Autosomal-dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease, caused by genetic mutations in PKD1 or PKD2, which leads to end-stage renal disease. Polycystins, the transmembrane proteins encoded by PKD1 or 2, are nonselective cation channels transporting calcium ions into the cells. Disruption of polycystic kidney disease (PKD) genes impairs intracellular calcium homeostasis and results in the development of numerous fluid-filled cysts from abnormally proliferating renal tubular cells. It is also accompanied by interstitial inflammation and fibrosis around the cyst-lining cells, ultimately reaching end-stage renal disease (ESRD) (1, 2).

The Hippo signaling cascades are essential to control organ size, differentiation, and tissue regeneration. These are highly coordinated processes in which more than 30 core proteins are involved in responding to the mechanical stimuli from the cellular microenvironment. Activated Hippo kinase cascades, including sterile 20-like kinase 1/2 (MST1/2) and large tumor suppressor 1/2 (LATS1/2), lead to the phosphorylation of their downstream effectors, YAP1/TAZ, followed either by their cytosolic retention or degradation, thus preventing their nuclear localization (3-5). Inactivation of the Hippo pathway increases the nuclear localization of YAP1 and TAZ, which interact with the TEAD family transcription factors and drive the expression of target genes, such as CTGF, CYR61, and c-MYC (4, 6).

Recently, the role of Hippo signaling pathway has emerged in the formation of cysts in ADPKD (7, 8). A previous study has reported that YAP1 and its transcriptional target, c-MYC, mediate cystic kidney pathogenesis in Pkd1-deficient mice, and a Rhoa–YAP–c-MYC axis was suggested to be involved in the pathogenesis of ADPKD (9-11).

However, the role of Hippo signaling pathway in cystogenesis in ADPKD remains unclear. TAZ may regulate the Wnt/β-catenin signaling. We previously reported that intrinsic activation of TAZ by genetic deletion of WW45, a component of the Hippo pathway, increased β-catenin activity in the kidneys (12). Furthermore, Wnt/β-catenin signaling or its target genes such as c-Myc may regulate cystogenesis in the mouse kidney (11, 13, 14). Therefore, we focused on deciphering the role of TAZ, a Hippo effector, in regulating cystogenesis in ADPKD.

Herein, we elucidate a mechanism by which TAZ promotes the activation of Wnt/β-catenin signaling in the kidney of Pkd1-deficient mice and show that it increases c-Myc transcript levels. Basal YAP1/TAZ expression levels were high around the cyst-lined cells in the kidneys of Pkd1-deficient mice and patients with ADPKD and were associated with high c-Myc and β-catenin expression levels. The loss of TAZ in Pkd1-deficient mice resulted in low levels of the c-Myc protein and was observed to delay the progression of PKD. In vitro studies revealed that PKD1 mostly interacted with TAZ in wild-type cells, but its absence allowed TAZ to strongly interact with AXIN1, thereby resulting in a weak interaction between β-catenin and AXIN1. We further observed that c-Myc expression, which causes cystogenesis in mouse kidney, was directly regulated by TAZ and β-catenin in PKD1-deficient cells. Overall, our results show that the TAZ–β-catenin–c-Myc axis is responsible for renal cystogenesis in Pkd1-deficient mice. Based on these findings, we
suggest that the TAZ-β-catenin-c-MYC axis is a potential therapeutic target for ADPKD.

Results

YAPI/TAZ and c-MYC Are Increased in the Kidney of Pkd1-Targeted Mice and Patients with ADPKD. We used renal collecting duct-specific Pkd1-knockout mouse as previously described (15). To determine whether the expression of TAZ correlated with those of β-catenin and c-MYC in Pkd1-null kidney, we first assessed protein levels of TAZ and c-MYC in the kidney of Pkd1-deleted mice and found that their levels, along with the level of active β-catenin protein, were highly up-regulated (Fig. 1A). The mRNA levels of c-Myc, a known target of β-catenin or YAPI/TAZ, were enhanced in the kidney of Pkd1-null mice (Fig. 1B). To ensure the collecting duct-specific deletion of Pkd1 that is followed by the development of renal cysts, we costained collecting duct-specific marker (DBA) with target proteins. We have confirmed that all of the cyst-lined cells were stained with DBA, and, furthermore, accumulation of TAZ and c-MYC was increased, in the Pkd1-deleted kidneys (Fig. 1 C–F). In addition, β-catenin was activated in both cyst-lined epithelia and peripheral cells around the cyst linings (Fig. 1G and H). In line with these experimental data, we examined TAZ, active β-catenin, total β-catenin, and c-MYC expression in normal human and ADPKD patient kidney. The indicated proteins were highly expressed in cyst-lining cells of ADPKD patient kidneys compared to normal human kidney tissues (Fig. 1I). These results indicated that the overexpression of TAZ was positively correlated to that of β-catenin and c-MYC in the kidneys of both Pkd1-deleted mice and patients with ADPKD.

