Loss of Polycystin-1 Inhibits Bicc1 Expression during Mouse Development

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Abstract

Bicc1 is a mouse homologue of Drosophila Bicaudal-C (dBic-C), which encodes an RNA-binding protein. Orthologs of dBic-C have been identified in many species, from C. elegans to humans. Bicc1-mutant mice exhibit a cystic phenotype in the kidney that is very similar to human polycystic kidney disease. Even though many studies have explored the gene characteristics and its functions in multiple species, the developmental profile of the Bicc1 gene product (Bicc1) in mammal has not yet been completely characterized. To this end, we generated a polyclonal antibody against Bicc1 and examined its spatial and temporal expression patterns during mouse embryogenesis and organogenesis. Our results demonstrated that Bicc1 starts to be expressed in the neural tube as early as embryonic day (E) 8.5 and is widely expressed in epithelial derivatives including the gut and hepatic cells at E10.5, and the pulmonary bronchi at E11.5. In mouse kidney development, Bicc1 appears in the early ureteric bud and mesonephric tubules at E11.5 and is also expressed in the metanephros at the same stage. During postnatal kidney development, Bicc1 expression gradually expands from the cortical and medullary and papillary regions, and it is highly expressed in the proximal tubules. In addition, we discovered that loss of the Pkd1 gene product, polycystin-1 (PC1), whose mutation causes human autosomal dominant polycystic kidney disease (ADPKD), downregulates Bicc1 expression in vitro and in vivo. Our findings demonstrate that Bicc1 is developmentally regulated and reveal a new molecular link between Bicc1 and Pkd1.

Introduction

Studies of animal mutant models for polycystic kidney disease (PKD) and of human PKD patients have identified over 20 genes whose mutations can induce PKD phenotypes [1,2,3]. The continued study of these cyst-associated genes and their products will help elucidate the disease mechanism of human inherited polycystic kidney diseases, such as autosomal dominant and recessive PKD (ADPKD and ARPKD).

Bicc1 is a mouse homologue of Drosophila Bicaudal-C (dBic-C), the orthologs of which are much conserved in many species, from C. elegans to humans [4,5,6,7,8]. Loss of dBic-C in Drosophila disrupts the direction of anterior follicle cell migration and affects anterior-posterior patterning, so that the resulting embryos lack heads and exhibit duplicated posterior segments instead [9,10]. The Xenopus homologue of Bicaudal-C (xBic-C) is one of the few molecules that, when microinjected ectopically, causes endoderm formation in the absence of mesoderm induction [11]. Knockdown of the zebrafish homologue of Bicaudal-C (zBic-C) induces cystic kidneys in vivo [12].

The gene product of Bicc1 (Bicc1) contains several conserved N-terminal KH domains and a conserved C-terminal SAM domain [13]. The KH domains bind target mRNAs [4]. Recent studies indicated that the KH domains enable Bicc1 to recruit specific miRNA precursors and associate with Dicer, to guide these nascent miRNAs to anchor their target mRNAs. The SAM domain is nonessential for mRNA binding, but it is required for the transfer of Bicc1-targeted miRNAs to P-body-associated AGO proteins for silencing [14]. Therefore, the gene product Bicc1 is thought to be an RNA-binding molecule that functions to regulate diverse proteins at the post-transcriptional level.

To study the functional role of Bicc1, some chemically-induced or natively occurring Bicc1-mutant mouse models (jcpk and bpk) have been identified and several gene-targeting mouse models have been established [5,6,15,16,17]. Although Bicc1 mutations in these models result from different mutant Bicc1 proteins, all the models exhibit cystic phenotypes in the kidney that are very similar to human polycystic kidney disease. These mouse models can provide insight into the functional roles of Bicc1 during mouse
development. Even though gene expression of Bicc1 during mouse development has been previously reported [5, 6, 9, 14], the developmental profiles of Bicc1 protein during mammalian development remain uncharacterized. Here we generated a polyclonal antibody against Bicc1 and used it to examine the distribution patterns of Bicc1 during mouse embryogenesis and organogenesis. In addition, we investigated the molecular relationship of Bicc1 to other human cystoproteins and discovered that loss of polycystin-1 (PC1), the gene product of Pkd1, whose mutation causes human ADPKD, reduced Bicc1 expression in vivo and in vitro. Our findings demonstrate that Bicc1 is developmentally regulated and indicate that its normal function may require normal PC1 expression.

