Acute kidney injury induces hallmarks of polycystic kidney disease

Almira Kurbegovic and Marie Trudel
Molecular Genetics and Development, Institut de Recherches Cliniques de Montréal, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada

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Kurbegovic A, Trudel M. Acute kidney injury induces hallmarks of polycystic kidney disease. Am J Physiol Renal Physiol 311: F740–F751, 2016.—Acute kidney injury (AKI) and autosomal dominant polycystic kidney disease (ADPKD) are considered separate entities that both frequently cause renal failure. Since ADPKD appears to depend on a polycystin-1 (Pc1) or Pc2 dosage-reduced mouse, transgenic models with a dosage-reduced Pkd1 allele, consistent with human ADPKD studies and a dosage-dependent pathogenetic mechanism (23).

A considerable number of AKI studies in animals have focused on the short-term molecular and cellular events from a couple of hours to a few days after ischemia-reperfusion injury (IRI) to study the damage and repair mechanisms. Renal regeneration from AKI in normal mice is reported to occur within a few days, probably via activation of a program linked to cellular proliferation, as in renal development (37). However, failure to switch off the repair mechanism may result in continuous activation of numerous genes and renal pathologic damage (54), consistent with frequent development of CKD in patients post-AKI. The few mid- and long-term AKI studies (>6 wk) in mice and rats reported kidney anomalies, including tubular atrophy, dilatations/cysts, interstitial fibrosis, and inflammation (4, 13, 34).

Presently, kidney injury is considered as a “modifier” in dosage-reduced Pkd1 murine models. Particularly, the variable progression or penetrance of cystogenesis in adult Pkd1 dosage-reduced kidneys can be overcome by additional stimuli such as toxic damage or AKI. Upon induction of bilateral injury, adult Pkd1-deficient kidneys acquire cysts more rapidly, one within 2 wk (44) whereas the others exhibit dilatations at 5–6 wk (1) and cysts by 10 wk (18). However, the exact mechanisms underlying this effect and the interconnection between AKI and Pkd1 dose-dependent cystogenesis remain to be elucidated.

The primary objective of this study was to investigate whether AKI is a modifier of cystogenesis from clinical-like and dosage-increased Pkd1 alleles as a step toward dissection of the global cellular and molecular interaction(s). In fact, transient IRI in a low Pkd1 dosage-increased mouse line, Pkd1TAG, in two Pkd1extra lines with slow PKD progression...
(23, 24), and in nontransgenic (non-Tg) controls reproducibly cause typical PKD cystogenesis independently of the genotype. This IRI cystogenic mechanism was associated not only with markedly elevated Pcl1 and Pcl2 levels but also with a persistent increase in hypoxia-inducible factor 1α (Hif1α) levels and stimulation of both the Wnt and mammalian target of rapamycin (mTOR) signaling pathways. Our studies provide evidence that AKI is sufficient to induce cystogenesis and reveal a novel cross talk with ADPKD signaling pathways.

METHODS

Unilateral IRI. Experiments were carried out in IRI-induced transgenic Pkd1extra/2/39 (n = 27), Pkd1TAC6 (n = 9), and their control non-Tg littersmates (n = 63) as well as in C57BL/6J sham-operated (n = 20) male mice. High-dose-increase Pkd1TAC6 transgenic mice were used as controls. All transgenic mice were backcrossed on the C57BL/6J background for several generations. Mice were anesthetized with isoflurane and placed on a heating pad during surgery. A vascular clamp was applied only on the left renal pedicle to induce transient moderate ischemia for 30 min. Protocols were approved by the Animal Care Committee and the Canadian Council on Animal Care.

Histopathological and morphometric analysis. Mice were euthanized at different stages of reperfusion, and kidneys were fixed in formalin. Paraffin-embedded blocks were sectioned and stained with hematoxylin and eosin or sirius red. Morphometric (cyst) analysis was performed, using hematoxylin and eosin sections of left IRI kidneys from non-Tg (n = 38), transgenic Pkd1extra/2/39 (n = 19), and Pkd1TAC6 (n = 8) mice. Images from a Leica MZ12 microscope were processed by Northern Eclipse software from Molecular Devices.