RNA-Seq Analysis Showed Significant Increase of Yap/Taz Target Gene Expression in Pkd1-Deleted Kidneys. For more in-depth analysis, alterations in the target genes expression were verified on an mRNA level based on RNA-seq data, which had been previously accomplished using kidney tissues from the same mice model (15). We first screened changes in Yap, Taz, and β-catenin levels and confirmed that expression of those genes insignificantly changed in Pkd1-deleted kidneys (Fig. 2A). Meanwhile, different expression patterns were observed in Yap/Taz target genes, which have been listed from public sources comprising 354 genes. We found that an enrichment score (ES) of the rank-ordered Yap/Taz target genes showed highly increased patterns by GSEA analysis (Fig. 2B). Of the Yap/Taz target genes, 129 genes were defined as leading-edge subset genes, which had significantly higher expression levels in Pkd1-deleted cystic kidneys, and the top 20 genes differentially expressed with log2 fold changes of greater than 0.795 were indicated (Fig. 2 C and D). Among them, high-ranking genes including c-Myc were validated using quantitative real-time PCR (qRT-PCR), and the expression level of those genes was indeed increased (Fig. 2E). These results further suggested that Yap/Taz activation might be positively implicated in the growth of renal cysts stimulated by Pkd1 deletion.

Deletion of Taz Inhibits Cyst Growth with Enhanced Renal Function in the Kidney of Pkd1-Targeted Mice. To elucidate the role of enhanced TAZ levels in renal cyst growth in the kidneys of Pkd1-targeted mice, we generated collecting duct-specific double Pkd1/Taz-knockout mouse (Pkd1 β/β; Taz β/β; HoxB7Cre). These mice were euthanized on postnatal day 13. Pkd1-deficient mice with Taz deletion showed highly reduced cyst development. The cystic area was quantified to indicate the changes in its size distribution, and it indeed revealed that the number of large cysts was significantly decreased in Pkd1/Taz double-knockout kidneys (Fig. 3A). In addition, the kidneys to total body weight (2KW/TBW) ratio, as well as blood urea nitrogen (BUN) level, in Pkd1/Taz double-knockout mice were significantly lowered compared to those in Pkd1-deleted mice, indicating that reduced TAZ expression enhanced renal function (Fig. 3B). Next, we examined whether the increased expression of active β-catenin and c-MYC in the kidney of Pkd1-null mice was modulated by the reduction in TAZ levels. As a result, TAZ mutation in Pkd1-deleted mice reduced the levels of active β-catenin and c-MYC in the kidney (Fig. 3C). Immunofluorescent analyses also revealed that the increased signals were significantly reduced in Pkd1/Taz double-knockout kidneys (Fig. 3 D–F). In addition, cell proliferation, which was hyperactivated around the cysts derived by Pkd1 deletion, was inhibited in double-knockout kidneys (Fig. 3G). Collectively, these results suggested that reduction in the TAZ expression alleviated the PKD phenotype and was accompanied by decreased expression of active β-catenin and c-MYC.

In Vitro Cystogenesis Is Stimulated by the Increase in TAZ Levels, and Wnt Inhibition Attenuates Its Effect. TAZ is one of the upstream regulators of c-MYC expression, both implicated in renal cystogenesis (2, 6). Since TAZ and c-MYC levels were increased in the kidney of Pkd1-null mice, we examined whether an increase in TAZ up-regulated the expression of c-MYC in Pkd1-silenced cells. Consistent with previous observations, Pkd1 silencing increased TAZ and c-MYC levels in IMCD cells (Fig. 4A and B). Next, we evaluated the protein levels of c-MYC in cells treated with a cell-permeable imidazole-[4,5-b] pyridine derivative, a TAZ nuclear-promoting chemical that enhances the nuclear localization of TAZ. The treatment had enhanced the amount of TAZ protein in a concentration-dependent manner with a concomitant increase in c-MYC expression (Fig. 4C). To further investigate whether changes in either the genetic expression or the activity of TAZ affected cyst growth in vitro, we performed 3D culture of IMCD cells. The cells were pretreated with siRNA targeting Pkd1 or Taz for 24 h and embedded in Matrigel (in a 1:1 ratio with the culture medium). Subsequently, DMEM F/12 medium with 5 μM forskolin was added, and cysts were observed on the fifth day of seeding. Forskolin increases the intracellular cAMP level, and thereby provides PKD-mimicking conditions and stimulates cyst growth. We observed the gradual development of cysts 2 to 3 d following forskolin treatment, while Pkd1 silencing led to an increased cystic area. Cysts developed from cells silenced with siRNAs targeting Pkd1 and Taz were smaller (Fig. 4D). We also observed the effect of TAZ overexpression on the growth of in vitro cysts. Forced expression of TAZ accelerated cystogenesis of Pkd1-silenced cells, as indicated by the increase in the cystic area compared to the cells transfected with the Pkd1-targeting siRNA alone (Fig. 4E). Next, we examined whether treatment with a drug regulating TAZ activity (TAZ modulator) affected cyst growth. Our results showed that the in vitro cysts were significantly increased by drug treatment and were associated with enhanced c-MYC levels (Fig. 4F). We further investigated whether the inhibition of β-catenin activity could attenuate the cyst-stimulating effect of the TAZ modulator. Endo IWR1, an AXIN stabilizer, was used to down-regulate β-catenin activity (16). Treatment with Endo IWR1 successfully inhibited active β-catenin, followed by a decrease in c-MYC level (Fig. 4G). A negative control Exo IWR1, conversely, did not affect either. Observations in 3D culture showed that in vitro cysts stimulated by TAZ modulator were significantly reduced by Endo IWR treatment (Fig. 4H). We additionally verified the effect of mTOR inhibitor, which was previously reported to reduce cyst growth in multiple preclinical models, on TAZ/β-catenin/c-MYC pathways. We first investigated whether rapamycin affects the TAZ/Wnt/β-catenin/c-MYC axis and found it to be significantly inhibited by drug treatment. In addition, treatment