Materials and Methods

Antibodies and reagents

A pGEX3 GST expression vector (Amersham) was used as the backbone for producing Bicc1 GST-fusion proteins. The cDNA of mouse Bicc1 (Q711-D858) was inserted in-frame into the vector. The mBicc1-fused plasmid was then transfected into Rosetta host cells (Novagen) to produce the GST-fusion antigen. The antigen was subcutaneously injected into New Zealand white rabbits, at 0.5 mg per injection. The anti-mBicc1 serum (mBicc1p) was generated as reported in our previous studies [18]. All the antiserum product and the preimmune serum (Pre-IM) were affinity-purified before performing experiments.

The following antibodies, staining materials, and reagents were used: anti-HA, and anti-Flag, anti-β-actin, and DAPI (Sigma-Aldrich Inc.); anti-Tamm-Horsfall glycoprotein antibody (THG) (Applied Biological Materials Inc.); fluorescein *lutea tetragonolobus* lectin (LTL) (Vector Laboratories); and anti-Aquaporin-2 (AQP2) antibodies (Abcam Inc.); anti-GST antibody and pBABE-Puro retroviral vector (Cell Biolabs Inc.); pCMV-tag4 expression vector (Stratagene Inc.); and pSico Lentiviral vector system (Addgene Inc.).

Mouse strains

Our *Pkd2*, *Pkd1*, and *Pkd1* mutant mice were described in detail previously [19, 20, 21]. All the mouse models used in this study were backcrossed (over 10 times) to the C57BL/6 inbred background. The animal protocol was approved by Vanderbilt University Institutional Animal Care and Use Committee (Permit Number: M/12/143).

Western blotting and quantitative PCR

Western blot analyses were performed using protocols similar to those described previously [18, 22]. Briefly, proteins from cultured cells or tissues were extracted in lysis buffer (0.5% NP-40, 5% Sodium deoxycholate, 50 μM NaCl, 10 μM Tris-HCl (pH 7.5), 1% BSA), homogenized and centrifuged. Protein samples were solubilized in protein loading buffer and denatured by boiling. The samples were electrophoresed in 10% SDS-PAGE gels. The membranes were incubated with 5% milk at room temperature for one hour and blotted with mBicc1p antibody at room temperature for 4 hours and then were incubated with peroxidase-conjugate secondary antibodies (Sigma) and detected with enhanced chemiluminescence (ECL) (Amersham).

Quantitative PCR was performed using the iCycler iQ Real-Time PCR Detection System with the iQ SYBR Green Supermix kit (Bio-Rad). The *Pkd1* primers were (forward) 5′-GCT AAG CTA CAC TTT TCC TTT ATC GG-3′ and (reverse) 5′-ACT TCT TGG CAT CTT TCA TCC CAC-3′. The *Pkd2* primers were (forward) 5′-GCC TGG TAG TAC CCT GCG AGT T-3′ and (reverse) 5′-CAC GAC AAT CAC AAG ATC C-3′. The *Pkd1* primers were (forward) 5′-CAA ATG CCA CAG CCC AAC AG-3′ and (reverse) 5′-CAG AAT GGT TAG GGG TGG GA-3′.

Histology, immunofluorescence (IF), immunohistochemistry (IHC), and confocal microscopy

The detailed procedures used for histology, IF, and IHC staining were published previously [18, 23]. For the microscopic analysis, a Zeiss Axioplan 2E research microscope and a Zeiss Axiostar 200 inverted microscope system with 10×, 20×, and 40× objectives were used.

