Polycystic Kidneys Caused by Sustained Expression of Cux1 Isoform p75*

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The transcriptional regulator Cux1 (CDP, Cutl1) is aberrantly expressed in mouse models for polycystic kidney disease. Here we show that p75–Cux1, the shortest isoform of Cux1, transcribed from an alternative promoter within intron 20, is also deregulated in polycystic kidneys derived from Pkd1 mutant embryos. To determine the role of the p75–Cux1 isoform in cystogenesis, we generated transgenic mice expressing p75–CUX1 in the kidneys and other tissues. Strikingly, these animals developed polycystic kidneys at variable penetrance and severity, correlating with transgene expression levels. Histological and marker analysis of p75–CUX1–derived polycystic kidneys revealed renal cysts derived from the tubular nephron, supporting a model of autosomal dominant polycystic kidney disease. Transgenic p75–CUX1 kidneys additionally showed an up-regulation of the protooncogene c-myc and a down-regulation of the cyclin-dependent kinase inhibitor p27. Chromatin affinity purification experiments confirmed the direct interaction of Cux1 with the c-myc and p27 promoters. These molecular alterations were accompanied by an increase in cilia length and in the proliferative index of epithelial cells lining the cysts. Together, these results identify an important role for the short isoform of CUX1 in polycystic kidney disease development.

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the development of renal cysts progressively leading to renal failure. ADPKD affects mostly adults with a frequency of ~1 in 800 (1). PKD1 encodes the membrane protein Polycystin-1 (2), whereas PKD2 encodes the Polycystin-2 calcium channel (1, 3, 4). Both proteins can interact at the cell membrane and form a complex acting as a mechanosensor leading to renal failure. ADPKD affects mostly adults with a frequency of ~1 in 800 (1).

CUX1 p200 isoform under the control of the CMV enhancer/cpk model (41). In addition, transgenic mice expressing the p75-CUX1 isoform were used in transgenic mouse experiments. In contrast, the p110 and p75 isoforms behave like classical transcription factors engaging in slow and stable interactions with DNA (37, 38). The p110 isoform contains three DNA binding domains and results from the proteolytic cleavage of the full-length protein, Cux1 overexpression is detected in polycystic kidneys of the congenital polycystic (cpk) (40) and Pkd1 null mouse models (41). The precise activity of Cux1 in these models remains unclear as Cux1 overexpression is associated with increased proliferation in the Pkd1 model and increased apoptosis in the cpk model (41). In addition, transgenic mice expressing the CUX1 p200 isoform under the control of the CMV enhancer/promoter displayed multiorgan (including renal) hyperplasia associated with glomerular abnormalities, interstitial fibrosis, and proteinuria (42, 43). Notably, p200-expressing transgenic animals did not harbor polycystic kidneys.
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A role for CUX1 in cell proliferation, specifically in the S phase of the cell cycle, has been inferred from a number of reports. The histone nuclear factor D (HinD-D), which was later found to include CUX1 as its DNA binding partner, was shown to be up-regulated in S phase in normal cells (44–48). Up-regulation of CUX1 DNA binding at the end of the G1 phase was found to result from at least two post-translational modifications: dephosphorylation of the Cut homedomain by the Cdc25A phosphatase (49), and proteolytic cleavage of p200-CUX1 (37, 39). Accumulating evidence now points to a crucial role of the shorter CUX1 isoforms in hyperproliferation and tumorigenesis. Proteolytic processing of CUX1 was found to be enhanced in many cancer cell lines (50), and constitutive expression of p110-CUX1 was shown to stimulate cell proliferation by accelerating S phase entry (51). Aberrant expression of p75-CUX1 mRNA or protein was observed in several breast cancer cell lines (38) and a significant association was established between higher p75-CUX1 expression and a diffuse infiltrative growth pattern in invasive tumors (38). In addition, MMTV:p75-CUX1 transgenic mice displayed increased susceptibility to a form of myeloproliferative leukemia (52).

The close association of the p75-CUX1 isoform with hyperproliferation led us to hypothesize that it could also contribute to the development of polycystic kidneys. In support of this, we find the p75-Cux1 isoform overexpressed in Pkd1+/−/− polycystic kidneys. To directly assess the role of this isoform in kidney cyst formation, we generated transgenic mice overexpressing p75-CUX1 in the kidneys. We show that p75-CUX1 transgenic mice develop polycystic kidneys in a dose-dependent manner. This phenotype is associated with increased cell proliferation and correlates with up-regulation of c-myc and down-regulation of p27 expression.