Cilia length was evaluated in formalin-fixed kidneys in cortical and medullary regions of IRI nontransgenic (n = 6, n = 3) and non-IRI non-Tg kidneys (n = 10, n = 5) as described in (23). Images of cilia length (83–165 cilia/kidney) by DM5500B microscope were measured by Volocity.

Transcriptional analysis. RNA was extracted from left kidneys using TRIzol at 16 and 23 days and/or 3 mo postsurgery (Invitrogen). RNA integrity was confirmed on formaldehyde/agarose gels. Primers used for analysis are Pkd1 forward 5′-TCAATGTGCTCGCGGCGCTGTT-3′, reverse 5′-CCAGGCTCGACGAGTGTGGTGGTGCCGCAG-3′; Pkd2 forward 5′-CCGAGGTCAAGGGACTCGCTTCCA-3′, reverse 5′-CCGAGTCCATCTGCTCGATTC-3′; Ankrd37 forward 5′-ACGAATTACCTGCAATGCAG-3′, reverse 5′-TAGAAACCCGTGAGGCGAGTCTGAG-3′; Ccl1 forward 5′-GCGGAGAAGCGGTGCTGAC-3′, reverse 5′-GGCATGCTGAGTCGGCTTG-3′; Ctnmb1 forward 5′-GCGCTTTCGTCACATGCTA-3′, reverse 5′-TGAGCCGCGCTGAGCGA-3′; C-Myc forward 5′-GGATGCGCTGCTGAGTCCCATC-3′, reverse 5′-GGATGCGTCGCTGATTCGCTT-3′; Gapdh forward 5′-GGCATGCTGAGTCGGCTTG-3′, reverse 5′-GCGTCTGGTCACATGCTA-3′; c-Myc forward 5′-GCGGAGAAGCGGTGCTGAC-3′, reverse 5′-GGCATGCTGAGTCGGCTTG-3′; and Hif1a forward 5′-TCAATGTGCTCGCGGCGCTGTT-3′, reverse 5′-CCAGGCTCGACGAGTGTGGTGGTGCCGCAG-3′; etc.

Proliferation was assessed with the Ki67 marker as reported (9, 10). A mean proliferative index was evaluated by quantification of positive signal over the total kidney surface (excluding cyst area) of the entire kidney section using Northern Eclipse software from Molecular Devices. Analysis of phospho-protein expression was performed by incubation overnight at 4°C, then incubation for 1,5–2 h with secondary anti-rabbit biotin-conjugated antibody and signal detection using a Vectastain ABC kit and peroxidase substrate DAB kit (Vector Laboratories).

Statistical analysis. Correlation between cystic surface and cystic number was assessed by a Pearson r-test. Data are represented as means ± SE, and the statistical significance of quantitative PCR and proliferation was analyzed using an unpaired two-tailed t-test. P < 0.05 was considered significant.

RESULTS

AKI causes renal cystogenesis. Since IRI in Pkd1 dosage-reduced mouse models was reported to promote cyst formation, we questioned whether cystogenesis could be accelerated in Pkd1 dosage-increased models and interrogated the molecular mechanism. Two sets of transgenic mouse lines were selected, one expressing Pkd1 full-length with mild dosage-increased Pkd1TAC (line 6) at ~1.3-fold endogenous levels that develop cysts at 9 mo (23), and the second a clinical-like mutant expressing Pkd1 extracellular domain, Pkd1extra, at low (line 39) and high (line 2) levels that exhibits cysts at ~15–16 mo (24). Unilateral left IRI was performed in 3-mo-old mice including control nontransgenic (non-Tg) littersmates (Fig. 1A). Mice were euthanized at three time points post-IRI, day 16, day 23, or 3–4 mo, before cyst detection in any of the three transgenic lines (Fig. 1B).