Renal epithelial cell lines and their cultures

Mouse inner medullary collecting duct (IMCD) cells were cultured using the conditions described in the American TypeCulture Collection manual. IMCD cells with *Bicc1*-silencing (IMCDΔBicc1) were reported in our previous study [24]. To generate *Bicc1*-overexpressable stable cell line (IMCDBicc1), we initially used Bicc1-pCMV-Tag4 clone (Fig. 1B) as backbone to insert mouse Bicc1 ORF cDNA into LZR5-GFP vector (Addgene). Resulting LZR5-GFP-Bicc1-flag vector and pBABE-Puro vector (Addgene) were co-transfected into HEK293 cells. At time points of cultured 48 and 72 hours, the viral-transfected supernatant was separately harvested and filtered with a 45 μm syringe filter. The 48-hour filtered supernatants were added on subconfluent cultured IMCD cells. After 24 hours, the infection was repeated with the 72 hour viral supernatants. One day later, puromycin was added for cell selection. Through a week puromycin-selected culture, the remaining cells were resuspended and seeded on 100 mm culture plates (Costar) at a cell density of 1000 per plate. Once Puromycin-selected colonies formed, single colonies were picked and expand the colony into 24-well plates (Costar). A cloned cell line with *Bicc1*-overexpression, IMCDBicc1, was identified by RT-PCR and confirmed by western blot analysis.

The null-*Pkhd2* (E8) cell line, its maternally-derived *Pkd2* heterozygous (D3) cell line, the null-*Pkd1* (M10H2) line, and its littermate-derived wildtype (W10B2) cell line were also described in our previous studies [23, 25].

The cells from null-*Pkd1* and its littermate-derived wildtype mice were generated by an approach similar to that reported in our previous studies [23, 25]. Briefly, the kidneys from E16.5 *Pkd1*−/− and wildtype littermate embryos with the C57BL/6 congenic background were finely minced with a scalpel, and the minced tissue was incubated with 0.5% collagenase type IV at 37°C for 45 minutes and pipetted vigorously. The digested tissue was removed by filtration through a 40 μm mesh filter. The remaining single cells and small organoids were washed three times with PBS containing 5 mM glucose. The cells were then incubated with 10 μg/ml biotinylated *Dolichos biflorus* agglutinin (DBA) (Vector, B-1035) at 4°C for 60 minutes. The cells were then washed again with PBS followed by incubation with 50 μl CELLectin Biotin binder Dynabeads (Dynal Biotech) at 4°C for 30 minutes. Since Dynabeads are superparamagnetic polystyrene beads, the incubated mixtures were then washed twice with PBS containing 5 mM glucose, using a magnetic rack. The cells were eluted with release buffer (Dynal Biotech) and were plated on 24-well dishes with LHC-9 Medium (Gibco) under 5% CO2 at 37°C overnight. The cells were then transferred to culture medium containing 5 units/ml murine IFN-γ (Peprotech Inc.), which was changed every other day, and placed in a 33°C incubator for at least 10 cell passages. The culture medium was then switched to 10% FCS DMEM/F12 (1:1) (Gibco), and the cells were cultured for at least another 10 cell passages under the same culture conditions. Since the isolated collecting duct cells were not further cloned, the pool of cells (pool cells) was used for the current study.
Figure 1. Specificity of the polyclonal antiserum mBicc1p for Bicc1. (A) The mBicc1-GST antigen was subjected to western blot analysis with the antibodies shown. The anti-Bicc1 (mBicc1p) and anti-GST antibodies, but not the preimmune serum (Pre-IM), recognized the mBicc1-GST antigen of the expected size (43 kD). (B) Schematic representation of the Bicc1-pCMV-Tag4 expression vector constructed by inserting the full-length ORF of Bicc1 into Flag-tagged pCMV-Tag4. (C) Bicc1-pCMV-Tag4-transfected HEK293 cell lysates were subjected to western blot analysis with the antibodies shown. The anti-Bicc1 (mBicc1p) and anti-GST antibodies but not the Pre-IM recognized the Bicc1-pCMV-Tag4 protein of the expected size (~110 kD). (D) Western analysis of duplicated protein lysates from wildtype, Bicc1-silenced (IMCDshC4C), and Bicc1-overexpressed IMCD cells (IMCDBicc1) were blotted with the mBicc1p antibody. Compared to the wildtype control, the immunoreactivity was significantly increased in Bicc1-overexpressed IMCD cells and was almost not detected in the Bicc1-silenced cells. (E) Normalized quantitative analysis of the densitometry values of the western analyses. Compared to wildtype IMCD and Bicc1-silenced IMCD (IMCDshC4C) cells, Bicc1-overexpressed IMCD (IMCDBicc1) cells showed significantly increased Bicc1 expression (*P<0.05). (F) Immunohistochemistry (IHC) staining of Bicc1 protein in the kidneys of E18.5 Bicc1+/+ and its wildtype littermates. Positive staining (arrows) were showed in the wildtype kidney (a), while no obvious positive staining was seen in the E18.5 Bicc1+/− kidney (b). (a'–b') Corresponding areas of a–b were stained by Bicc1p IHC without counterstaining. Data presented are representative of two to three independently replicated experiments. "cy" = renal cysts. Bars: 25 μm in F. doi:10.1371/journal.pone.0088816.g001
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**Results**