EXPERIMENTAL PROCEDURES

Transgenic and Knock-out Mice—The p75-CUX1 transgenic construct included the cmv immediate early enhancer and the chicken β-actin promoter, as present in the pTriEx 2.1 vector (Novagen), the coding sequences for amino acids 1062–1505 of the human CUX1 protein (GenBank accession number M74099) in-frame with an HA tag and followed by a rabbit β-globin polyadenylation signal. The final construct was excised from the vector and injected into FVB/N pronuclei. Two lines of p75-CUX1 transgenic mice were obtained: Tg157 and Tg166. Mouse colonies were maintained by breeding transgenic mice with wild-type FVB mice or as homozygous transgenic mice with wild-type FVB mice or as homozygous transgenic mice with wild-type FVB mice. Genotyping was performed by PCR analysis of genomic DNA prepared from mouse-tail biopsy using primers in the cmv promoter 5′-GCTGCTGGTTATGTGCTCTCAT-3′, and CUX1 cDNA sequence 5′-TCCACATTGTGGGT- CGTTC-3′. Pkd1+/−/− mice (53) were genotyped using the following primers: 5′-CTAGCGTCTGGATCCAGGCGG-3′, 5′-GATCTCCITGATCTCACCTGCTG-3′ and 5′-GGGA- GGAGGAGGAGCTG-3′. All experiments involving animals were conducted in accordance with McGill University animal care guidelines.

In Situ Hybridization—Kidneys were dissected and fixed overnight in 4% paraformaldehyde, then immersed in 30% sucrose prior to embedding in tissue freezing medium (Triangular Biomedical Sciences). The intron 20-specific probe was prepared with the following primers: 5′-GCAAGAATTGGCTGC- TCACACATATC-3′ and 5′-AAACAGTCCCAAGGTCTAC- GACG-3′, and the full-length CUX1-specific probe was prepared with the following primers: 5′-CAGCGTATTGTGGGAGACCC-3′ and 5′-TGGAAACATTGTAGCGTGT-3′. In situ was performed as previously described (54) using digoxigenin-labeled cRNA probes.

Reverse Transcription-Polymerase Chain Reaction Analysis—RNA was extracted by homogenizing tissues in TRIzol reagent (Invitrogen). RNA extracts were digested with DNase I (1 unit per reaction for 45 min), purified and reverse-transcribed with the Superscript II RNase H-reverse transcriptase kit (Invitrogen) following the manufacturer’s instructions. The following primer pairs were used to perform Real-Time PCR using the Qiagen SYBR Green PCR kit: c-myc, 5′-CGGACACACAACCGTCTTGGA-3′, 5′-CAACTGTTCCTGTCTTTCC-3′, p27 5′-GACTGCTTTCTCATATCCGGACGT-3′, 5′-CCGGCCGAGGAGGAGGTACAGTCAAACTAT-3′, Pkd1 5′-AGACACCGCTCAACTCTCATGTTG-3′, 5′-TTCCTCTCCTCCAGCCTCGTTC-3′.

Immunoblotting—Total protein extracts were prepared by homogenizing 100 mg of tissue in 200 μl of lysis buffer (500 mM NaCl, 50 mM Tris, pH 8.0, 1% Nonidet P-40, 10% glycerol, 0.5 mM dithiothreitol, 2 mM EDTA, Protease inhibitor mixture tablet (Roche), sodium fluoride (10 mM), leupeptin (1 μg/ml), pepstatin (1 μg/ml), sodium vanadate (1 mM), phenylmethylsulfonyl fluoride (1 mM), iodoacetamide (1 mM)) and mixing for 30 min at 4 °C. The extracts were centrifuged for 15 min at 4 °C and the supernatants collected. Protein concentration was calculated using the BCA protocol ( Pierce). Western blot analyses with anti-HA (Covance; 1:4000), anti-actin (Santa Cruz Technology; 1:4000), and anti-CUX1 1300 (1:1000) were done as previously described (37).