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Fig. 1. High expression of TAZ, c-MYC, and active β-catenin in the kidneys of Pkd1-deleted mice and patients with ADPKD. (A) Screening of the basal levels of YAP/TAZ, c-MYC, and active β-catenin in the kidneys of Pkd1-deleted mice at postnatal day 13 (p13). (B) Increased c-Myc levels in mRNA samples from Pkd1-deleted kidneys. (C–H) Immunofluorescent (IF) staining of DBA, TAZ, c-MYC, and active β-catenin (ABC) around the cyst-lining cells in the kidneys of Pkd1-deleted mice. Fluorescent signals were quantified by the green histogram analyses using ImageJ. (I) Immunohistochemistry of normal and ADPKD human kidney. Immunohistochemical (IHC) staining of TAZ, β-catenin, active β-catenin (ABC), and c-MYC. TAZ, β-catenin, active β-catenin (ABC), and c-MYC were substantially increased around the cyst-lining cells of ADPKD patients compared to the normal kidney. (Scale bar, 100 μm.) Two wild-type and four Pkd1-null mouse kidneys were used to test basal expression levels of TAZ, c-MYC, and active β-catenin. Statistical analysis was performed using two-tailed t tests. A value of P < 0.05 was considered statistically significant (*P < 0.05, **P < 0.01, ***P < 0.001).
with rapamycin combined with the Wnt inhibitor showed significant decrease of in vitro cyst growth compared to that observed in the rapamycin-treated group (SI Appendix, Fig. S1). Therefore, we believe that TAZ and its downstream signaling pathways mediated by c-MYC negatively impact cyst development and thus PKD progression.