**Generation and characterization of anti-Bicc1 antiserum**

A purified mouse Bicc1-GST fusion protein (mBicc1p), encoding residues Q711 to D858 of Bicc1, was used to produce a rabbit polyclonal antiserum (mBicc1p) against the Bicc1 gene product, Bicc1. The antiserum was generated and affinity-purified as described in our previous studies [18].

To test the specificity of mBicc1p, we performed western blot analyses of the antigen using the Pre-IM, mBicc1p, or an anti-GST antibody. No immunoreactive band was seen with Pre-IM, although strong positive bands of the expected size (~43 kD) were detected with mBicc1p and the anti-GST antibody (Fig. 1A). The mBicc1p-positive band could be competed away with the mBicc1-GST antigen (data not shown), suggesting that the mBicc1p antiserum was specific for Bicc1.

To further confirm the specificity, we constructed a Bicc1-expression vector Bicc1-pCMV-Tag4, in which a Flag-tag was fused in-frame with the full-length ORF Bicc1 cDNA (Fig. 1B). Western analyses showed that the transient transfection of HEK293 cells with Bicc1-pCMV-Tag4 yielded bands of the expected size (~110 kD) that were immunoreactive with the mBicc1p and anti-Flag antibodies, but not with Pre-IM (Fig. 1C). We also used the mBicc1p antiserum to detect Bicc1 in lysates of IMCD cells, the stably Bicc1-overexpressed cell line IMCD\textsuperscript{Bicc1} and the Bicc1-silencing stable cell line IMCD\textsuperscript{Bicc1}\textsubscript{KO} [24]. The ~110-kD Bicc1 band was readily detected in wildtype IMCD cells and highly strong band was showed in the IMCD\textsuperscript{Bicc1} cells, whereas almost no band was seen in the IMCD\textsuperscript{Bicc1}\textsubscript{KO} cells (Fig. 1D–E).

In addition, renal tissues from Bicc1 knockout mice were also used for testing the specificity of mBicc1p antiserum. Strong immunohistochemistry (IHC) staining can be observed in E18.5 wildtype kidney, while there is no positive staining in its Bicc1\textsuperscript{-/-} littermate (Fig. 1F). These results strongly indicated that the mBicc1p antiserum was specific for Bicc1.

**Bicc1 expression during mouse development**

To elucidate the patterns of Bicc1 protein expression during embryogenesis and primary organogenesis in mammals, mBicc1p was used for IHC analyses in developing mouse tissues. We found clear positive staining in the epithelial cells of the neural tube on embryonic day 8.5 (E8.5) (Fig. 2A), and then in the myocardial wall of the heart at E9.5 (Fig. 2B). By E10.5, the epithelial cells in the primordial gut and immature hepatocytes in the liver exhibited positive staining in their cytosol (Fig. 2C–D). By E11.5, Bicc1 expression was seen in the epithelia of the main bronchi, aorta, and early ureteric bud and mesonephric tubules, as the bud penetrated into the metanephrogenic mesenchyme (Fig. 2E–G).