Histology, Immunohistochemistry, and TUNEL Stainings—Formalin-fixed organs were embedded in paraffin and cut in sections of 5 μm. Sections were rehydrated and stained with hematoxylin and eosin (H&E) according to standard procedures for histology analysis. Alternatively, sections were prepared for immunohistochemistry by antigen retrieval with 10 mM sodium citrate solution (pH 6) at 95 °C for 10 min. After blocking for 1 h in normal goat serum, slides were incubated overnight at 4 °C with the following primary antibodies: anti-HA (1:500; Covance), anti-CUX1 1300 (1:500), anti-calbindin (1:300; Chemicon), anti-proliferating cell nuclear antigen (1:500; Dako), and anti-acetylated α-tubulin (1:1000; Sigma) or with biotinylated lotus tetragonolobus agglutinin (1:500; Vector). Sections were then incubated either directly with a fluorescence-coupled secondary antibody, or with a biotin-linked secondary antibody followed by the ABC elite reagent (Vector). For immunohistochemistry, the DAB reagent (Vector) was used prior to counterstaining with hematoxylin and mounting. For immunofluorescence, staining with 4′,6-diamidino-2-phenylindole (200 ng/ml) was performed prior to mounting. Acetylated α-tubulin staining was visualized using a Zeiss LSM 510 confocal microscope. TUNEL assay was performed using the ApopTag Fluorescein In Situ Apoptosis Detection Kit (Chemicon), according to the manufacturer’s instructions.
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Classification of Polycystic Kidneys—Polycystic kidneys were classified as mild or moderate to severe. H&E stainings of adult kidney sections were scored using the following system: mild or moderate to severe. H&E stainings of adult kidneys (Fig. 1, A–C). This increase in p75 expression paralleled the transcriptional up-regulation of the full-length Cux1 (Fig. 1, D–F). It is thus plausible that the p75-Cux1 isofom plays a role in kidney cyst formation.

Generation of p75-CUX1 Transgenic Mice—To directly address the role of p75-CUX1 in cystogenesis, transgenic mice specifically expressing this shorter isofom were generated. For this, the sequence coding for amino acids 1062–1505 of the human CUX1 protein (precisely corresponding to the p75 isoform) was fused to protein A and CBP tags (pREV/TRE, Clontech) and stably expressed in Hs578T breast cancer cells. The cells were synchronized by a single thymidine block for 18 h, cross-linked and lysed. Fifty percent of the cell extract was used to purify p75-CUX1-bound DNA by ChAP as described (55). The remaining 50% was used to measure nonspecific binding by performing ChAP with Sepharose beads instead of IgG Sepharose at the first purification step. The following primers were used to amplify promoter regions: c-myc 5′-ATCTCTCTCTGCTA-ATCTCCGC-3′, 5′-TCCCTCCC- TCCGTTCTTTTTC-3′, p27 5′- AATGTTCAATGCTTTAAGCTGT- 3′, 5′-GAAGCGAAAGGAGACT- TCTT-3′, Pkd1 5′-TCTCACCAT- CAACCTCCCCATTG-3′, 5′-TATG- TTCCAGCTCCGC-3′, and G6PDH 5′-GAGTGATCCTGAAAAT- TCATCG-3′, 5′-AGGTCAGGTT- CTCCACCTTG-3′.

RESULTS

Expression of Cux1 Isoform p75 Is Increased in the Kidneys of Pkd1 Null Mice—Overexpression of the full-length Cux1 in Pkd1−/− polycystic kidneys was reported previously (41). To determine whether p75, a more transcriptionally active isoform of Cux1 was also expressed in Pkd1−/− kidneys, a cRNA probe was designed to recognize the p75-Cux1-specific region within intron 20 (38). In situ hybridization stainings in Pkd1−/− and control kidneys revealed a significant up-regulation of p75-Cux1 mRNA expression in these polycystic kidneys (Fig. 1, A–C). This increase in p75 expression paralleled the transcriptional up-regulation of the full-length Cux1 (Fig. 1, D–F). It is thus plausible that the p75-Cux1 isoform plays a role in kidney cyst formation.