Renal histological analysis was performed in transgenic and non-Tg mice. All IRI kidneys of Pkd1extra (n = 27) and Pkd1TAC (n = 9) mice displayed tubular dilatations and cysts (Fig. 1C), consistent with IRI promoting cystogenesis in dosage-increased models as in dosage-reduced mouse models. Cysts were detected in the cortex, corticomedullary junction, and papilla of left transgenic kidneys. Noticeably, left kidneys of non-Tg mice (n = 63) compared with sham-operated mice (n = 20) also showed tubular and glomerular dilatations and cysts at days 16 and 23, and by 3 mo post-IRI some were overly cystic whereas sham-operated kidneys were unaffected (Fig. 1, D and E). Non-Tg and transgenic left kidneys displayed epithelial hyperplasia, infiltrates, tubular damage/atrophy, protein hyaline casts, and fibrosis. To determine whether transgenic clinical-like and/or dosage-increased Pkd1 lines are prone to more severe cystogenesis relative to non-Tg mice, the cystic surface (% of total area) was quantified in kidneys at 3 mo postreperfusion. Non-Tg and transgenic mouse lines had similar proportions of mice in each cystic severity subgroup (Fig. 1F, left). Non-Tg and transgenic IRI kidneys were also monitored for the mean number of cysts per surface area and then subdivided
into subgroups. The proportion of mice in each subgroup was relatively comparable for each genotype, suggesting no major difference in the initiation of cyst formation (Fig. 1F, right). Notably, the cystic surface significantly correlated with the mean number of cysts for each subgroup (non-Tg r = 0.90, P < 0.0001; Pkd1 extra r = 0.72, P < 0.0005; Pkd1 TAG r = 0.92, P < 0.001). These quantifications indicate that the transgenes had minimal effect on cystic phenotype initiation and/or progression.
Based on our observation of renal epithelial hyperplasia and stimulated proliferation in ADPKD (26) and cystic mouse models (9, 23, 48), we evaluated cell proliferation in IRI kidneys. Since the transgenic lines showed a similar range of cystic severity as non-Tg kidneys, we focused our analysis on non-Tg kidneys at 3 mo post-IRI (Fig. 1, G and H). Proliferation was calculated as the number of Ki67-positive nuclei per surface area, corrected for the cystic surface. Non-IRI kidneys displayed few Ki67-positive cells sparsely within the parenchyma. In contrast, IRI kidneys displayed significantly increased Ki67-positive cells by three- to sixfold relative to non-IRI non-Tg (n = 3; P < 0.05). Interestingly, a positive signal was detected in dilated and cystic tubules often in cell clusters but also in glomeruli and in the parenchyma, possibly resulting from a long-term repair program.

To assess renal repair processes, we monitored fibrosis and interstitial scarring in kidneys at 3 mo post-IRI. Both IRI non-Tg and transgenic Pkd1extra left kidneys showed intense staining over the corticomedullary junction in the outer medulla, the most susceptible region to ischemia, as well as in the cortex and papilla (Fig. 1I). Evidence of increased fibrosis was also evaluated for levels of collagen type IV by immunoblotting (Fig. 1J). Fibrosis and collagen deposits provide evidence of maladaptive repair mechanism in both non-Tg and transgenic AKI kidneys.

Because cilia anomalies are associated with renal cysts and the stress response (5), we monitored cilia length in non-Tg kidneys at 3 mo post-IRI. The distribution of epithelial cilial length in the cortical and medullary regions was determined according to small intervals. The cilia length was similar in cells from the cortical region of IRI and non-IRI kidneys. In contrast, the medullary region of IRI kidneys showed a significantly higher proportion (10.9%) of cells with longer cilia (4–5 μm) than non-IRI left kidneys (6.2%) (Fig. 1K), suggesting that ischemia also elicits mild stimulation of ciligenesis.

**Stimulation of Pc1/Pc2 signaling pathways.** To investigate IRI molecular cystogenic mechanisms, Pkd1/Pc1 and Pkd2/Pc2 expression was evaluated since their dysregulation can induce cystogenesis (23, 27, 36, 38, 46). Analysis of IRI kidneys from non-Tg mice by real-time PCR showed a substantial increase in Pkd1 expression levels by about fourfold relative to the levels in sham non-Tg left kidneys (P < 0.005) (Fig. 2A). Consistently, IRI kidneys showed a major increase in Pc1 expression (~3–4 fold) at 16 days post-IRI (Fig. 2B) that was sustained at 23 days and 3 mo post-IRI. In contrast, contralateral right kidneys exhibited similar Pc1 expression levels to non-IRI mice at these time points. The Pkd1extra39 displayed as expected, from the transgene, elevated expression 1.3- to 2-fold Pc1 (Pc1extra) in the right kidneys (23, 24). Pc1 expression was further increased (~3–6 fold) in left IRI kidneys compared with contralateral kidneys at 23 days and 3 mo post-IRI. Similarly, the low Pkd1 dosage-increased Pkd1TAG6 line had clearly higher Pc1 levels in IRI left kidneys (~3–5 fold) (Fig. 2B, right). Of significance, both IRI non-Tg and Pkd1TAG6 kidneys display stimulated Pc1 expression in the same range but without reducing levels obtained in non-ischemic high dosage-increased Pkd1TAG26 kidneys (right) (23).