Clear positive staining continued to be observed in the renal comma-shaped body and the S-shaped body by E12.5 (Fig. 2H). At this stage, strong positive immunoreactivity could also be seen in the pancreatic primordium and posterior root ganglia (Fig. 2I–J). At E16.5, strong Bicc1 expression was observed in fasciculated cells in the developing adrenal cortex and in olfactory cells (Fig. 2K–L). By E16.5, the Bicc1 expression appeared to decrease in the cardiomycocytes and primordial gut (data not shown). The finding of Bicc1 protein profiling highly coincides with the studies by Bicc1 mRNA in situ hybridization [5,6,9].

**Expression of Bicc1 in the postnatal mouse kidneys**

Using the mBicc1p antibody, we next performed IHC analyses of the kidneys in newborn mice. Bicc1-positive staining was predominantly observed in the developing tubules of the kidney (Fig. 2M). By the age of 1 month, the Bicc1 expression was concentrated at the juxta-medullary region, with relatively weak expression in the cortical and medullary regions (Fig. 2N). At 3 months, although staining pattern was similar to that seen at 1 month, increased Bicc1 expression was seen over the cortical region of the kidney (Fig. 2O). At 6 months, the Bicc1 expression also extended into the medullary region (Fig. 2P). We also examined the kidneys of 9- and 12-month-old mice with the same antibody, but found no significant Bicc1 expression changes. The staining was mainly seen in the cytoplasm of the positive cells, suggesting that Bicc1 has a cytosolic distribution in vivo (Fig. 2M–P). Collectively, these results showed that Bicc1 expression is regulated during renal development.

**Bicc1 is expressed in multiple nephron segments of the adult mouse kidney**

To explore the molecular relationship between Bicc1 and the other known human ADPKD and ARPKD causal genes, Pkd1, Pkd2, and Pkd1/pkd2, we first examined the Bicc1 mRNA levels in Pkd1\textsuperscript{-/-}, Pkd2\textsuperscript{-/-}, or Pkd1/pkd2 cells using qPCR and compared them to the levels in their littermate-generated wildtype counterparts [23,25]. Interestingly, only the cells lacking Pkd1 exhibited a significant downregulation of Bicc1 (Fig. 4A), suggesting that the loss of Pkd1 inhibits Bicc1 gene expression. To confirm this finding,
Figure 2. Expressional profiles of Bicc1 protein during embryogenesis and kidney development. By immunohistochemistry (IHC) staining (n=3), (A) positive labeling is observed in the neural tube (arrow) at E8.5, * indicates the perineural tube mesenchymal tissue; (B) cardiomyocytes in the myocardial wall (arrows) at E9.5, * indicates the pericardo-peritoneal canal; (C) the primordial gut (arrow), * indicates the peritoneal cavity at E10.5; (D) immature hepatocytes (arrow) at E10.5; (E) epithelia of the main bronchi (arrows), + indicates the pericardo-peritoneal canal and * indicates the lung bud tissue; (F) the aortic wall (arrow), * indicates the cardinal vein; and (G) early ureteric bud (lower arrow)/mesonephric duct (upper arrow) at E11.5; (H) the renal comma-shaped body (arrows), (I) epithelia of the pancreatic primordium (arrows), * indicates hepatic tissue and (J) the posterior root ganglions at E12.5, * indicate vertebral bodies. Bicc1 appeared in (K) the adrenal cortex (lower arrow) and cortex of the kidney (upper arrow), * indicates the liver at E16.5; (L) olfactory epithelia (arrows) at E16.5, + indicates the primordial turbinate bone and * indicate the nasal cavities. (M) IHC with mBicc1p antiserum showed positive labeling (arrow) in the newborn mouse kidney. (N) In the 1-month-old kidney, positive staining (arrow) was observed in the juxta-medullary region of the kidney. (O) In the 3-month-old kidney, clear-cut Bicc1 expression (arrow) was seen a similar region of the kidney, but some positive labeling also appeared over the cortical region. (P) Besides of the juxta-medullary and
we used the mBicc1p antibody in western blots of cell lysates from the littermate-derived, paired cell lines Pkd2+/−/Pkd2−/− (E8) [23], wildtype (W10B2)/Pkd1+/− [25], or wildtype/Pkd1+/−. We found no differences in Bicc1 expression between the lysates of the Pkd2+/−/Pkd2−/− and wildtype/Pkd1+/− cells (Fig. 4B–C). By comparison, however, there was a marked decrease in Bicc1 expression in the lysates of the Pkd1−/− cells (Fig. 4D). The mRNA and western results together indicated that Pkd1−/− cells had approximately half the Bicc1 levels of their wildtype littermates (Fig. 4A, E), and suggested that both the mRNA and protein of Bicc1 are downregulated by the lack of Pkd1.