Chromatin Affinity Purification (ChAP)—CUX1 amino acids 1062–1505 (corresponding to p75-CUX1) was fused to protein A and CBP tags (pREV/TRE, Clontech) and stably expressed in Hs578T breast cancer cells. The cells were synchronized by a single thymidine block for 18 h, cross-linked and lysed. Fifty percent of the cell extract was used to purify p75-CUX1-bound DNA by ChAP as described (55). The remaining 50% was used to measure nonspecific binding by performing ChAP with Sepharose beads instead of IgG Sepharose at the first purification step. The following primers were used to amplify promoter regions: c-myc 5′-ATCTCTCTCTGCTA-ATCTCCGC-3′, 5′-TCCCTCCC- TCCGTTCTTTTTC-3′, p27 5′- AATGTTCAATGCTTTAAGCTGT- 3′, 5′-GAAGCGAAAGGAGACT- TCTT-3′, Pkd1 5′-TCTCACCAT- CAACCTCCCCATTG-3′, 5′-TATG- TTCCAGCTCCGC-3′, and G6PDH 5′-GAGTGATCCTGAAAAT- TCATCG-3′, 5′-AGGTCAGGTT- CTCCACCTTG-3′.

FIGURE 1. Increased expression of p75-Cux1 mRNA in Pkd1 null polycystic kidneys. In situ hybridization on E16.5 Pkd1+/− (A and D) or Pkd1−/− (B, C, E, and F) embryos using a cRNA probe recognizing all Cux1 isoform transcripts (D and E) or a p75-Cux1-specific cRNA probe (A and B). Sense probes failed to detect any signal (C and F).

FIGURE 2. Generation of the p75-CUX1 transgenic mice. A, transgenic construct consisting of the p75-CUX1 mRNA under the control of the cytomegalovirus/β-actin enhancer promoter. The p75-CUX1 isoform contains one cut repeat (CR) DNA-binding domain (CR3), a homeodomain (HD), and two repression domains. Epitopes for anti-CUX1 antibody 1300 and for the HA tag are indicated. B, Western blot analysis on kidney extracts from wild-type mice (numbers 1, 4, 8, and 9), heterozygous transgenic mice from lines Tg166 (numbers 2, 6, 12, and 13) and Tg157 (numbers 3, 5, 10, and 11), and homozygous transgenic mice from line Tg166 (numbers 7, 14, and 15). Anti-HA recognizes the transgenic p75-CUX1 protein, whereas anti-CUX1–1300 recognizes endogenous and transgenic p75-CUX1. Anti-actin was used as loading control.

p75-CUX1 Expression in MDCK Cells—MDCK cells were maintained in culture in Dulbecco’s modified Eagle’s medium with 10% serum. Cells were infected with pRev empty vector or p75-CUX1HA-containing vector and selected with hygromycin (350 μg/ml) to generate stable cell lines. Retrovirus production was performed as described (51).
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| Wild-type | Tg157 +/- | Tg166 +/- | Tg166 +/- 16mo | Tg166 +/- 12mo |
|-----------|-----------|-----------|----------------|---------------|
| ![A](image1) | ![B](image2) | ![C](image3) | ![D](image4) | ![E](image5) |

**FIGURE 3.** *p75-CUX1 transgenic mice develop polycystic kidneys.* Whole organ (A–E) and hematoxylin and eosin histological stainings (F–J) of kidneys from 18-month-old wild-type (A and F), heterozygous Tg157 (B and G), and heterozygous Tg166 (C and H), as well as homozygous Tg166 kidneys at 16 (D and I) and 12 months (E and J). Arrows point to cystic structures.

revealed strong expression of p75-CUX1 in heterozygous transgenic line 166 (Tg166) (Fig. 2B, mice numbers 2, 6, 12, and 13), and a weaker expression in transgenic line 157 (Tg157) (Fig. 2B, mice numbers 3, 5, 10, and 11). The highest p75-CUX1 expression levels were obtained by breeding Tg166 to homozygosity (Fig. 2B, mice numbers 7, 14, and 15). An antibody detecting both the endogenous and transgenic Cux1 (anti-1300, identified in Fig. 2A) revealed a marked overexpression of p75-CUX1 in Tg166 in comparison to endogenous protein expression levels (Fig. 2B). In contrast, Tg157 expression showed a more modest overexpression (Fig. 2B).

In addition to their expression in the kidney, analysis of protein expression in other organs showed high activity of Tg166 in the spleen, mammary gland, liver, heart, lungs, and brain, whereas Tg157 expressed the transgene in the heart and brain (data not shown). However, no gross phenotypic abnormalities were observed in these organs, suggesting a higher sensitivity of the kidney to p75-CUX1 expression.