To monitor whether the increased Pc1 protein by IRI was processed as the endogenous native Pc1, the most abundant GPS/GAIN-cleaved Pc1 form was analyzed for N-glycan modification with endoglycosidase H (endoH, E) treatment. Figure 2C shows a strong Pc1 endoH-resistant band (top, Pc1NTF570), which is the mature GPS-cleaved form, and a weaker Pc1 endoH-sensitive (bottom, Pc1NTF750) band, the immature form. A similar proportion of Pc1 endoH-resistant and -sensitive bands was detected in kidneys at day 16 and 3 mo post-IRI, suggesting that increased Pc1 in IRI kidneys is processed like the native endogenous Pc1.

We next assessed whether Pkd2/Pc2 expression was modulated in IRI renal cystogenesis in parallel with Pkd1/Pc1. IRI kidneys of non-Tg mice showed a mild but significant increase in Pkd2 expression level (P ≤ 0.02) (Fig. 2D). Notably, analysis of non-Tg mice showed increased Pc2 expression by 8- to 15-fold in IRI kidneys at all time points compared with non-IRI non-Tg left kidneys (Fig. 2E). Contralateral kidneys displayed similar Pc2 expression levels in non-Tg and transgenic mice. Similar to the IRI non-Tg kidneys, Pc2 expression levels in IRI kidneys were increased from ~3 to 6-fold in Pkd1TAG6 kidneys relative to non-IRI kidneys (Fig. 2F).
Fig. 2. Upregulation of Pkd1/Pc1 and Pkd2/Pc2 in post-IRI non-Tg and transgenic kidneys. A: Pkd1 expression normalized to Gapdh in IRI (+) and non-IRI (−) sham-operated non-Tg left kidneys was carried out at 3m postsurgery by real-time PCR. Histogram depicts 4-fold induction of Pkd1 in IRI kidneys (n = 7) relative to sham (n = 6) set at 1. **P < 0.005. B: Pc1 immunoblots of IRI non-Tg, Pkd1extra39, and Pkd1TAG26 left (L) kidneys compared with contralateral right (R), non-IRI left, and Pkd1TAG26. Pc1 levels were evaluated at onset (0 time point), end of experiment [3m (3–4 mo) post-IRI], with mid-time points (16d and 23d). Histograms below immunoblots show Pc1 fold-induction of IRI to non-IRI left kidneys (non-IRI non-Tg set at 1; left and right) and ratio (or mean ratio) of Pc1 to Gapdh that compares right to left kidneys (middle). IRI non-Tg kidneys exhibit increased Pc1 levels compared with right and with non-IRI left kidneys (all panels) at all postreperfusion time points. IRI Pkd1extra39 and Pkd1TAG26 kidneys displayed even more pronounced Pc1/Pc1extra, expression levels than right kidneys. C: glycosylation status of Pc1 in IRI left kidneys using endoH (E). IRI and non-IRI non-Tg kidneys showed similar proportion of Pc1 endoH resistant to sensitive bands (Pc1NT449 and Pc1NT579). −, Untreated and E, endoH-treated protein samples. D: Pkd2 expression normalized to Gapdh in IRI non-Tg left kidneys at 3m postreperfusion by real-time PCR. Histogram depicts modest but significant induction of Pkd2 in IRI (n = 7) relative to non-IRI sham-operated left kidneys set at 1 (n = 6). **P < 0.05. E: Pc2 immunoblots on IRI left non-Tg and Pkd1extra kidneys exhibit strikingly elevated Pc2 (~130 kDa) expression at 23d and 3m post-IRI relative to baseline levels and contralateral kidneys. Histograms depict Pc2 fold-induction of IRI to non-IRI left kidneys (non-IRI non-Tg at 0 and 3m set at 1; left) and expression of Pc2 (mean ratio relative to Gapdh) for right and left kidneys of transgenic and non-Tg at 3m postsurgery (right).
kidneys of \( Pkd_{extra} \) transgenic lines were markedly increased. These data on IRI kidneys revealed long-term and strong dosage-increase levels of both Pc1 and Pc2 following stress conditions.

