Polycystic kidney disease in Han:SPRD Cy rats is associated with elevated expression and mislocalization of SamCystin

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Nagao S, Morita M, Kugita M, Yoshihara D, Yamaguchi T, Kurahashi H, Calvet JP, Wallace DP. Polycystic kidney disease in Han:SPRD Cy rats is associated with elevated expression and mislocalization of SamCystin. Am J Physiol Renal Physiol 299:F1078–F1086, 2010. First published August 18, 2010; doi:10.1152/ajprenal.00504.2009.—Polycystic kidney disease (PKD) in Han:SPRD Cy rats is caused by a missense mutation in Anks6 (also called Pkdr1), leading to an R823W substitution in SamCystin, a protein that contains ankyrin repeats and a sterile alpha motif (SAM). The cellular function of SamCystin and the role of the Cy (R823W) mutation in cyst formation are unknown. In normal SPRD rats, SamCystin was found to be expressed in proximal tubules and glomeruli; protein expression was highest at 7 days of age and declined by ~50–60% at 45–84 days of age. In Cy/+ and Cy/Cy kidneys, expression of SamCystin was lower than in +/+ kidneys at 3 and 7 days but became elevated at 21 days. Immunohistochemical analysis revealed that SamCystin was distributed on the brush border of proximal tubules in normal rat kidneys. In Cy/+ kidneys, there were robust SamCystin staining in cyst-lining epithelial cells and loss of apical localization, and increased number of PCNA-positive cells in cyst-lining epithelia. Verapamil, an L-type Ca2+ channel blocker, accelerated PKD progression in this model and caused a further increase in the expression and abnormal distribution of SamCystin. We conclude that aberrant expression and mislocalization of R823W SamCystin lead to increased cell proliferation and renal cyst formation.

ADPKD; proximal tubule; protein expression; calcium channel blocker

AUTOSOMAL DOMINANT POLYCYSTIC kidney disease (ADPKD) is the most common inherited renal disorder and is characterized by the formation of innumerable fluid-filled cysts. Although cysts are benign, their unrelenting growth causes extensive nephron loss, interstitial fibrosis, and progressive loss of renal function (1, 8, 9). ADPKD is caused by mutations in PKD1 or PKD2, genes that encode polycystin-1 (PC-1) and polycystin-2 (PC-2), respectively. The polycystins interact with each other and possibly other proteins to form a multifunctional protein complex involved in intracellular Ca2+ regulation and the morphological development of epithelial structures within the kidneys, liver, pancreas, and other tissues and organs (reviewed in Ref. 1). Many of the proteins associated with cystic disease, including PC-1, PC-2, fibrocystin, polaris, nephrocystins 1, 3, 4, and 5, cystin, and kinesin-II, localize to the primary cilium, a microtubule-established organelle that extends from the surface of renal epithelial cells into the tubule lumen. The role of the primary cilium is unknown, but in vitro studies suggested that it functions as a mechanosensor and/or chemosensor to transmit changes in the tubule fluid into intracellular Ca2+ signals. One hypothesis is that dysregulation of intracellular Ca2+ due to functional loss of ciliary proteins is the underlying cellular basis for the proliferation phenotype of cystic cells.

The Han:SPRD Cy rat is a well-documented animal model of PKD that has several features in common with human ADPKD, including renal hyperplasia, thickened basement membranes of the cystic epithelia, interstitial fibrosis, and azotemia (6, 18–20). Heterozygous Cy/+ rats develop slowly progressive PKD with a gender difference in disease severity between male and female rats by 8 wk of age (13). Male Cy/+ rats have a more rapid disease progression and often die of uremia at 1 yr, whereas female rats live to ~2 years of age. Homozygous Cy/Cy rats develop massive bilateral polycystic kidneys, marked azotemia, and rapid progression to renal failure within 3 wk of age.