*p75-CUX1 Transgenic Mice Develop Polycystic Kidneys with Long Latency*—In contrast to the innocuous extrarenal expression sites, p75-CUX1 expression in the kidney led to cyst formation in both Tg157 and Tg166 heterozygous mice (Fig. 3, A–C and F–H). The severity of the disease was graded as mild, moderate, or severe based on the number and size of kidney cysts (see “Experimental Procedures” for grading system). The weaker expressing Tg157 line generated 22% of mice with moderate to severe polycystic kidneys at 18 months of age (n = 66) (Fig. 3, B and G). This represents a significant increase in cystogenesis from the 9% spontaneous cyst formation observed in control mice at this age (n = 67) (p value < 0.05). The percentage of mice with moderate to severe cyst formation increased to 29% in the higher expressing heterozygous Tg166 (p value < 0.01) (Fig. 3, C and H), consistently with a dose-dependent effect of the transgene (n = 56). To confirm the dosage effect of p75-CUX1, we characterized the kidneys of homozygous Tg166 mice. At 16 months of age, homozygous Tg166 mice showed very advanced polycystic kidneys, with both kidneys being enlarged, pale, and filled with cysts (Fig. 3, D and I). Such disease levels were never observed in heterozygous Tg166 or Tg157 mice. In addition, all homozygous Tg166 mice sacrificed at 12–13 months displayed kidney anomalies such as renal tubule degeneration, in marked contrast to wild-type controls. Some of these mice had initiated renal tubular hyperplasia with cystic dilation of the lumen (Fig. 3, E and J). Hence, the sustained overexpression of the p75-CUX1 isoform in the kidney leads to polycystic kidney disease in a dose-dependent manner.

*The Cyst-lining Cells Express the Transgene and Display Enhanced Proliferation*—To better characterize the origin of p75-CUX1 cysts, we initially localized p75-CUX1 transgenic expression by anti-HA immunostaining on control and homozygous Tg166 kidneys. In Tg166 samples, HA was detected in most epithelial cells lining the developing cysts. Expression was additionally detected in glomeruli but was largely absent from the stroma (Fig. 4B). As expected, wild-type kidneys were negative for HA staining (Fig. 4A). Anti-CUX1 immunostaining was used to confirm the increased CUX1 expression in cystic epithelia compared with non-transgenic kidney samples. In agreement with HA immunostainings, CUX1 was detected at the highest level in the epithelial cells lining the forming cysts (Fig. 4, C and D).

As renal cysts from ADPKD typically arise from both nephron and collecting duct epithelia, whereas the recessive form of the disease (ARPKD) mostly generates collecting duct-derived cysts, we sought to identify the epithelial origin of p75-CUX1 cysts. For this, homozygous Tg166 and control kidneys were stained with the proximal nephron marker lotus tetragonolobus agglutinin. Interestingly, a proportion of p75-CUX1 cysts were found positive for lotus tetragonolobus agglutinin, whereas others were negative (Fig. 4, E and F). These results are thus consistent with an ADPKD-type cystic disease.

We then verified whether cyst formation was accompanied by alterations in proliferation or apoptosis indexes in homozygote Tg166 kidneys. Proliferation was assessed by immunohistochemistry with an anti-proliferating cell nuclear antigen antibody, which showed high expression in the majority of cells lining the developing Tg166 cysts (Fig. 5B). This is in striking contrast to wild-type tubular epithelia, which showed very low or no expression (Fig. 5A). No significant difference in cell death was observed between wild-type and Tg166 transgenic kidneys (Fig. 5, C and D). Together these results indicate that
the expression of p75-CUX1 in both renal epithelia promotes cyst formation at least partially through increased proliferation.

**Effect of p75-CUX1 on the Primary Cilium**—To determine whether p75-CUX1-mediated cyst formation was associated with primary cilium defects we visualized cilia in normal and cystic kidneys with an anti-acetylated α-tubulin antibody. This immunolabeling revealed that cystic epithelial cells generally had mean cilium length about twice that of normal epithelial cells \((p < 0.0001)\) (Fig. 6, A and B). However, this difference was not observed in healthy tubules of p75-CUX1 transgenic mice (data not shown), suggesting that the change in cilium length does not precede cyst formation. As the cilium defect may be a consequence rather than the cause of cyst formation we next assessed whether cilium defects were observable in MDCK cells stably overexpressing a control vector (C) or p75-CUX1-expressing vector (D). E, quantification of cilia length on kidney sections and MDCK cells.