**Regulation of β-Catenin Activation by PKD1 through TAZ and AXIN1.** We observed that the kidneys of Pkd1-deleted mice showed an increase in the levels of TAZ along with high levels of active β-catenin and c-MYC proteins (Fig. 2A and B). TAZ is known to induce the expression of c-MYC mRNA overlap with the target gene of Wnt/β-catenin signaling (6). Next, we determined the TAZ–β-catenin–c-MYC downstream signaling of PKD1 in detail. For this, we first investigated whether PKD1 or TAZ depletion or a codepletion affected the expression of active β-catenin. PKD1 depletion induced a slight increase in TAZ levels and significantly increased the levels of active β-catenin. Further, this increase was reduced to a level comparable to that in control cells upon transfection of PKD1 siRNAs in TAZ shRNA-expressing cells (Fig. 5A). Conversely, the feeble expression of active β-catenin in control cells was abrogated by the overexpressed Flag-PKD1, whereas the expression of HA-β-TAZ markedly increased the active β-catenin levels in a dose-dependent manner, irrespective of the forced expression of Flag-PKD1 (Fig. 5B). In line with these results, we further investigated whether the transcriptional activity of β-catenin was regulated under the same conditions as presented in Fig. 5A. The depletion of PKD1 led to a strong induction of the transcriptional activity of β-catenin, which was reduced by the knockdown of TAZ in PKD1-depleted cells (Fig. 5C). In contrast, PKD1 expression slightly decreased the transcriptional activity of β-catenin, which was enhanced to the level comparable with TAZ-expressing cells upon coexpression of PKD1 and TAZ (Fig. 4D). Collectively, these results suggest that TAZ-dependent PKD1 expression negatively regulates the activation of β-catenin. β-Catenin is degraded by an AXIN1-containing destruction complex. Therefore, we examined whether AXIN1 participated in the PKD1-mediated regulation of β-catenin activation. Our results showed that AXIN1 depletion restored the levels of active β-catenin in PKD1-expressing cells (Fig. 5E). Furthermore, AXIN1 depletion also abrogated the reduction of the transcriptional activity of β-catenin in PKD1-expressing cells (Fig. 5F). These data indicate that AXIN1 may be a downstream mediator of PKD1 in regulating the β-catenin activity in PKD1-depleted cells. We further determined the epistatic relationship between TAZ and AXIN1 underlying the PKD1 function in the regulation of the transcriptional activity of β-catenin. The expression of TAZ shRNA reduced the transcriptional activity of β-catenin, which was significantly increased by AXIN1 silencing (Fig. 5G). Furthermore, AXIN1 expression led to a strong reduction in β-catenin activation induced by TAZ expression (Fig. 5G). These results suggest that TAZ acts upstream of AXIN1 to regulate the transcriptional activation of β-catenin. To confirm whether TAZ-dependent PKD1 expression affected the expression of the target genes of β-catenin, we examined the effects of PKD1 silencing alone or of PKD1 and TAZ silencing on the expression of AXIN, c-MYC, and CCND2 mRNAs; the expression levels of these genes were elevated in PKD1-depleted cells but not in TAZ-deficient cells expressing PKD1 (Fig. 5H). These data indicate that the regulation of β-catenin–mediated transcriptional activation of AXIN2, c-MYC, and CCND2 by PKD1 depends on TAZ.
A Strong Interaction of AXIN1 with TAZ in the Absence of PKD1 Causes the Activation of β-Catenin. β-Catenin activation is regulated by PKD1, which in turn depends on TAZ and AXIN1 (Fig. 5). TAZ is known to associate with the β-catenin destruction complex (17) or to interact with PKD1 (18). We therefore tested the possible interaction of TAZ with AXIN1 and PKD1 using PKD1-silenced cells transfected with HA-TAZ. Our results showed that PKD1 depletion did not change the amount of total HA-TAZ, which was thought to be saturated in cells. HA-TAZ coprecipitated with endogenous PKD1, whereas it interacted with AXIN1 in the absence of PKD1 (Fig. 6A). These observations suggest that TAZ interacts more strongly with PKD1 than with AXIN1, whereas the absence of PKD1 triggers the interaction of TAZ with AXIN1. We assumed that the interaction of TAZ with AXIN1 in the absence of PKD1 influences the association of AXIN1 with β-catenin. To confirm this, Myc-AXIN1 was pulled down with the Myc antibody from the lysates of Myc-AXIN1–expressing cells transfected with si-PKD1 and si-control. Immunoblotting of the Myc-AXIN1 immunoprecipitated with anti-β-catenin antibody revealed that β-catenin coprecipitated

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Fig. 4. Changes in c-MYC levels by controlling of TAZ, followed by in vitro cyst growth. (A and B) Screening of TAZ and c-MYC expression levels in Pkd1-silenced mouse inner medullary collecting duct (IMCD) cells. (C) Effect of TAZ modulator, which enhances TAZ activities via translocation into the nucleus. Cells were treated with the drugs at different concentrations from 1 to 10 μM. The level of TAZ was increased at both low and high concentrations and was followed by increased expression of c-MYC. (D–F) The effect of controlling TAZ levels on in vitro cystogenesis, either by genetic regulation or treatment of TAZ modulator. siRNA successfully targeted TAZ, and, conversely, the TAZ-expressing vector overexpressed the TAZ protein with consequent increase in the expression of c-MYC. Cells pretransfected for 24 h were embedded in Matrigel, and culture medium with 10 μM forskolin was added on the next day. The medium was changed daily. In vitro cysts were observed at 5 d after embedding. Taz silencing decreased the in vitro enlargement of cysts, whereas overexpression or enhancement of TAZ activity stimulated in vitro cystogenesis. (G) The changes in c-MYC level by Wnt inhibition. Either Exo IWR1 (a negative control of Endo IWR1) or Endo IWR1 (the AXIN stabilizer) was administered at 10 μM for 24 h. (H) The effect of Wnt inhibitors on in vitro cysts stimulated by TAZ modulator. Pretransfected cells were embedded in Matrigel, three-dimensionally cultured as described above, and finally observed at 5 d after seeding. All data were obtained from a minimum of three independent experiments. Statistical analysis was performed using two-tailed t tests. A value of *P < 0.05, **P < 0.01, ***P < 0.001 was considered statistically significant. 

