosphorylation of LATS, which induces the phosphorylation and sequestration of the ultimate pathway target YAP or TAZ in the cytoplasm (19, 25, 46, 75). In this sense, an “active” Hippo pathway is one in which YAP/TAZ are phosphorylated and expressed in the nucleus where they bind one of four TEAD transcription factors (TEAD1-4). The YAP/TAZ-TEAD transcription complex in turn mediates context-dependent transcriptional gene expression such as ctgf and others involved in cell survival, cell polarity, and cell fate determination (74, 77, 78). Several components of the pathway have been implicated in mammalian models of tumorigenesis. Nf2 is a tumor suppressor gene responsible for neurofibromatosis type II. Nf2 loss is associated with a wide range of tumors, including osteosarcoma and hepatocellular carcinoma (39). Lats1 deficiency results in soft tissue sarcomas and ovarian tumors (62). Mst-deficient mice develop hepatocellular carcinoma, cholangiocarcinoma, and colonic adenoma (37, 60, 80). Mob1 mutant mice develop skin, liver, and breast cancer as well as osteosarcomas and fibrosarcomas (44). Yap is an oncogene that is amplified in hepatocellular and squamous cell carcinomas, gastrointestinal dysplasia, and pancreatic duct metaplasia (4, 12, 55, 73).
Kidney Development

Kidney development requires a complex, well-orchestrated signaling interplay between the epithelial ureteric bud (UB) and the surrounding metanephric mesenchyme (11, 13, 36, 52). The mesenchyme promotes UB branching, resulting in renal collecting duct formation. Cap mesenchyme (CM) cells are in turn induced by the UB to form a pretubular aggregate (PA), which are mesenchymal preepithelial cells that form the renal vesicle (RV). The RV, the most primitive stage of nephron development, results in subsequent elongation to a comma-shaped and then S-shaped body, making contact and fusing with the distal ureteric bud. The S-shaped body undergoes elongation and differentiation in association with invasion of endothelial cells to give rise to the glomerulus and various nephron segments. The signaling mediators critical in this intricate morphogenetic process remain poorly understood.

The role of the Hippo pathway in nephrogenesis has been the subject of recent attention. Constitutive Yap deletion results in embryonic lethality at embryonic day 8.5 (E8.5) with a failure to develop an organized yolk sac vascular plexus (42). Conditional gene silencing approaches have therefore been utilized to define the role of YAP and TAZ at various stages in kidney development. Animals in which Yap is silenced at the CM do not survive beyond 48 h postbirth (49). The kidneys from these animals show a multitude of anatomical and histological anomalies: hypoplastic kidneys and an empty bladder, suggesting failure to produce urine; smaller papilla and a reduced nephrogenic zone; indistinguishable convoluted renal tubules and glomeruli in the inner cortex; a medulla mainly composed of collecting ducts; a dramatic reduction in the number of detectable glomeruli and proximal tubules; abnormal structures in the glomeruli; defects in Henle’s loop and distal tubule formation; and abnormal morphogenesis of S-shaped bodies. The loss of Yap also significantly reduced the number of ureteric bud tips at P0, revealing a late-onset function of Yap in branching morphogenesis in the developing nephron. These data support the conclusion that Yap-depleted CM cells are unable to undergo normal nephrogenesis and morphogenesis during renal development. Interestingly, the loss of the Rho-GTPase Cdc42 from the CM results in reduced YAP activation and decreased Yap-dependent gene expression that phenocopies Yap\textsuperscript{CM−/−} kidneys (49).

In contrast to Yap\textsuperscript{CM−/−} kidneys, deletion of Taz in the CM does not result in dysplastic kidneys or defective nephrogenesis. Taz\textsuperscript{CM−/−} do, however, display highly cystic cortical tubules. These investigators also silenced Taz in Yap\textsuperscript{CM−/−} mice, generating double Yap\textsuperscript{CM−/−}, Taz\textsuperscript{CM−/−} mutants, which did not have an exacerbation of the Yap\textsuperscript{CM−/−} phenotype. Yap and Taz therefore appear to have divergent but critical roles in nephrogenesis (49).