The Cy mutation is caused by a spontaneous missense mutation in Anks6 (formally called Pkdr1) on rat chromosome 5 that led to an R823W substitution in SamCystin, a novel protein that contains 10 tandem ankyrin repeats in the NH2 terminus and a sterile alpha motif (SAM) in the COOH terminus (2, 3, 16, 23). These domains are thought to be involved in RNA-binding and/or protein-protein interactions (10, 12). Replacement of the arginine with a tryptophan in the SAM domain is likely to affect the ability of SamCystin to interact with other signaling molecules critical for its function. The cellular distribution and function of SamCystin and the role of the Cy (R823W) mutation in cyst formation are unknown. In the present study, we show that changes in expression and mislocalization of mutant SamCystin correlate with an accelerated rate of renal cystic disease progression in the Cy rat.

METHODS

Animals. Han:SPRD Cy rats were originally derived from a strain of Sprague-Dawley rats in Hanover, Germany; descendants of this colony have been maintained at the Education and Research Center of Animal Models for Human Diseases at Fujita Health University. Normal, Cy/+, and Cy/Cy male rats were anesthetized with pentobarbital sodium (Scherin-Plough, Kenilworth, NJ), and both kidneys were removed rapidly, causing lethal exsanguination. Body weight and total kidney weight were measured. Half of the left kidney was homogenized in lysis buffer to extract proteins and the other half was rapidly frozen in liquid N2 for RNA extraction. Half of the right kidney was embedded in 4% carboxy methyl cellulose (CMC:FINETEC, Tokyo, Japan) after sequential incubations in 10, 20, and 30% sucrose,
and the other half of the right kidney was fixed overnight in 4% paraformaldehyde, embedded in paraffin, and sectioned for immunohistochemistry. Concurrently, the brain, testis, and liver were homogenized in lysis buffer to extract proteins. In some experiments, male rats were treated with 20 mg/kg verapamil (VP; Sigma, St. Louis, MO) by gavage every 12 h from 5 to 12 wk of age, as described previously (15). The protocol for the use of these animals was approved by the Animal Care and Use Committee at Fujita Health University.

*Genotyping.* Mutational analysis revealed that a C to T transition in the *Anks6* (*Pkd1*) gene was responsible for PKD in the Han:SPRD rats (3). Normal, Cy/+, and Cy/Cy rats were genotyped using a PCR restriction fragment length polymorphism method as described previously by Brown et al. (3). Briefly, DNA was extracted from a small piece of ear of each rat and PCR products were obtained from each DNA sample using the Applied Biosystems GeneAmp PCR System 9700. The PCR reaction (T_m = 55°C) was linear for 35 cycles using genomic DNA. Primers for rat SamCystin were as follows: sense primer: CTA GAA GCC TCA GTG ACC CC; anti-sense primer: GAC TAC AGC TCA AAG GTA GG. Amplification products were digested by MspI (TAKARA BIOINC., Tokyo, Japan) at 37°C for 1 h. Digested PCR products were resolved on an agarose gel, producing bands at 157 and 85 bp; 242, 157, and 85 bp; and 242 bp for +/+, Cy/, and Cy/Cy rats, respectively.

*Production of anti-SamCystin polyclonal antibodies.* Anti-rat SamCystin IgG polyclonal antibodies were generated using two antigenic peptide sequences (peptide #1: amino acids 511 to 527, AKDSPGPNPRREKDDVL; and peptide #2: amino acids 868 to 884, CPSMAGWVRPEETVSSSR) homologous to rat SamCystin (NP_001015028) determined by Epitope Adviser Lite software (MBL, NAGANO, Japan). Both peptides were injected six times into two rabbits (3.0 kg body wt, MBL) to optimize the titer and the animals were bled out.

Antibodies were affinity-purified using a SulfoLink Immobilization Kit (#44895; Pierce, Rockford, IL).