**Persistent ischemic response following postreperfusion.**

Based on similar responses of IRI non-Tg and transgenic kidneys stimulating Pc1 as well as Pc2 expression, we focused our studies on non-Tg kidneys. To determine whether AKI induced a persistent hypoxic and/or repair response, we assessed Hif1\( \alpha \) transcript levels from 16 days post-IRI (Fig. 3A). Transcriptional expression analysis of Hif1\( \alpha \) showed a significant approximately threefold increase in IRI non-Tg kidneys (\( P < 0.0001 \)) relative to sham left kidneys (Fig. 3A), confirming that a Hif1\( \alpha \) signal is triggered by IRI. Noticeably, Hif1\( \alpha \) levels remained elevated at 23 days and \( \sim 3 \) mo by more than twofold (Fig. 3A). Consistently, Ankrd 37, a Hif1\( \alpha \) transcriptional target, was also significantly stimulated (Fig. 3B). Since Hif1\( \alpha \) protein is reported short lived (49), we monitored cellular localization of Hif1\( \alpha \) in IRI kidneys by immunostaining. Figure 3C shows a strong Hif1\( \alpha \) signal on the entire IRI non-Tg renal sections. In particular, a markedly intense nuclear signal was detected in cystic and in dilated tubules compared with a very weak and cytoplasmic signal observed in non-IRI kidneys. This persistent ischemic response points to activation of several regulatory pathways.

**AKI as a modulator of the mTOR cascade.**

Since cystogenesis by deregulation of Pc1 and Pc2 respond to mTOR inhibitors (43), we interrogated regulators of the mTOR pathway in AKI-induced cystogenesis. We first investigated the activation status of Erk. Both P-Erk (T202/Y204) and total Erk expression was substantially elevated in IRI kidneys of non-Tg and transgenic mice at all ages post-IRI (Fig. 4, A and B), suggesting that IRI modulates their transcriptional regulation. The ratio of P-Erk to total Erk of non-Tg left IRI kidneys was not significantly increased relative to non-IRI kidneys, suggesting that Erk negative control on mTOR is not a major regulator of IRI cystogenesis. We then monitored whether Akt activation could regulate mTOR through negative regulation. Total Akt levels were consistently increased (~10-fold) in left IRI compared with non-IRI kidneys. Interestingly, P-AktT308 and P-AktS473 levels were at 16 and 23 days post-IRI equivalent or slightly increased in IRI non-Tg left kidneys relative to non-IRI kidneys (Fig. 4, C and D). At 3 mo post-IRI, both P-AktT308 and P-AktS473 displayed higher levels. While absolute P-Akt levels may play a role in the late post-IRI stage, the lack of increase in P-Akt to total Akt ratio would suggest otherwise.

We then determined whether the mTOR effector tuberin, a known key cystogenic factor and interacting partner of Pc1, is dysregulated. In fact, tuberin expression was not decreased as expected for a role in cystogenesis but strongly stimulated (7- to >20-fold) from early tubular dilatation stages onward in left IRI kidneys relative to contralateral non-IRI kidneys (Fig. 4E). Similarly, \( Pkd_{extra} \) IRI kidneys exhibited increased tuberin expression (~8- to 11-fold) at 3 mo post-IRI (Fig. 4F; re-probed from Fig. 4B).

To determine whether mTOR is activated, we analyzed the phosphorylation status of the downstream effector ribosomal subunit protein S6K (P-S6K1, Thr389) and total S6K. Levels of total S6K were similar in both IRI and non-IRI kidneys (Fig. 4G). By contrast, P-S6K1T389 levels were strongly induced (6- to 11-fold) in left IRI kidneys relative to contralateral and non-IRI kidneys. These data indicated substantial activation of the mTOR complex 1 (mTORC1) pathway in response to AKI.