**Deregulation of c-myc and p27 Expression in p75-CUX1 Transgenic Mouse Kidneys**—We next investigated the molecular mechanisms of p75-CUX1-mediated cyst formation. As these cysts were associated with increased proliferation, we
examined a number of genes associated with proliferation control and with polycystic kidney formation using quantitative-PCR assays. Two of these genes were significantly altered in p75-CUX1 transgenic mouse kidneys. The protooncogene c-myc was up-regulated over 2-fold (and up to 9-fold) in 6 of 9 samples analyzed (Fig. 7A). Conversely, the cyclin-dependent kinase inhibitor gene p27 was expressed at significantly lower levels in polycystic kidneys, compared with controls (over 2-fold reduction in 5 of 9 samples analyzed) (Fig. 7B). Interestingly, Pkd1 was also deregulated in several cystic kidneys analyzed with 4 of 9 samples showing significant down-regulation or even absence of Pkd1, whereas 2 of 9 samples showed over a 2-fold increase in Pkd1 expression levels (Fig. 7C). Other candidates such as β-catenin, PKD2, inversin, and N-Myc were found unaffected in this assay (data not shown).

We then verified whether p75-CUX1 could bind the c-myc, p27, and Pkd1 promoter regions. Because there are no antibodies specifically recognizing p75 but not p200 or p110 CUX1, we expressed physiological levels of the p75-CUX1 isoform fused to two different tags in Hs578T cells and purified p75-CUX1-bound chromatin by ChAP. This method has recently been described and validated for p110-CUX1 (55). Interestingly, the promoter region of all three genes, c-myc, p27, and Pkd1, was enriched in the chromatin purified from p75-CUX1 expressing cells as compared with the control (Fig. 7D). In contrast, the G6PDH promoter region was not enriched in p75-CUX1-purified chromatin (Fig. 7D). Taken together, these results are consistent with a direct effect of p75-Cux1 on regulators of cell proliferation during renal cyst formation.

DISCUSSION

Cux1 has previously been implicated in renal cyst formation as its expression was shown to be elevated in both Pkd1−/− and cpk mouse models of polycystic kidney disease (40, 41). However, which Cux1 isoform(s) was overexpressed could not be assessed through immunohistochemical assays because there are no antibodies that specifically recognize the short isoforms without detecting the full-length protein. Moreover, whether high Cux1 expression played a causal role in the disease or was the consequence of it remained an opened question. In the first transgenic mouse model that was generated, expression of the full-length CUX1 isoform (p200) did not promote kidney cyst formation (42). In the present study, using in situ hybridization we confirmed that Cux1 is overexpressed in polycystic kidneys of Pkd1−/− mice. Importantly, using a probe from the 3′ end of intron 20, which is specific for the mRNA that codes for the p75 isoform, we discovered that this specific isoform is overexpressed in polycystic kidneys of Pkd1−/− mice. Moreover, sustained expression of p75-CUX1 in transgenic animals lead to renal cyst formation in a dose-dependent manner. We further
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This study reveals a striking difference in the activity of p75-CUX1 in comparison to p200-CUX1 activity previously reported in transgenic overexpression experiments (42). In p75-CUX1 transgenic animals, we observe renal cyst formation with relatively long latency (12–18 months), whereas p200-CUX1 overexpression caused neonatal glomerular defects and kidney hyperplasia at 6 months of age (42). These abnormalities were not observed in p75-CUX1 transgenic animals. The expression levels or target tissue are unlikely to be responsible for these phenotypic differences as both transgenes were expressed under 

\[ \text{cmyv} \]

regulation and displayed comparable expression levels (this study and Ref. 42). Instead, the intrinsic properties of the two isoforms are a more likely cause for the phenotypic differences. The p200-CUX1 isoform is a complex transcription factor containing three cut repeats and a homeodomain DNA-binding motif. With a transcription initiation site located within intron 20, the p75-CUX1 isoform only contains one cut repeat domain and the homeodomain (38). These two isoforms have highly divergent DNA-binding properties as p75 harbors higher DNA binding stability and different specificity (36, 38, 56). Moreover, whereas p200-CUX1 was reported to function as a repressor (57–61), p75 and p110-CUX1 were found to mediate transcriptional repression or activation depending on promoter context (37, 62). This difference in activity likely explains the differential effect of p75 and p200 on c-myc regulation. The activation by p75-CUX1 observed in this study indeed contrasts with the repression of c-myc transcription previously reported in reporter assays (63).

