Periostin promotes renal cyst growth and interstitial fibrosis in polycystic kidney disease

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In renal cystic diseases, sustained enlargement of fluid-filled cysts is associated with severe interstitial fibrosis and progressive loss of functioning nephrons. Periostin, a matricellular protein, is highly overexpressed in cyst-lining epithelial cells of autosomal-dominant polycystic disease kidneys (ADPKD) compared with normal tubule cells. Periostin accumulates *in situ* within the matrix subjacent to ADPKD cysts, binds to $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins, and stimulates the integrin-linked kinase to promote cell proliferation. We knocked out periostin (*Postn*) in *pcy/pcy* mice, an orthologous model of nephronophthisis type 3, to determine whether periostin loss reduces PKD progression in a slowly progressive model of renal cystic disease. At 20 weeks of age, *pcy/pcy:Postn*$^{-/-}$ mice had a 34% reduction in kidney weight/body weight, a reduction in cyst number and total cystic area, a 69% reduction in phosphorylated S6, a downstream component of the mTOR pathway, and fewer proliferating cells in the kidneys compared with *pcy/pcy:Postn*$^{+/+}$ mice. The *pcy/pcy Postin* knockout mice also had less interstitial fibrosis with improved renal function at 20 weeks and significantly longer survival (51.4 compared with 38.0 weeks). Thus, periostin adversely modifies the progression of renal cystic disease by promoting cyst epithelial cell proliferation, cyst enlargement, and interstitial fibrosis, all contributing to the decline in renal function and premature death.

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Polycystic kidney disease (PKD) is a family of hereditary disorders characterized by the formation and progressive expansion of numerous fluid-filled cysts that cause massively enlarged kidneys.[1] Renal cysts are benign neoplasms that ultimately cause renal insufficiency through extensive nephron loss and replacement of normal parenchyma with fibrosis.[2] Autosomal-dominant PKD (ADPKD) is the most common inherited renal disorder with a frequency of 1:500 individuals and accounts for 7–9% of patients on renal replacement therapy. ADPKD is caused by mutations in *PKD1* or *PKD2*, genes that encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively.[3–6] Autosomal-recessive PKD (ARPKD) is less common ($\sim$1:20,000 births) and is caused by mutations in *PKHD1*, a gene that encodes fibrocystin. ARPKD is characterized by rapid disease progression, often leading to renal failure within the first year of life. Several signaling pathways, including those regulated by cAMP, growth factors, B-Raf/MEK (mitogen-activated protein kinase kinase)/ERK (extracellular signal–regulated kinase), mammalian target of rapamycin (mTOR), integrins, and Akt have been implicated in aberrant cell proliferation and the relentless expansion of PKD cysts.[7–16]

In addition to intrinsic defects in PKD cells, factors secreted into the extracellular environment may also contribute to cyst growth and development of interstitial fibrosis. We and others have shown that mRNA levels of genes involved in tissue remodeling and extracellular matrix (ECM) production are highly elevated in cystic cells compared with normal renal cells.[12,17] In fact, periostin was one of the most highly differentially expressed genes in human ADPKD cells compared with normal tubule cells.[12] Periostin is a secreted protein that binds to the components of the ECM, including type I collagen and fibronectin, and has been implicated in collagen fibrillogenesis.[18–20] Periostin, as well as other 'matricellular' proteins, transmits signals from the ECM to the cell by binding to cell surface integrins, leading to changes in cell adhesion, migration, proliferation, survival, and tissue angiogenesis.[21–26] Although periostin is expressed in several tissues during embryonic development,[27,28] its expression in adults is restricted to collagen-rich tissues and can be

upregulated by mechanical stress.[29–31] Periostin has also been found to be elevated in a variety of cancers, including breast, lung, and colon, where it promotes cell proliferation and survival.[21,22,25,32–36]

In ADPKD, periostin is secreted by cyst-lining cells and accumulates within the ECM adjacent to cysts.[12] Periostin binds $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins and stimulates integrin-linked kinase (ILK), leading to an acceleration of cell proliferation and *in vitro* cyst growth of ADPKD cells within a collagen matrix. By contrast, periostin does not stimulate the proliferation of normal renal cells, suggesting that periostin is a novel autocrine mitogen secreted by cystic cells.[12] In this study, we show that upregulation of periostin expression is not limited to ADPKD but rather is a common feature of inherited renal cystic epithelia, regardless of the underlying genetic defect. To determine whether periostin contributes to PKD progression, we knocked out periostin (*Postn*) expression in *pcy/pcy* mice, a well characterized model orthologous to human nephronophthisis type 3, a recessive form of PKD that typically causes renal failure in children and adolescents.[37,38] In *pcy/pcy* mice, cystic kidneys enlarge to several times the normal size and are associated with extensive renal interstitial fibrosis by 18 weeks of age and the development of azotemia.[37] Previously, we monitored PKD progression in a *pcy/pcy* mouse by measuring kidney volume by magnetic resonance imaging. Kidney volume increased exponentially up to 20 weeks of age, after which there was a plateau as renal parenchyma was replaced with fibrosis.[39] In this study, we determined if loss of *Postn* expression reduced kidney weight (KW), cystic index, cell proliferation, and fibrosis in 20-week-old *pcy/pcy* mice and extended their survival. The results indicate that periostin and its associated signaling pathways may be viable targets for therapy in PKD.