Lower urinary tract. A crucial step in urinary tract matura-

Fig. 1. Hippo-mutant phenotypes in Drosophila and mice. Electron micro-

graphs of wild-type (A) and homozygous mutant Hippo (Hpo) gene (B) are shown. (C, D) mouse liver from a wild-type animal at 2 mo of age. (C) mouse liver from a Mst1\textsuperscript{−/−} Mst2\textsuperscript{−/−} conditional knockout animal. This image was reproduced from Ref. 18 with permission (Company of Biologists, Ltd., 2011).

Fig. 2. Dysregulation of the mammalian Hippo pathway leads to tumorigenesis in vivo. A: liver from an ApoE/rtTA-YAP mouse raised on Dox for 8 wk,

starting at 3 wk after birth. Note the presence of discrete nodules scattered throughout the liver (arrowheads). B: liver from an ApoE/rtTA-YAP mouse raised on Dox for 3 mo, starting at birth. Note the widespread development of hepatocellular carcinoma throughout the liver. This image was reproduced from Ref. 12 with permission (Elsevier, 2012).
mally, suggesting that YAP expression is sufficient in the absence of TAZ. However, silencing of Taz in YapND−/− mice severely worsens the phenotype, with kidneys being dysplastic, showing no ureters at E18.5, aberrant branching morphogenesis, blind-ending NDs, a defective ND-cloaca connection, and markedly increased apoptosis (48). These findings highlight the crucial role YAP and TAZ play in lower urinary tract development and lay the groundwork for further diagnostic, mechanistic, and therapeutic approaches to CAKUT. The synergistic roles of Yap and Taz in lower urinary tract development also contrast with their distinct ones in nephrogenesis and highlight the cell- and context-dependent nature of their signaling properties.

**Glomerulus**

To date, however, on Hippo signaling in glomerular visceral epithelial cells or podocytes. Podocytes are terminally differentiated cells that form the final barrier to urinary protein loss, and podocyte injury is usually associated with proteinuria (17, 33, 34). Persistence of podocyte injury results in loss of podocytes, then leading to irreversible glomerulosclerosis and kidney failure (29, 32, 67). The roles of YAP and KIBRA have been explored in podocyte homeostasis. YAP is present in dual compartments in podocytes, with abundant baseline nuclear and notable cytoplasmic expression (5). YAP inhibits podocyte apoptosis by binding to and inhibiting the proinjury signaling molecule dendrin. Yap gene silencing makes cultured podocytes more susceptible to adriamycin- and staurosporine-induced injury (5). In vivo, podocyte-specific Yap deletion results in proteinuria between 5 and 6 wk of age and histological lesions characteristic of FSGS at 12 wk (Fig. 4) (57). Yap-deficient podocytes undergo apoptosis, resulting in progressive podocyte depletion (57). Taken together these findings high-

Fig. 3. The core Hippo pathway. KIBRA/NF2 promote phosphorylation and activation of MST 1/2 and Sav1. This in turn phosphorylates and activates LATS1/2 and MOB1. LATS 1/2 phosphorylates YAP/TAZ, leading to its cytoplasmic sequestration, inactivation, and degradation. When YAP/TAZ are dephosphorylated, they enter the nucleus to induce gene transcription by interacting with TEAD1-4 transcription factors.
light the role of YAP in maintaining an intact adult glomerular filter. The cell- and context-dependent consequence of YAP silencing is once again reinforced where Six2-mediated Yap deletion in the CM impairs nephrogenesis without evident apoptosis (49) while podocin-Cre-mediated Yap deletion results in normal development but a later onset podocytopathy associated with apoptosis and podocyte depletion (57).

KIBRA (“kidney brain”) protein, an upstream regulator of YAP via activation of the Hippo kinases, is important in podocyte homeostasis. KIBRA was initially characterized by a yeast two-hybrid screen as a putative binding partner for dendrin (31). KIBRA shares dendrin’s role as a growth inhibitor and promoter of apoptosis as initially shown in Drosophila where KIBRA overexpression in imaginal disks results in decreased size of the adult Drosophila eye (72). Similar to other upstream members of the mammalian Hippo pathway, KIBRA antagonizes YAP function. In the MCF-10A human breast cancer cell line, KIBRA inhibits YAP-dependent growth and migration in these cells, suggesting that it may serve as a tumor suppressor (40). Two studies have investigated KIBRA signaling in podocytes. The first, by Duning et al. (14) in 2008, identified KIBRA’s presence in podocytes of the glomerular tuft and in renal tubular cells. This study demonstrated that KIBRA interacts with the actin-bundling protein synaptopodin and the cell polarity protein PATJ. The functional impact of these interactions was highlighted by the finding that silencing the Wwcl gene that encodes KIBRA in human podocytes resulted in impaired directed migration based on the reduced ability of knockdown podocytes to close a wound in a wound-healing assay (14). Despite increased velocity, the KIBRA-knockdown podocytes had impaired polarity that disrupted their capacity for directed cell migration. Further studies will be needed to clarify the role of KIBRA in the context of podocyte actin cytoskeleton integrity and its interaction with modulators of podocyte motility and polarity (15, 71).