In general the activity of p75-CUX1 more closely resembles that of p110-CUX1, which results from proteolytic cleavage of p200. However, the presence of an additional cut repeat domain and weaker DNA-binding stability of p110 suggest that the p75 and p110 isoforms may also have slightly different properties (38). Interestingly, p200 did not seem to be significantly cleaved to generate the p110 isoform in the p200-CUX1 transgenic expression experiment (42).

Several lines of evidence suggest an implication of p75-Cux1 in ADPKD-like disease development. The mouse model we present here shows a direct correlation between p75-CUX1 expression levels and disease severity. A transgenic line (Tg157) with low p75-CUX1 expression harbored cystic kidneys at low penetrance, whereas the higher expression levels of p75-CUX1 in Tg166 resulted in a corresponding increase in disease penetrance and severity. In homozygous Tg166 mice, one cannot exclude a contribution of the transgene insertion site to disease progression. However, the phenotypic correlation with p75 dosage in the heterozygous animal and low probability of such fortuitous effect argue against it. Strikingly, all p75-CUX1 animals generated cysts with relatively long latency (12–18 months), a feature typical of ADPKD. In humans, the disease appears with variable age and penetrance but typically during adult life. In further support of an ADPKD-like disease model, a number of cysts observed in p75-CUX1 mice were derived from the nephron tubular epithelium. Hence, p75-CUX1 transgenic mice may represent an interesting model to study ADPKD development.

The disease latency observed in p75-CUX1 transgenic mice implies that it is the sustained pressure of p75-CUX1 on tubular epithelia that leads to renal cyst formation and thus suggests that other cellular/genetic events are necessary for cystogenesis to occur. We identified p27 and c-myc as two primary genes regulated by CUX1 that may play a role in disease progression. In p75-CUX1 polycystic kidneys, p27 was consistently down-regulated, whereas c-myc was up-regulated in most samples analyzed. The cyclin kinase inhibitor gene p27 has been previously identified as a target of CUX1 and reporter assays demonstrated that CUX1 was able to repress transcription from the p27 gene promoter (41, 42, 52). Our data corroborate this finding by identifying a direct interaction between CUX1 and the p27 promoter region in vivo using affinity purification. Similarly, our results extend previous studies on the regulation of c-Myc and suggest that short CUX1 isoforms can activate transcription from the c-Myc promoter (63). This finding identifies a key candidate for p75-CUX1-driven cystogenesis. The role of c-myc in polycystic kidney development was indeed clearly established in transgenic models (15), and correlates with the increase in c-myc expression in human ADPKD (64). Together, the down-regulation of p27 and up-regulation of c-myc are also in perfect line with the strong increase in cell proliferation index observed in p75-CUX1 polycystic kidneys.

Interestingly, Pkd1 itself was consistently deregulated in p75-CUX1 cystic kidneys, however, the deregulation we observed was inconsistent, as several samples showed a down-regulation of Pkd1, whereas some had more than 2-fold higher expression and a minority of samples displayed relatively normal Pkd1 expression levels. Although results from affinity purification suggest a direct regulation of Pkd1 by CUX1, the complex expression pattern we observed cannot be reconciled with a simple model whereby CUX1 would activate or repress Pkd1. Clearly, Pkd1 expression responds to a more complex regulatory network. Interestingly, both down-regulation and up-regulation of Pkd1 have been associated with renal cyst development (9–13). We can envisage that alternative disease progression paths lead to the opposite effect on Pkd1 expression. How higher and lower Pkd1 expression can contribute to the disease remains to be elucidated at the mechanistic level.

In conclusion, our study has uncovered a direct role for p75-CUX1 in kidney cystogenesis. In this context, it will be crucial to identify the regulatory elements located within intron 20 leading to p75-CUX1 up-regulation in ADPKD-like models.