To further validate this finding, we have restored the entire human PC1 COOH-terminus (PC1-CT) into Pkd1−/− cells by lentivirus system to determine if re-expression of PCK1 can rescue the downregulation of Bicc1 in the null-Pkd1 cells. The western blots of cell lysates from the littermate-derived cell lines: wildtype, Pkd1+, Pkd2+, or Pkd1−/− mice. Compared to wildtype, the Bicc1 expression was significantly decreased in the kidney of the Pkd1−/− litters at E14.5 (Fig. 5Aa vs. b). However, the Bicc1 expression levels in E14.5 kidneys of Pkd1−/− and Pkd2−/− mice seem similar to their wildtype littersmates (Fig. 5Ac vs. d and Fig. 5Ae vs. f).

Finally, we have also performed western analyses from tissue lysates of E14.5 null-Pkd2; null-Pkd1 and null-Pkd1embryos, and their wildtype-littermates as well. Compared to wildtype, Bicc1 is also significantly downregulated in the null-Pkd1 tissues. However, there are no any Bicc1 expression changes between the tissue lysates with and without Pkd2 and Pkd1 (Fig. 5B–C). These in vitro results provide further evidence that the loss of Pkd1 downregulates Bicc1 expression.

**Discussion**

Studies using chemically-induced or natively occurring Bicc1-mutant mouse models (jcpk and bpk) and other Bicc1-gene-targeted mouse models have recently demonstrated that the disruption of Bicc1 can induce polycystic kidney disease with phenotypes very similar to human ADPKD [5,6,17]. Although gene expression of Bicc1 during mouse development has been previously reported [5,6,9,14], the characteristics of Bicc1 gene product, Bicc1, during embryogenesis and organogenesis in mammals has not been completely explored. In this study, we generated a new polyclonal antibody, mBicc1p, against the Bicc1 gene product, Bicc1, and used it to study the expression of Bicc1 during mouse development. By using this antibody, we also discovered that the loss of Pkd1 downregulates Bicc1 expression in vitro and in vivo, revealing a molecular relationship between Bicc1 and Pkd1, a known causal gene for human ADPKD. Our findings demonstrate that Bicc1 expression is developmentally regulated and that its normal function may require PCK1 expression.

To investigate the functional role of Bicc1 during mammalian development, several studies have examined mouse Bicc1 gene expression patterns using in situ mRNA hybridization [6,9]. By whole-mount in situ hybridization, Bicc1 mRNA expression is first detected at the rostral tip of the primitive streak around E7.5 and in neural tissues at E8.5. At E13.5, strong Bicc1 mRNA labeling is detected in the bone, heart, and lung tissues. In mouse kidney development, Bicc1 mRNA is detected at the metanephros and the first branch of the ureteric bud tree at E11.5, and then at the metanephros and the comma- and S-shaped bodies at E13.5. At birth, Bicc1 mRNA is mainly seen in the proximal tubules of the...
mouse kidney. These Bicc1 mRNA patterns and tissue distributions highly correspond to our findings with anti-Bicc1 antibody staining, further demonstrating the specificity of the mBicc1p antibody for Bicc1, and supporting protein expression profile described in this study.