**AKI stimulates the Wnt canonical signaling pathway.**

Since tuberin and Pc1 can cross talk with GSK3 of the Wnt canonical pathway (21, 22), we evaluated \( \beta \)-catenin regulation. As shown in Fig. 5A, transcription of the Ctnnb1 gene encoding \( \beta \)-catenin was stimulated by 1.5- to 2-fold in 3 mo post-IRI relative to sham-operated left kidneys (\( P \leq 0.02 \)). Analysis of IRI non-Tg kidneys showed a striking increase in total \( \beta \)-catenin expression of >10-fold compared with the almost undetectable levels in contralateral kidneys from early stages of tubular dilatations to 3 mo post-IRI (Fig. 5B; re-probed from Fig. 4B). Most interestingly, levels of active \( \beta \)-catenin were even more enhanced than total \( \beta \)-catenin in IRI kidneys with a ratio of active to total \( \beta \)-catenin of 2-8-fold (Fig. 5B, top). Similarly, IRI Tg kidneys showed stimulation of active and total \( \beta \)-catenin expression (Fig. 5B, bottom). To determine the cellular localization of \( \beta \)-catenin expression, IRI kidneys at 3 mo postreperfusion were analyzed by immunostaining (Fig. 5C). Compared with the weak signal detected in non-IRI kidneys, a strong \( \beta \)-catenin signal was detected over the epithelium of cystic and...
dilated tubules basolaterally as well as in the cytosol and nuclei in IRI non-Tg renal sections. Interestingly, β-catenin expression was also very noticeable in parietal cells of glomeruli of IRI non-Tg kidneys. AKI appears to stimulate markedly the Wnt canonical pathway.

A major downstream target of the Wnt canonical pathway, c-Myc, was then evaluated since c-Myc renal expression is increased in human ADPKD and high Pkd1 dosage-increased mice (23, 26, 46). Expression analysis of c-Myc transcript levels by quantitative PCR in IRI kidneys from non-Tg mice.
showed a 5- to 10-fold stimulation compared with sham-operated kidneys ($P \leq 0.0002$) (Fig. 5D). Consistently, c-Myc protein expression was markedly upregulated (3- to 5-fold) in IRI non-Tg and transgenic kidneys while barely detectable in control kidneys (Fig. 5E; reprinted from Fig. 4B). Noticeably, the additional $-42$-kDa band likely corresponding to c-Myc cytoplasmic cleaved-form, Myc-nick (7), was also elevated by 2- to 5-fold. We then monitored c-Myc cellular localization in the left kidneys post-IRI. A more pronounced and generalized c-Myc signal was detected on the entire IRI non-Tg compared with non-IRI kidneys (Fig. 5F). Interestingly, a c-Myc signal was observed in both cytoplasmic and nuclei, consistent with expression of both the cleaved and full-length forms. As determined for β-catenin cellular localization, the most intense c-Myc expression was detected in dilated to cystic tubules and in parietal cells of glomeruli. These results suggest that β-catenin and/or c-Myc levels and localization could be cystogenic in AKI.

**DISCUSSION**

The original intent of these studies was to determine whether AKI is a modifier of cystogenesis in late-onset clinical and mild dosage-increased *Pkd1* allelic with a focus on progressive and long-term outcomes. Our study shows that ischemia can be an inducer and not only a modifier of cystogenesis in non-Tg and in two late-onset *Pkd1* mouse models. This AKI-induced cystogenic mechanism cross talks with Pc1 and Pc2 and targets activation of the mTOR and Wnt pathways. We uncovered that AKI shares similar cellular responses and a global molecular regulatory network with PKD.

Particularly striking is the 100% penetrance of renal tubular and glomerular cystogenesis in the transgenic and non-Tg IRI kidneys along with tubular atrophy, inflammation, fibrosis, and longer cilia. The comparable range of cystic severity from the transgenic as well as non-Tg mice indicates that the transgene does not significantly potentiate the phenotype. This finding may appear inconsistent with the reported accelerated cysts in older animals at or beyond 1 yr of age (14). Nonetheless, Hif1α-stimulated expression could be a significant modifier of or contributor to the IRI cystogenic pathway.