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REFERENCES

1. Wilson, P. D. (2004) *N. Engl. J. Med.* **350**, 151–164
2. Hughes, I., Ward, C. J., Peral, B., Aspinwall, R., Clark, K., San Millan, J. L., Gamble, Y., and Harris, P. C. (1995) *Nat. Genet.* **10**, 151–160
3. Mochizuki, T., Wu, G., Hayashi, T., Xenopoulos, S. L., Veldhuisen, B., Saris, J. J., Reynolds, D. M., Cai, Y., Gabow, P. A., Pierides, A., Kimberling, W. J., Breuning, M. H., Deltas, C. C., Peters, D. J., and Somlo, S. (1996) *Science* **272**, 1339–1342
4. Koulou, P., Cai, Y., Geng, L., Maeda, Y., Nishimura, S., Witzgall, R., Ehrlich, B. E., and Somlo, S. (2002) *Nat Cell Biol.* **4**, 191–197
5. Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., Elia, A. E., Lu, W., Brown, E. M., Quinn, S. J., Inger, D. E., and Zhou, J. (2003) *Nat Genet.* **33**, 129–137
6. Yoder, B. K., Hou, X., and Guay-Woodford, L. M. (2002) *J. Am. Soc. Nephrol.* **13**, 2508–2516
7. Tsokas, L., Kim, E., Arnaudt, T., Sukhatme, V. P., and Walz, G. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 6965–6970
8. Qian, F., Germino, F. J., Cai, Y., Zhang, X., Somlo, S., and Germino, G. G. (1997) *Cell. Biol.* **171**, 1722–1728
9. Lu, W., Peissel, B., Babakhani, H., Pavlova, A., Geng, L., Fan, X., Larson, C., Brent, G., and Zhou, J. (1997) *Nat Genet.* **17**, 179–181
10. Thivierge, C., Kurbegovic, A., Couillard, M., Guillaume, R., Cote, O., and Trudel, M. (2006) *Mol Cell Biol.* **26**, 1538–1548
11. Lantinga-van Leeuwen, I. S., Dauwerse, J. G., Baelde, H. J., Leonhard, P. C., Perez-Atayde, A. R., Rennke, H. G., and Zhou, J. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 6965–6970
12. Stocklin, E., Botteri, F., and Groner, B. (1993) *J Cell Biol.* **122**, 199–208
13. Vees, D. J., Sorenson, C. M., Shutter, J. R., and Korsmeyer, S. J. (2005) *Kidney Int.* **67**, 1710–1722
14. Veis, D. J., Sorenson, C. M., Shutter, J. R., and Korsmeyer, S. J. (1993) *Cell* **75**, 229–240
15. Wu, X., Van Wijnen, A. J., Van Gurp, M. F., de Ridder, M. C., Tufarelli, D., Last, T. J., Birnbaum, M., Vaughan, P. S., Giordano, A., Krek, W., Neufeld, E. J., Stein, J. L., and Stein, G. S. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 11516–11521
16. Coqueret, O., Berube, G., and Nepveu, A. (1998) *EMBO J.* **17**, 6480–6494
17. Harada, R., Silvestri, P., Bellacosa, A., Bogyo, M., Weber, E., Chauhan, S. S., and Nepveu, A. (2007) *Mol Cancer Res.* **5**, 899–907
18. Harada, R., Vanden Heuvel, G. B., Bodmer, R., Liu, B., Bellacosa, A., and Nepveu, A. (2006) *Mol Cell Biol.* **26**, 2441–2455
19. Cadieux, C., Fournier, S., Peterson, A. C., Bedard, C., Bedell, B. J., and Nepveu, A. (2006) *Cancer Res.* **66**, 4992–5001
20. Wu, G., Tian, X., Nishimura, S., Markowitz, G. S., D'Agati, V., Park, J. H., Yao, L., Li, L., Geng, L., Zhao, H., Edelmann, W., and Somlo, S. (2002) *Hum Mol Genet.* **11**, 1845–1854
21. Henrique, D., Adam, J., Myat, A., Chitnis, A., Lewis, J., and Ish-Horowicz, D. (1995) *Nature* **375**, 778–790
22. Nishio, H., and Walsh, M. J. (2004) *Proc Natl Acad Sci U S A* **101**, 11257–11262
23. Zhuo, Q., Maitra, U., Johnston, D., Lozano, M., and Dudley, J. P. (2004) *Mol Cell Biol.* **24**, 4810–4823
24. Truscott, M., Raynal, L., Premdas, P., Goulet, B., Liu, B., Berube, G., and Nepveu, A. (2003) *Mol Cell Biol.* **23**, 3013–3028
25. Dufort, D., and Nepveu, A. (1994) *Mol Cell Biol.* **14**, 4251–4257
26. Lanoix, J., D’Agati, V., Szabolcs, M., and Trudel, M. (1996) *Oncogene* **13**, 1153–1160