Primary cilia are found on diverse cell types ranging from fibroblasts to epithelia [27]. These cilia are generally thought to function as extracellular sensors, for regulating cell behavior [28]. Many human and rodent cystoproteins, the mutants of which cause PKD phenotypes, have been reported to co-localize with the primary cilium/basal bodies of renal epithelia [1,29]. However, previous reports indicate that Bicc1 was distributed to the P-bodies of renal epithelial cells [5,6,14]. This novel subcellular localization of Bicc1 indicated that the primary cilium/basal bodies in renal epithelial cells may not be the only targeted organelle for cystogenesis of the kidney.

We recently established stable IMCD (mouse inner medullary collecting duct) cell lines, in which Bicc1 is silenced by short hairpin

Figure 4. Loss of PC1 downregulates Bicc1 expression in vitro. (A) qPCR analysis of cultured cell lines with or without Pkd1, Pkd2, or Pkhd1 showed no change in the Pkd2 or Pkhd1 mRNA expression, while Bicc1 was significantly downregulated in the cells lacking Pkd1 compared to the wildtype control (*P<0.05) (n = 3). (B) Western blot analysis of protein lysates from null-Pkd2 cells (E8) and Pkd2-heterozygous cells (D3) using the mBicc1p antibody suggested that PC2 loss did not affect Bicc1 expression. (C) Similar western blots for null-Pkhd1 cells (M10H2) and Pkhd1-wildtype cells (W10B2) showed that loss of Pkhd1 also did not affect Bicc1 expression. (D) Western blot analysis for the null-Pkd1 cells and their wildtype littermate cells showed that loss of Pkd1 markedly downregulated the Bicc1 protein expression. (E) Normalized quantitative analysis of the densitometry values of the western analyses. Compared to null-Pkd2 or -Pkhd1 cells, only the null-Pkd1 cells showed significantly reduced Bicc1 expression (*P<0.05). (F) With the wildtype littermate cell control, western blot analyses for the null-Pkd1 pool cells and their PC1-CT transfected cells showed that the restoration of PC1 COOH-terminus markedly rescued the downregulation level of Bicc1 expression in the null-Pkd1 cells. (G) Normalized quantitative analysis of the densitometry values of the western analyses. Compared to the null-Pkd1 cells, the cells with PC1-CT transfection can partially restored Bicc1 expression in the null-Pkd1 cells (*P<0.05). Data presented are representative of two to three independently replicated experiments.

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RNA (shRNA) expression. Our previous results showed that the inhibition of Bicc1 disrupts normal tubulomorphogenesis and induces the cystogenesis of IMCD cells grown in three-dimensional culture [24]. These cells also have a significant defect in E-cadherin-based cell-cell adhesion, along with abnormalities in actin cytoskeletal organization, cell-extracellular matrix interactions, ciliogenesis, and cell proliferation/apoptosis. Interestingly, these defects are also seen in epithelial cells lacking Pkd1 and Pkd2, mutations that can cause human ADPKD [23,30,31]. We therefore assume that the cystogenesis resulting from the downregulation of Bicc1 may be associated with a disruption in the normal Pkd1 or Pkd2 expression.

As predicted, a recent study demonstrated that the inhibition of Bicc1 disrupts normal tubulomorphogenesis and induces the cystogenesis of IMCD cells grown in three-dimensional culture [24]. These cells also have a significant defect in E-cadherin-based cell-cell adhesion, along with abnormalities in actin cytoskeletal organization, cell-extracellular matrix interactions, ciliogenesis, and cell proliferation/apoptosis. Interestingly, these defects are also seen in epithelial cells lacking Pkd1 and Pkd2, mutations that can cause human ADPKD [23,30,31]. We therefore assume that the cystogenesis resulting from the downregulation of Bicc1 may be associated with a disruption in the normal Pkd1 or Pkd2 expression.