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with Myc-AXIN1 in the si-control cells, whereas a lesser coprecipitation of β-catenin and AXIN1 was observed in PKD1-depleted cells (Fig. 6B). Moreover, Myc-AXIN1 slightly coprecipitated TAZ in si-control cells, but strongly interacted with TAZ in the absence of PKD1 (Fig. 6B). We also tested the effects of excess PKD1 on the association of AXIN1 with β-catenin or TAZ. We observed that AXIN1 communoprecipitated well with TAZ or β-catenin in the control cells but interacted weakly with TAZ and had stronger interaction with β-catenin (Fig. 6C). These results indicated that PKD1 depletion renders TAZ prone to interaction with AXIN1, resulting in a weak interaction of β-catenin with AXIN1. On the contrary, excess PKD1 did not allow TAZ to interact with AXIN1, consequently enhancing the interaction with β-catenin. To further assess whether abundant TAZ levels affected the interaction of AXIN1 with β-catenin, we carried out IP with HA-TAZ–expressing cells. Immunoprecipitation of endogenous AXIN1 revealed β-catenin, whereas forced expression of HA-TAZ induced a weak interaction of AXIN1 with β-catenin compared to the control (Fig. 6D). β-Catenin released from the destruction complex localizes to the nucleus to activate the target genes. We therefore determined whether β-catenin unbound from AXIN1 was present in the nucleus of HA-TAZ–expressing cells. Cells used for the experiments presented in Fig. 6D were used for nuclear fractionation. β-Catenin was observed in the nuclear fraction of HA-TAZ–expressing cells. Notably, HA-TAZ was also present in the same fraction (Fig. 6E). To determine whether TAZ interacts with intact AXIN1 in Pkd1-null mouse kidney, we prepared tissue lysates from Pkd1−/− or Pkd1−/−;HoxB7cre mice kidney. TAZ or AXIN1 was communoprecipitated and then blotted for AXIN1, PKD1, and active β-catenin (Fig. 6F and G). Consistent with previous results, a weak interaction of TAZ with AXIN1 was shown in wild type mouse kidney, whereas Pkd1−/−;HoxB7cre mouse kidney revealed increased interaction between AXIN1 and TAZ (Fig. 6F). Conversely, AXIN1 immunoprecipitates in Pkd1 knockout mouse kidney revealed a significant interaction with TAZ compared to that of the wild type kidney. Interestingly, the interaction between β-catenin and AXIN1 was decreased to a lesser extent in Pkd1 mutant mouse kidney (Fig. 6G). Taken altogether, deficiency of PKD1 leads to a strong interaction of TAZ with AXIN1, and β-catenin is thereby released from the destruction complex and is transcriptionally activated. Colocalization of TAZ and AXIN1 in Pkd1-Silenced Cells. Polycystin-1 and YAP/TAZ have been proposed to be mechanosensing proteins. A recent study suggested that overexpression of PKD1 decreased nuclear localization of YAP (19). We hypothesized that TAZ localization is regulated by the PKD1 aspect of their role in mechanosensing. At 50% cell density, TAZ is mostly localized in the cytoplasm, with some in the nucleus (Fig. 7A). Meanwhile, PKD1-silenced cells displayed an increase of nuclear TAZ (Fig. 7A). It suggested that the presence of PKD1 retained TAZ in the cytoplasm. Previously, we found that the interaction of TAZ with AXIN1 is increased in the absence of PKD1. We examined whether TAZ is colocalized with AXIN1 in the absence of PKD1. Ectopically expressed HA-TAZ was detected in the cytoplasm of control cells, whereas PKD1-silenced cells showed an increased HA-TAZ nuclear translocation, implying PKD1-dependent cytoplasmic localization of TAZ (Fig. 7B). PKD1 knockdown increased the interaction of TAZ with AXIN1. We investigated whether HA-TAZ expression colocalized with Myc-AXIN1 in PKD1-silenced cells. PKD1 knockdown led the colocalization of HA-TAZ with Myc-AXIN1 in the cytoplasm (Fig. 7B), suggesting that PKD1 depletion induced colocalization of HA-TAZ with Myc-AXIN1.
c-MYC Transcription Is Increased by TAZ and β-Catenin in PKD1-Depleted Cells. We found that silencing of PKD1 in cells led to the transcriptional activation of β-catenin and that expression of c-MYC was increased in the kidney of *Pkd1*-null mice. Furthermore, c-MYC is a known target gene of β-catenin or YAP/TAZ and is implicated in the pathogenesis of cystic kidney (9, 11). Thus, we proposed that the deletion of PKD1 contributed to the induction of c-MYC expression during cystogenesis. We first investigated whether β-catenin or TAZ was localized to the nucleus to regulate the transcription of the c-MYC gene. We performed cellular fractionation of PKD1-depleted cells (Fig. 8A). As expected, the amount of active β-catenin protein was high in the nuclear fraction of PKD1-depleted cells (Fig. 8A). Interestingly, TAZ was partially present in the nuclear fraction of PKD1-depleted cells, and TAZ phosphorylated at serine 89 was consistently decreased slightly in the cytosolic fraction of these cells (Fig. 8A). Our findings indicate that PKD1 depletion induces the translocation of active β-catenin or TAZ into the nucleus, suggesting that this may participate in an increase in the expression of c-MYC. To test this hypothesis, we performed qRT-PCR to determine the expression of c-MYC mRNA in PKD1- or TAZ-depleted cells or in cells codepleted for PKD1 and TAZ. The silencing of PKD1 increased the expression of c-MYC mRNA, which was markedly reduced in PKD1/TAZ-codepleted cells and in TAZ-depleted cells (Fig. 8B). Consistent with these results, c-MYC levels were significantly higher in PKD1-silenced cells but were reduced in the codepleted cells or in TAZ-depleted cells (Fig. 8C). These results suggest that TAZ is responsible for the expression of c-MYC in PKD1-depleted cells. We further tested whether increased β-catenin activity in PKD1-silenced cells contributed to the expression of the c-MYC gene. As expected, increased c-MYC expression under PKD1-deficient condition was reduced by the knockdown of β-catenin (Fig. 8D). Moreover, the effect of β-catenin knockdown on c-MYC expression was less than that of TAZ depletion, indicating that the expression of c-MYC mRNA remained regulated by TAZ.