Recently, it was demonstrated that overexpression of KIBRA in human podocytes activates LATS kinase, inducing the phosphorylation and cytoplasmic sequestration of YAP (66). Treatment of podocytes with latrunculin B, which disrupts the actin cytoskeleton, led to similar changes in podocytes as KIBRA overexpression, namely, an increase in the levels of phosphorylated LATS and the cytoplasmic redistribution and phosphorylation of YAP. KIBRA overexpression in podocytes was also associated with increased levels of apoptosis (66). This study further highlights the existence of canonical Hippo signaling in podocytes and its role in podocyte survival and homeostasis. KIBRA, like its binding partner dendrin, promotes podocyte injury through enhanced apoptosis.

**Tubules and Interstitium**

**Hippo in cystic kidney disease.** Hippo signaling is also relevant in cystic kidney disease. A study by Hossain et al. (24) from 2006 demonstrated that constitutive Taz null adult mice presented with cystic kidney disease by 8 wk of age, without evidence of cyst formation in other organs (24). Interestingly, most of the renal cysts were of glomerular origin, with a minority derived from the collecting duct and proximal and distal tubules. These knockout mice developed progressive renal insufficiency by 4–6 mo of age. The renal cysts were...
characterized by abnormalities of the epithelial cilia, including shortened ciliary length and structural abnormalities, and some epithelial cells lining the cystic or dilated ducts lacked cilia completely. The importance of TAZ/Wwtr1 to ciliary integrity and cyst formation was further suggested by the corresponding decreased expression of PKD-associated genes that have also been linked to the renal ciliary system, including khd1, Tg737, Kif3a, and well as Tsc1 (tuberous sclerosis complex), which has been associated with glomerular cyst development (24).

The link between Taz deletion and the development of cystic renal disease was further demonstrated with a Taz-null/lacZ knockin mouse (38). These animals also developed cystic renal disease, with cysts mainly derived from glomeruli and proximal tubular epithelia, as well as dilated air spaces in the lung resembling human pulmonary emphysema. As observed in human PKD, these Taz-null/lacZ knockout mutants also had urinary concentration defects, polyuria, and hydropnephrosis. Interestingly, here there were no changes in expression levels of genes involved in human polycystic kidney disease (PKD), including autosomal dominant PKD (ADPKD)-associated polycystin-1 (Pkd1) and polycystin-2 (Pkd2), and Pkd1, which has been linked to ARPKD (autosomal recessive PKD).

Taz serves as a transcriptional coactivator of Glis3, a subfamily of Kruppel-like zinc finger proteins that contain a DNA binding domain and regulate gene transcription (2, 28). Similar to Taz knockout mice, Glis3-deleted mice develop renal cysts mostly from glomeruli but also from proximal and distal tubules and collecting ducts. In addition, Glis3 localized to primary cilia, and a significant number of epithelial cells in renal cysts either lacked primary cilia or had truncated primary cilia. Thus it is possible that TAZ interacts with Glis3 to suppress cyst formation.

Another interesting observation lies with the signaling properties of cysts in Taz null mice. Wnt/β-catenin signaling has been implicated in cyst formation in PKD. Activated mutant β-catenin gene expression results in a phenotype that mimics human ADPKD (51). Like cysts in humans with ADPKD (35), kidney cysts in Taz null mice display enhanced Wnt/β-catenin signaling (65). This provides evidence of cross talk between Wnt and Hippo signaling pathways that has been further validated in other model systems such as colorectal carcinoma and in osteogenesis (30, 58). It also provides a potential mechanistic explanation for cyst formation in Taz-null mice.