Of importance, the persistent and substantial renal transcriptional and translational upregulation of *Pkd1/Pc1* in control and transgenic mice as well as major stimulation of Pc2 indicate that AKI controls Pc1 and Pc2 levels via two mechanisms, transcriptional activation and protein stability, respectively. Previous reports observed stimulation of Pc2 and/or Pc1 downstream target expression suggest an absence of switching off the repair process, maladaptive repair, or activation of injury-associated secondary cascades (54). Hif1α strong nuclear sublocalization supports a regulatory role in the cystogenic process. Interestingly, increased expression of Hif1α was detected in late stages of human ADPKD and also in PKD animal models (2, 3). The sustained increase in Hif1α expression alone is unlikely responsible for injury-induced cystogenesis in our IRI control and transgenic mice, since overexpression of Hif1α causes renal cysts in older animals at or beyond 1 yr of age (14). From the sustained and high levels of Pc2 detected for several weeks are likely to have cystogenic potential since mild *Pkd2/Pc2* upregulation in transgenic mice can cause cysts by 6–18 mo of age (36). Concurrently, in
creased levels of Pkd1 and the Pc1 mature form as detected in IRI kidneys can also trigger cystogenesis as shown in Pkd1 transgenic mice targeted systemically or specifically to the kidney (23, 46). The same range of Pc1 increased expression in non-Tg and transgenic could explain the similar phenotypic progression. Our data show that acute ischemic injury upregulates both polycystin responsible for ADPKD after reperfusion which could individually or together act as important modifiers or account for the cystogenic phenotype.

Our studies show dysregulation of the mTOR signaling cascade in ischemic kidneys as observed in cystic mouse models and human ADPKD tissues (15, 40, 42, 45).
increased levels of phospho and total Erk in all and Akt in late-stage postreperfusion provide a potential regulatory connection to the mTORC1 pathway. This pattern of P-Erk and P-Akt patterns in IRI kidneys mimic that reported in a renal cystic mouse model (15). Of interest, such AKI mice with physiological overexpression of Pc1 and Erk also indicate that Pc1 does not or is not sufficient to inhibit Erk expression or signaling, as suggested from in vitro data (12). Tuberin activity may also be modulated by alternative factors of this cascade since it was reported that exogenous Pc1 can interact with tuberin and the mTOR pathway become activated (42). Accordingly, activation of S6K, the mTORC1 downstream target, by ischemic stress is possibly a repair response as more pronounced tubular damage is triggered by IRI in mTORC1-deficient kidneys and in rapamycin-treated animals (17, 28). Our findings in renal ischemic stress are also consistent with mTOR cascade activation and delayed cystogenesis by mTOR inhibitors in several cystic non- and orthologous Pkd1 mouse models (15, 42, 43). An important insight into our analysis is that in the face of markedly elevated Pc1, mTORC1 pathway is not downregulated but in fact is upregulated in vivo. Coactivation of mTOR and Hif1α in response to renal IRI and downregulation of Hif1α with inhibitors of mTOR (20, 31) indicate that Hif1α signals into the mTOR pathway to adapt cell metabolism and protein synthesis responses, as shown in the proposed model (Fig. 6). Collectively, our data clearly suggest that the mTOR pathway is implicated in repair and/or progression of ischemic renal cystic pathology.

Our studies determine that the Wnt canonical pathway in AKI is likely a key and critical mechanism and possibly a modulator of the mTORC1 cascade in the ischemic-induced phenotype. In IRI kidneys, Wnt signaling in adulthood becomes activated possibly through Pc1 stimulation that can inhibit GSK3 activity (22). Consistently, the strong and sustained activation of β-catenin from early to late postreperfusion shows that the Wnt canonical pathway is stimulated during renal injury and/or repair. This finding correlates with a more severe AKI response in β-catenin-deficient animals (53). However, persistent upregulation of β-catenin can be responsible for development of the cystogenic phenotype, as previously observed (41). Conversely, cystic mouse models and human ADPKD tissues upon Pc1 dysregulation induce activation of the Wnt/β-catenin pathway (25, 30, 35) (Trudel M and Kurbegovic A, unpublished observations). Significantly, the strong induction of c-Myc, a downstream effector of β-catenin (19), supports implication of the Wnt canonical pathway. Upregulation of both full-length c-Myc and Myc-nick could play a role following ischemic stress in transcriptional regulation and in cell survival/metabolism, respectively (7, 16). Moreover, elevated c-Myc levels were shown in several cystic mouse models (11, 52), including renal or systemic dosage-increase Pkd1 mouse models (23, 46) and in human ADPKD tissues (26). Most importantly, renal c-Myc overexpression in transgenic mice was shown to induce PKD (47), pointing to c-Myc as a potential causative IRI cystogenic factor. Together, these results suggest that the Wnt canonical pathway implicated in ADPKD is also determinant in the AKI-induced signaling network.