Overall, based on our results, it can be suggested that the depletion of TAZ in PKD1-silenced cells reduces the expression of c-MYC mRNA via TAZ-mediated regulation of β-catenin or TAZ (Fig. 9).

Discussion

We have demonstrated a role for TAZ in the regulation of β-catenin in the pathogenesis of ADPKD. Highly accumulated TAZ was observed in cyst-lining epithelium of the collecting duct-targeted *Pkd1* knockout mice kidneys with high expression of c-MYC and active β-catenin. The deficiency of TAZ rescued...
renal cyst formation with reduced active β-catenin and c-MYC in Pkd1-null mice. Likewise, the formation of in vitro cysts stimulated upon forskolin treatment was reduced by Taz depletion in Pkd1-silenced cells. Our results prove that TAZ participates in the activation of β-catenin, as PKD1 deficiency renders TAZ prone to interact with AXIN1, resulting in a weak interaction of β-catenin with AXIN1. β-Catenin and TAZ were detected in the nucleus, where they regulated the c-MYC expression. Consequently, we suggest two independent roles of TAZ in the regulation of c-MYC mRNA implicated in cystogenesis in the kidney of Pkd1-deleted mice: 1) β-catenin activation via the TAZ-AXIN1 axis or 2) transcriptional activation of nuclear TAZ itself.

YAP and TAZ, the paralogous effectors of the Hippo pathway, are known to be involved in PKD; however, their roles are complicated. Both have been reported to be commonly activated around the cystic epithelium and are involved in regulating cyst growth (8, 20, 21). A recent study showed that loss of YAP in Pkd1-deleted mice alleviated the PKD phenotypes, and LARG-RhoA-ROCK signaling module mediated the increase in YAP/TAZ, followed by c-MYC activation in the Pkd1-deleted model. These authors also observed that transgenic expression of YAP enhanced the cell proliferation and dilation of tubules in the kidneys (9). This is in contrast to the observation that renal cysts appeared in Taz-deficient mice with tubule dilation. Inactivation of TAZ results in the development of renal cysts predominantly originating from the glomerulus cells, finally leading to end-stage renal disease (ESRD) (22). In another study on Taz-deficient mice, multicystic kidneys with other pathologic phenotypes, including urinary concentration defects, were observed (23). In addition to the conflicting effects of the lack of either YAP or TAZ on the formation of renal cysts in PKD rodent models, increasing evidence has highlighted the independent roles of each protein (24–26). Our observations support the notion that TAZ contributes to the disease progression in Pkd1-depleted kidneys based on the following evidence: 1) TAZ was increased in Pkd1-knockout kidneys; 2) Pkd1 knockout mice developed polycystic kidneys, but this was rescued by the deletion of Taz; and 3) increased cystic growth in Pkd1-silenced cells under forskolin treatment was restored to the basal levels by TAZ knockdown.

YAP and TAZ are believed to play distinct roles in regulating the expression of target genes despite their functional redundancy and similarities in their protein sequence. For example, loss of TAZ leads to cystic proximal tubule in a normal-sized kidney whereas the YAP mutant shows reduced nephrogenesis (27). RNA-seq analyses revealed that many of the genes that are differentially expressed either by YAP or TAZ are key transcription factors or growth factors (26). It is therefore believed that context-dependent binding to DNA-binding partners, such as Pax3, TBX-5, TTF1, and PPAR gamma for TAZ (28–31) and ErbB4 and p73 for YAP (32, 33), endows YAP and TAZ with their respective regulatory traits. It has been reported that YAP negatively regulates TAZ at the protein level, indicating that TAZ might influence other downstream signaling pathways in the cytoplasm irrespective of YAP (24, 34, 35). In our study, the levels of TAZ proteins were more faithfully increased than YAP in the kidneys of Pkd1-null mice. This evidence provides a strong clue indicating a potential link between TAZ and PKD1 in renal cystogenesis in comparison with the link between YAP1 and PKD1. As expected, TAZ depletion alleviated the Pkd1 mutant phenotype and inhibited the increase in c-MYC expression in the kidneys of Pkd1-deleted mice or Pkd1-silenced cells. We concluded that TAZ functions as a downstream signal of PKD1, although the role of YAP remains to be determined.