Epithelial-specific deletion of the Pkd1 gene in mice, which results in renal cystogenesis, is associated with the development of aberrations in Four-jointed 1 (Fjx1), a regulator of the Hippo pathway (20, 68). Fjx1 is also a member of the planar cell polarity (PCP) signaling pathway, which is normally active during the repair phase of epithelial injury but displays abnormalities in the setting of Pkd1 deletion (20). Specifically, Fjx1 expression is reduced in the kidneys of Pkd1-deleted mice in the precystic stages but later increased significantly in the cystic kidneys. The same investigators found that in adult conditional Pkd1-deleted mice (cKO), nuclear localization of YAP was present in the cystic epithelial cells that developed in the proximal tubules 10 wk after injury with the nephrotoxin 1,2-dichlorovinylcysteine (DCVC). Strong nuclear YAP expression was detected in cystic epithelial cells using an alternate model, the Pkd1 null mice, which develop renal cysts derived mostly from the distal tubules or collecting ducts by 3 wk of age. Analysis of gene expression levels of YAP target genes revealed significant upregulation of baculoviral IAP-repeat-containing-3 (Birc-3) and inhibit β-A (Inhba) in the cystic stages of the cKO mice and in cystic kidneys of the Pkd1 null mice. The findings from animal studies turned out to be relevant to human pathology, as evidenced by YAP staining of tissue from renal biopsies. Nuclear accumulation of YAP was found in cystic epithelial cells in human ADPKD and ARPKD, as well as in cysts associated with various renal tumors, including clear-cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma, and renal mixed epithelial and stromal tumors. In contrast, no nuclear YAP was present in the proximal tubular epithelial cells from normal human biopsy renal tissue (21).

In summary, there is emerging evidence that the Hippo signaling pathway plays a role in the development of PKD. Cross talk between the Hippo and Wnt signaling pathways appears to regulate cyst growth. The expression of TAZ and the nuclear/cyttoplasmic distribution of YAP are key regulatory determinants of cystogenesis.

Diabetic kidney disease. Diabetics are at high risk for renal disease progressing to end-stage renal disease (3). The pathogenesis of disease and factors that distinguish progressors from nonprogressors remain unclear. Like the Hippo pathway, the epidermal growth factor receptor (EGFR) pathway was initially proposed as a target for cancer therapy (54). It has since been implicated in diabetic kidney disease. Prolonged EGFR activation in diabetic animals enhances TGF-β-mediated renal injury, while attenuation of EGFR signaling is protective (8, 47, 50, 53). A recently published study establishes a link between EGFR and Hippo signaling in the diabetic kidney (9). YAP expression and phosphorylation were found to be increased in animal models of type 1 (streptozotocin-induced) and type 2 (db/db) diabetes, as well as in proximal tubule-like epithelial cells exposed to high glucose for 24 h. These effects were attenuated by in vivo and in vitro silencing of EGFR expression as well as treatment of mice and cells with the EGFR inhibitor erlotinib. In mice with proximal tubule EGFR deletion, TAZ expression was decreased, indicating differential upstream regulation of the paralogs YAP and TAZ. Another interesting finding with respect to the pathogenesis of diabetic kidney disease was the observation that expression of CTGF and another YAP transcriptional target gene, amphiregulin (AREG), was increased after high-glucose treatment. The high-glucose effects on CTGF and AREG were blunted after either Egfr or Yap was silenced by their specific small interfering (si) RNA and also after treatment with verteporfin, which is an inhibitor of YAP-TEAD interaction (9). These findings suggest that the diabetic milieu promotes differential YAP expression, phosphorylation, and target gene expression in an EGFR-dependent manner.

Renal cell carcinoma. Aberrant Hippo pathway signaling resulting in hyperactive YAP function has been detected in various human cancers, including those affecting the kidney. Nuclear overexpression of YAP has been detected in a subset of patients with ccRCC (56). Yap silencing also reduces proliferation, migration, and anchorage-independent growth of ccRCC cells (56). Yap mRNA and protein expression levels are also increased in ccRCC tissue and cell lines. Furthermore, Yap silencing in 786-0 ccRCC lines results in cell cycle arrest and increased apoptosis (6). Targeted deletion of the Nf2 gene that encodes the upstream Hippo pathway regulator Merlin in proximal convoluted epithelium results in intratubular neopla-
sia that progresses to invasive carcinoma (43). Interestingly, early lumen-filling lesions in this model exhibited hyperactive EGFR signaling with EGFR inhibition halting tumor cell proliferation (43). These findings highlight the potentially deleterious effects of unrestrained YAP signaling and provide further confirmation of cross talk between the Hippo and EGFR pathways.