Figure 5. Loss of PC1 downregulates Bicc1 expression in vivo. (A) Positive mBicc1p IHC staining (arrows) in the mouse embryonic kidneys. The E14.5 wildtype (a) and its littermate Pkd1−/− (b) kidneys were stained by mBicc1p antibody (n=3). In comparisons of kidney tissues with and without Pkd1, the null-Pkd1 kidney showed significantly reduced Bicc1 expression. However, there are no Bicc1 expression difference between the age-matched wildtype (c and e) and their littermate Pkd1−/− (d) or littermate Pkd2−/− (f) kidneys, respectively. "cy" = renal cysts; "G" = glomerulus. (B) Compared to its wildtype littermate, western blot of duplicated tissue lysates from E14.5 embryos showed that loss of PC1 markedly downregulates Bicc1 protein level (left panel). A similar western blot showed equal immunoreactivities between the tissue lysates from E14.5 Pkd2−/− and its wildtype littermate (middle panel) and between the E14.5 Pkd1−/− and its littermate wildtype embryos (n=3). β-actin for protein loading control. (C) Normalized quantitative analysis of densitometry values of the western analyses. Compared tissues with and without Pkd1, Pkd2 or Pkd1, only null-Pkd1 tissue shows significantly reduced Bicc1 expression (*P<0.05). Bars: 50 μm in A. doi:10.1371/journal.pone.0088816.g005

Polycystin-1 Regulates Bicc1 Expression

RNA (shRNA) expression. Our previous results showed that the inhibition of Bicc1 disrupts normal tubulomorphogenesis and induces the cystogenesis of IMCD cells grown in three-dimensional culture [24]. These cells also have a significant defect in E-cadherin-based cell-cell adhesion, along with abnormalities in actin cytoskeletal organization, cell-extracellular matrix interactions, ciliogenesis, and cell proliferation/apoptosis. Interestingly, these defects are also seen in epithelial cells lacking Pkd1 and Pkd2, mutations that can cause human ADPKD [23,30,31]. We therefore assume that the cystogenesis resulting from the downregulation of Bicc1 may be associated with a disruption in the normal Pkd1 or Pkd2 expression.

As predicted, a recent study demonstrated that the absence of Bicc1 in cells promotes miR-17’s binding to the 3’UTR of Pkd2 and disrupts the stability of the Pkd2 mRNA [6]. This finding indicates that Bicc1 acts as a posttranscriptional factor upstream of Pkd2 and reveals the molecular relationship between Bicc1 and Pkd2, a causal gene of human ADPKD. In the present study, we showed that another human ADPKD causal gene, Pkd1, is involved in the regulation of Bicc1 expression in vitro and in vivo. This finding indicates that Bicc1 is not only associated with Pkd2, but also with Pkd1 expression. The association between Bicc1 and polycystins implies that a disruption of Bicc1 induces cystic phenotypes through the polycystin pathway.

In summary, we have newly generated a polyclonal antibody, mBicc1p, that is specific for the Bicc1 gene product Bicc1. Using this antibody, we demonstrated the developmental profile of Bicc1 protein during embryogenesis and organogenesis in mammals. We found that the Bicc1 protein is expressed as early as E8.5 in the mouse neural tube and appears at the ureteric bud and nephronic tubules by E11.5. After birth, Bicc1 expression extends into the proximal tubules of the kidney, and thereby is predominately expressed in this nephron segment. Moreover, we discovered that normal Bicc1 expression requires PC1 expression. These findings together indicate that Bicc1 is a key protein for embryogenesis and
organogenesis in mammals and uncover a new molecular link between Bicc1 and Pkd1, whose mutation causes human ADPKD.

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Author Contributions

Conceived and designed the experiments: GW. Performed the experiments: PL AL YL HL D. Liang BH D. Lin. Analyzed the data: TJ GM DQ GW. Contributed reagents/materials/analysis tools: PL AL YL HL D. Liang BH D. Lin. Wrote the paper: GM DQ GW.