Previous studies have suggested the possibility that the upstream regulator of YAP/TAZ could affect the activation of Wnt/β-catenin signaling. Some studies have reported that the Hippo pathway YAP/TAZ antagonizes the Wnt/β-catenin signaling via sequestering the β-catenin in the destruction complex (17, 36, 37). In colorectal cancer, cytosolic YAP/TAZ has been
reported to inhibit the nuclear activity of β-catenin either via direct binding or by association with the destruction complex comprising AXIN1 (17, 38). However, our experiments with Pkd1/Taz-double-knockout mice suggested that TAZ triggered the β-catenin activation via interaction with AXIN1, thereby leading to an increase in the expression of c-MYC. In line with our findings, evidence exists to support that YAP/TAZ activates the Wnt/β-catenin signaling. Sav and Mst1/2 depletion in neonatal heart enhanced the expression of target genes controlled by the Wnt/β-catenin signaling (36). Also, we previously reported that the activation of TAZ in Sav1-knockout mice was accompanied by elevated β-catenin expression (12). Recently, inflammation-mediated regeneration of intestinal epithelial cells increased the nuclear colocalization of YAP and β-catenin (38). Therefore, a tissue-specific or context-dependent relationship between β-catenin and YAP/TAZ is anticipated.

Wnt/β-catenin signaling has been reported to be involved in cystogenesis in renal cystic disease. However, the role of Wnt/β-catenin signaling in renal cystogenesis remains unclear. High levels of β-catenin were reported to induce cyst formation in the kidney (13, 39). Our findings also indicated that the activation of β-catenin by an interaction of TAZ and AXIN1 was responsible for renal cystogenesis in Pkd1 knockout mice. Therefore, we postulated that the TAZ/AXIN1/β-catenin axis could be a therapeutic target against ADPKD. Similar to our suggestions, a study reported that inhibition of Wnt signaling by Wnt/b-catenin inhibitor LGK974 delays the progression of renal cystogenesis (40). Our data also revealed that Endo IWR1, which inhibited β-catenin activity via AXIN stabilization, reduced in vitro cyst growth in Pkd1-silenced cells. It also showed a significant decrease in in vitro cysts when Endo IWR1 was coadministered with rapamycin, which previously had been reported to inhibit renal cysts in preclinical models of ADPKD (41). These data suggest a potential therapeutic strategy that combines Wnt-targeting drugs with mTOR inhibition to delay disease progression in ADPKD.

In conclusion, our data show that the PKD1–TAZ–Wnt–β-catenin–c-MYC signaling axis regulates renal cystogenesis, which might be a potential therapeutic target against ADPKD.

Materials and Methods

Animals. Pkd1-floxed mice containing loxP sites flanking the exons 2 to 5 of Pkd1 gene were obtained from Stefan Somlo, Yale University, New Haven, CT (42). Taz-floxed mice, containing Taz mutant allele in which exon 3 is replaced by one loxP site, established at Korea Advanced Institute of Science and Technology, were provided by Daesik Lim, Korea Advanced Institute of Science and Technology, Daejeon, Korea, and transferred and bred in specific pathogen-free animal facilities at Sookmyung Women’s University (10, 43). HoxB7Cre transgenic mice, which drive cre-recombinase activity specifically in the renal collecting duct, were used. All mice were maintained in C57BL6 genetic background and bred in an animal facility under optimal conditions (12-h light/dark cycle at 20 °C) following the approval of the institutional animal care and use committee at Sookmyung Women’s University.

Human Kidney Specimens. As a control group, normal regions of human kidneys, confirmed by histologic examination, were acquired from renal cell carcinoma patients undergoing surgical treatment. All biological samples and data were deidentified prior to use in this study. Informed consent was waived because of the retrospective nature of the study and because the analysis used anonymous clinical data. The study was approved by the medical ethics committee of St. Vincent’s Hospital (VC20SISI0136).

Histology and Immunofluorescence Analyses. Paraffin-embedded mice kidneys were cut into 6-μm sections and stained with hematoxylin and eosin (H&E) for histological analysis. Tissue slides were also probed with antibodies against TAZ (Abcam), c-MYC (Santa Cruz, sc-764), β-catenin (Millipore, 05–665), DAB (Vector, FL-1031), Ki-67 (Abcam, ab16667), and collagen IV (Southern Biotech, 1340–01) to monitor the expression of the respective target proteins.
**Immunofluorescence of IMCD Cells.** Cells were fixed for 20 min, permeabilized with 0.4% Triton X-100/PBS for 20 min, and blocked with 5% BSA in PBS (PBS of 0.5% Tween-20) for 30 min at room temperature. Monoclonal primary antibodies (anti-HA mouse mono antibody and anti-Myc rabbit poly antibody) were applied for 1 h at room temperature, washed with PBS at least three times, and visualized using Alexa Fluor 488 donkey anti-mouse or anti-rabbit 568-conjugated secondary antibodies (Invitrogen) used at 1:200 for 1 h at room temperature. Preparations were mounted in Dako mounting medium containing DAPI (Vector Laboratories).