**Renal fibrogenesis.** Renal fibrosis, arising as a result of numerous divergent processes, culminates in the deposition of extracellular matrix that classically accompanies chronic kidney disease (23). Stiff extracellular matrix enhances TGF-β-induced profibrotic Smad signaling in a process mediated by YAP and TAZ (63). In the unilateral ureteral obstruction (UUO) model of renal fibrosis, YAP/TAZ display nuclear expression unlike sham-operated controls. Furthermore, verteporfin, a drug that inhibits YAP-TEAD interaction and YAP transcriptional activity, reduces interstitial YAP/TAZ staining, associated nuclear Smad 2/3 nuclear accumulation, and renal fibrosis (63). An antibody that recognized both YAP/TAZ was used here, so a distinction cannot be made regarding divergent functions of the two molecules. This study identified a potential therapeutic strategy to target both classic Smad 2/3 and mechanosensitive YAP/TAZ in renal fibrogenesis.

**Conclusion**

Limited but compelling evidence exists that the Hippo signaling pathway has a significant role in kidney and urinary tract development, podocyte homeostasis, fibrotic, and cystic and diabetic kidney disease. In the kidney as in other model systems, the relationship between YAP and its paralog TAZ is cell and context dependent. The Hippo pathway communicates with EGFR in diabetic kidney disease and Wnt/β-catenin in cystic kidney disease. Further studies are needed to determine the relationship between Hippo signaling and other established interacting cascades such as TGF-β and Notch. The potential utility of pharmacological manipulation of the Hippo pathway in developmental and chronic kidney disorders is yet to be determined.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**Table 1. Hippo pathway modulators of kidney disease**

<table>
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<tr>
<th>Hippo Mediator</th>
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<td>KIBRA</td>
<td>1. KIBRA silencing impairs human podocyte polarity; 2. KIBRA overexpression promotes podocyte apoptosis.</td>
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<td>Lats</td>
<td>1. LATS promotes YAP phosphorylation in podocytes. 2. High glucose induces LATS phosphorylation in proximal tubular epithelial cells.</td>
<td>?</td>
<td>?</td>
<td>66</td>
</tr>
<tr>
<td>YAP</td>
<td>1. Promotes podocyte survival. 2. Proximal tubule epithelial cells exposed to high glucose → increased YAP expression. 3. Rat fibroblasts grown of stiff matrix → increased nuclear YAP/TAZ.</td>
<td>1. Constitutive YAP KO → embryonic lethal. 2. Cap mesenchyme YAP deletion → Death at 48 h postbirth, hypoplastic kidneys, empty bladder, dramatic reduction in detectable glomeruli and proximal tubules. 3. Nephric duct YAP deletion → death within 24 h; hydronephrotic kidneys with blind-ending megaureters at birth. 4. Podocyte-specific YAP deletion → FSGS. 5. Streptozotocin-induced (type 1) and db/db diabetic models: YAP expression and phosphorylation increased.</td>
<td>1. FSGS: reduced podocyte YAP. 2. ADPKD and ARPKD: nuclear YAP. 3. Renal carcinoma (clear cell, papillary, renal mixed epithelial and stromal): nuclear YAP.</td>
<td>5, 9, 48, 49, 56, 57, 63</td>
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<tr>
<td>TAZ</td>
<td>Rat fibroblasts grown of stiff matrix → increased nuclear YAP/TAZ.</td>
<td>1. Cap mesenchyme TAZ deletion → cystic cortical tubules. 2. Nephric duct deletion → normal development but double YAP/TAZ deletion in nephric duct → no ureters at E18.5. 3. Constitutive TAZ null mice → cystic kidney disease. 4. Streptozotocin-induced (type 1) and db/db diabetic models: TAZ expression decreased.</td>
<td>?</td>
<td>9, 48, 49, 63</td>
</tr>
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</table>

KO, knockout; FSGS, focal segmented glomerulosclerosis; ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease.
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AJP-Renal Physiol • doi:10.1152/ajprenal.00500.2015 • www.ajprenal.org

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