RESULTS

Periostin expression in *pcy/pcy* kidneys

Periostin (*Postn*) mRNA is highly overexpressed in human ADPKD cyst epithelial cells compared with normal human kidney cells.[12] Similarly, we found that *Postn* is overexpressed in ARPKD and several animal models of cystic disease (Table 1), suggesting that aberrant periostin expression is a general feature of PKD, regardless of the underlying genetic mutation.

To determine whether periostin was increased in cystic kidneys of *pcy/pcy* mice, we compared *Postn* expression with wild-type (WT) mice at 1, 10, and 20 weeks. Kidney volume of *pcy/pcy* mice was elevated at 1 week and continued to increase to 20 weeks (Figure 1a), as described previously.[39] By contrast, body weight (BW) of *pcy/pcy* mice was reduced compared with WT mice (Figure 1b). At 20 weeks, periostin mRNA (Figure 1c) and protein (Figure 1d, Supplementary Figure S1 online) were elevated in *pcy/pcy* kidneys compared with age-matched WT kidneys, confirming that periostin is overexpressed in this model of slowly progressive renal cystic disease.

Table 1 | Periostin mRNA levels in recessive models of PKD

	N	Fold increase
Human ARPKD versus NHK cells	3	19.6
cpk/cpk mouse kidney versus WT tissue (3 weeks)	3	9.3
jck/jck mouse kidney versus WT tissue (20–25 weeks)	3	>50

Abbreviations: ARPKD, autosomal-recessive polycystic kidney disease; NHK, normal human kidney; WT, wild-type.

Effects of periostin on body and kidney mass and renal cystic disease

Periostin knockout mice have been reported previously.[40] Consistent with this report, sex-matched *Postn*$^{-/-}$ mice were similar in general appearance to WT (*Postn*$^{+/+}$) littermates (not shown). Also as reported, 20-week-old *Postn*$^{-/-}$ mice exhibited a moderate reduction in BW compared with WT littermates (36.8 ± 1.1 g for WT vs. 31.6 ± 0.9 g for the *Postn*$^{-/-}$, $P < 0.005$) (Figure 2a). However, there was no difference in total KW (both kidneys) between *Postn*$^{-/-}$ and WT mice when corrected for BW (%BW) (Figure 2b).

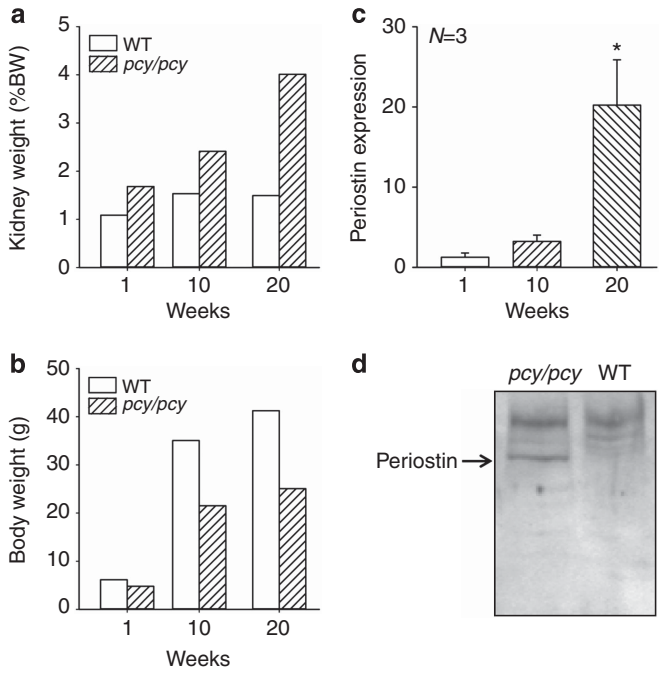

Figure 1 | Kidney and body weight and periostin expression in *pcy/pcy* and wild-type (WT) mice. Male *pcy/pcy* mice and WT mice (2–3 mice per group) were killed at 1, 10, and 20 weeks for determination of (**a**) total kidney weight, represented as a percentage of body weight (%BW) and (**b**) body weight. (**c**) Renal periostin (*Postn*) mRNA expression between *pcy/pcy* and WT mice was determined by quantitative real-time reverse transcriptase–PCR. Periostin expression was normalized to GAPDH (glyceraldehyde 3-phosphate dehydrogenase), and the expression in *pcy/pcy* kidneys was represented as fold change compared with WT kidneys, calculated from ΔC_t ($N = 3$ per group). Data represent mean ± s.e. Statistical analysis was determined by analysis of variance and Student–Neuman–Keuls post test. *$P < 0.05$. (**d**) Immunoblot for periostin protein (90 kDa) in kidneys of 20-week old *pcy/pcy* and WT mice. Expression of periostin protein was confirmed in two additional pairs of *pcy/pcy* and WT mice (Supplementary Figure S1 online).