**Cell Culture and Reagents.** Mouse inner medullary collecting duct (IMCD) cells were cultured in Dulbecco’s modified Eagle’s medium F/12 (DMEM F/12; Welgene) supplemented with 10% fetal bovine serum (FBS). For in vitro analysis of cysts, 2.0 to 3.0 × 10^5 cells/mL of DMEM F/12 were plated on Matrigel (Corning, no. 354230) at a 1:1 ratio and cultured with DMEM F/12 without FBS for 5 to 6 d. The drugs, including forskolin (Sigma-Aldrich, no. F3917), forskolin modulator (Merck, no. 530959), Exo IWR1 (Tocris, no. 2087), c-MYC (no. 9402), phospho-CREB (no. 9198), Histone H3 (no. 60538), and LSD1 (Lysine-specific demethylase 1; no. 2139; all from Cell Signaling); β-actin (A2228; Sigma); β-catenin (no. 60153) and TAZ (no. 560235; both from BD Biosciences); and anti-β-catenin (ABC 05-865; Millipore). For immunofluorescence, anti-HA tag antibody (ab18181; Abcam) and anti-Myc tag rabbit (sc-2278; Cell Signaling) were used.

**Gene Knockdown, Expression Constructs, and Transfection.** The cells were transfected either with a scrambled siRNA (Santa Cruz) or with a siRNA targeting Pkd1 (Santa Cruz, sc-40862), TAZ (Santa Cruz, sc-38569), or AXIN1 (Santa Cruz, sc-41449) using Lipofectamine RNAiMAX (Invitrogen) for at least 24 h. Flag-Pkd1 was purchased from Addgene (cat. no. 31793). HA-TAZ plasmid was a gift from Dae-Sik Lim. HA-β-catenin and Myc-AXIN1 were provided by E.J. The cells were transfected with these expression plasmids using Lipofectamine 2000 for 24 h.

**Western Blot Analysis and Immunoprecipitation.** Protein lysates from mouse tissues were purified either by using NucleoSpin RNA/Protein column (Macherey-Nagel) or nucleus/cytosol fractionation kit (Thermo). Subcellular fractionation was obtained using a homogenizer as previously described (44). Western blot and immunoprecipitation (IP) with cell lysates were performed in at least two independent experiments as previously described (44). Pkd1Tas and Pkd1TasFoxOBl/cre mouse kidney were used for Axin or Taz immunoprecipitates. Briefly, mouse kidney tissue fragments were homogenized using a pestle in 200 μL of cold lysis buffer containing 1% Triton X-100. Homogenates were centrifuged at 12,000 rpm (20 min, 4 °C), and the supernatant was used for preclearing in IP buffer containing normal rabbit IgG and A/V agarose beads. Pre cleared supernatant was used for IP.

**Antibodies.** The sources and catalog numbers of the antibodies used were as follows: PCT (sc-130554), c-MYC (sc-7939), AXIN1 (sc-293190), β-catenin (sc-7963), p-TAZ (Ser89; sc-17610), and alpha tubulin (sc-5286; all from Santa Cruz Biotechnology); HA (ab96911), Myc (ab9106), and Flag (sc-316; both from BD Biosciences); and anti-β-catenin (ABC 05-865; Millipore). For immunofluorescence, anti-HA tag antibody (ab18181; Abcam) and anti-Myc tag rabbit (sc-2278; Cell Signaling) were used.

**RNA-Seq Data Analysis.** For identification of transcriptome levels in Pkd1-deleted kidney tissues compared to controls, RNA sequencing data from GSE86509 was reanalyzed, which had been done in our previous study and published on GEO public database (https://www.ncbi.nlm.nih.gov/geo/) (15). GSEA (gene set enrichment analysis) was conducted to assure differentially expressed gene levels between Pkd1-deleted kidney tissues and controls (45).

**Luciferase Assay.** pTOP reporter constructs and pTK-Renilla (transfection control; ratio of DNA amounts was 1:10) were cotransfected into IMCD cells expressing the indicated plasmid or siRNA oligonucleotides. Luciferase activity was measured using the Promega luciferase assay reagents as described by the manufacturer.
Viral Vectors. For knockdown of human TAZ or human β-catenin, shRNA vectors against TAZ or β-catenin were delivered using a retroviral vector containing a puromycin resistance cassette. The viral particles were produced as previously described (17). shRNA against β-catenin and TAZ retroviral vectors were provided by E.J. and Dae-sik Lim.

Statistical Analysis. All of the data were obtained from a minimum of three independent experiments and were statistically analyzed as mean ± SD. Unpaired t test was performed using GraphPad Prism 5.0 (GraphPad software). Results with a P value less than 0.05 was considered statistically significant.

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Data Availability. All study data are included in the article and supporting information.

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