Particularly remarkable is the parallel response and long-term signaling of AKI and ADPKD. These similarities suggest that ischemic stress plays an important role in ADPKD. Re-

![fig5](http://ajprenal.physiology.org/)

**Fig. 5. IRI induces Wnt signaling pathway activation in kidneys.** A: expression of Ctnnb1 transcript normalized to Gapdh in IRI and non-IRI sham-operated non-Tg left kidneys was performed at 3m post-surgery using quantitative PCR. Ctnnb1 expression in IRI kidneys (n = 7) was increased ~2-fold relative to sham kidneys (n = 6) set at 1. *P < 0.05. B: unphosphorylated active and total β-catenin in IRI non-Tg left kidneys were markedly enhanced from 16d onward (top) whereas the contralateral and non-IRI kidneys were barely detectable. Similarly, active and total β-catenin in IRI left kidneys of non-Tg and Pkd1−/− mice (line 39; bottom) were strongly stimulated at 3m post-IRI. Adjacent histograms show fold-induction of active/total β-catenin ratio of IRI left kidneys at all time points relative to non-IRI set at 1 and ratio of active/total β-catenin for right and left kidneys. C: cellular localization of β-catenin by immunostaining in IRI non-Tg left kidneys at 3m post-surgery compared with non-IRI non-Tg kidneys. An intense β-catenin signal was readily detectable in epithelial cells of cystic/dilated tubules (T) and of glomeruli (G) of IRI non-Tg kidneys compared with a weak signal in non-IRI kidneys. Scale bars = 100 μm. D: c-Myc expression analysis in IRI and non-IRI left non-Tg kidneys compared by quantitative PCR. IRI kidneys exhibit ~6-8-fold increased c-Myc expression relative to non-IRI sham kidneys (set at 1), **P < 0.005. The c-Myc expression pattern correlates with activation of β-catenin. E: c-Myc full-length (~60 kDa) and, probably, Myc-nick ~42-kDa levels are increased from 16d post-IRI in non-Tg (top) kidneys relative to contralateral and baseline kidney levels. Similarly, expression of c-Myc is importantly increased in IRI left kidneys of non-Tg and Pkd1−/− mice (line 39; bottom) same gel as in Fig. 2E). Adjacent histogram displays fold-induction of c-Myc in IRI relative to non-IRI non-Tg left kidneys (set at 1) for all time points. F: analysis of c-Myc cellular localization by immunostaining in IRI non-Tg left kidneys at 3m post-surgery compared with non-IRI non-Tg kidneys. Consistent with β-catenin cellular localization, the most intense signal for c-Myc was in the cytosol and nuclei of epithelial cells of cystic and dilated tubules and of glomeruli of IRI non-Tg kidneys. Scale bars = 100 μm.
cently, AKI and chronic kidney disease were proposed to be interconnected human syndromes (6). Our findings suggest that recurrent injury in ADPKD and Pkd1 dosage-dependent mouse models trigger cystogenesis at least in part via chronic kidney ischemia. Molecularly, the convergence of several causative cystogenic/PKD factors like Pc1, Pc2, Hipp1α, β-catenin, and c-Myc induced a few weeks after reperfusion supports cross talk between AKI and ADPKD signaling pathways.

In conclusion, we demonstrated that AKI in mice remarkably reproduces several of the typical ADPKD phenotypic features and signaling pathways. Most importantly, our study warrants future prospective cohort studies on the AKI prognostic outcome with ADPKD effectors.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

A.K. and M.T. provided conception and design of research; A.K. performed experiments; A.K. and M.T. analyzed data; A.K. and M.T. interpreted results of experiments; A.K. and M.T. prepared figures; A.K. and M.T. drafted manuscript; A.K. and M.T. edited and revised manuscript; A.K. and M.T. approved final version of manuscript.

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