*Western blot analysis.* Kidney lysates were prepared for immunoblot as described previously (15). Lysis buffer contained 20 mM Tris (pH 7.4), 137 mM NaCl, 25 mM β-glycerophosphate, 2 mM EDTA, 1 mM sodium orthovanadate, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, and 5 μg/ml leupeptin with 1% Triton X-100. Proteins (20 μg protein/lane) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Membranes were blocked with 5% milk in TBS-T (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, and 0.05% Tween 20) for 1 h at room temperature and then incubated overnight at 4°C in primary anti-SamCystin antibody (1:1,000) or GAPDH (1:10,000, ab8245 Abcam Cambridge, CB4 OFW, UK) in 2% milk in TBS-T. Membranes were then washed three times with TBS-T and incubated with secondary antibody conjugated to horse-radish peroxidase diluted 1:2,000–5,000 in 2% milk in TBS-T. Specific antibody signals were detected using an enhanced chemiluminescence system (ECL or ECL Advance Western Blotting Detection System; Amersham Life Sciences, Arlington Heights, IL). Images of the blots were captured, and the intensity of the protein bands was quantified using a CS Analyzer 2.0 with a CCD camera (ATTO, Tokyo, Japan). Relative band intensity was compared with +/+ kidneys (set to 100%).

*Immunofluorescence and immunohistochemistry.* Kidney sections (4-μm thick) were incubated with primary antibody for SamCystin (1:1,000), calbindin-D28K (1:1,000 C9848, Sigma), biotinylated *Phaseolus Vulgaris* Ethyroleucocitin (1:1,000 PHA-E; B-1125 Vector Laboratories, Burlingame, CA), or mouse IgG calbindin-D28K (1:1,000 C9848, Sigma) overnight at 4°C. After the sections were rinsed, they were incubated with Alexa 568 anti-rabbit IgG for SamCystin, Alexa 486 anti-mouse IgG for calbindin-D28K, or fluorescein-conjugated streptavidin (SP-4040 Vector) for PHA-E. Nuclei were stained with DAPI using VECTASHIELD Mounting Medium (Vector Laboratories).

Immunohistochemistry was used to detect SamCystin abundance and localization in kidneys from untreated and VP-treated Cy/+ rats as described previously (15). Briefly, 4-μm paraffin sections were incubated with 0.1% trypsin at 37°C for 30 min. Endogenous peroxidase was destroyed by incubating sections in 0.3% H₂O₂ in methanol and sections were incubated with SamCystin antibody (1:1,000) in PBS containing 1% BSA plus 0.05% NaN₃ overnight at 4°C. Primary antibodies for PCNA (P8825; Sigma) were also used for immunohistochemistry. Sections were incubated with secondary antibody HISTOFINE MAX-PO (MULTI: for anti-mouse/rabbit IgG+IgA+IgM) obtained from NICHIREI Biosciences. Immune reaction products were developed using 3,3′-diaminobenzidine (ENVISION kit HRP Dako Cytochemistry K3466). To evaluate the effect of VP on SamCystin localization, the number of cystic cells with basolateral SamCystin staining was determined per Cy/+ kidney section by a naive observer. The number of cells with basolateral SamCystin staining above background was counted in 38–40 fields per kidney section (at ×200 magnification) within the renal cortex of vehicle-treated and VP-treated 84-day-old Cy/+ rats.

*Statistics.* Data are represented as means ± SE. Statistical significance was determined by one-way ANOVA and Student-Newman-Keuls posttest for multiple comparisons or unpaired t-test for comparison between control and treated groups (Instat; Graphpad Software, San Diego, CA).

**RESULTS**

*SamCystin expression in Cy/+ and Cy/Cy rat kidneys.* Proximal tubule cysts develop within the kidneys of both Cy/+ and Cy/Cy rats as early as 3 days of age (Supplemental Fig. 1; the online version of this article contains supplemental data) and the cystic area corresponds to Cy gene dosing (6). We used a polyclonal antibody against two peptide sequences of rat SamCystin to determine the relative abundance of SamCystin in +/+, Cy/+, and Cy/Cy rats. The antibody detected a
110-kDa band in kidneys of +/+ and Cy/+ rats by immunoblot analysis, consistent with the predicted molecular weight of SamCystin (Fig. 1A). The band was eliminated by incubation of the antibody with excess competing peptide. This antibody also detected SamCystin protein in the brain, kidney, and testis, but not the liver of 21-day-old +/+, Cy/+, and Cy/Cy rats (Fig. 1B), consistent with the SamCystin mRNA expression profile by PCR (3).

To determine the temporal expression of SamCystin in kidneys of +/+, Cy/+, and Cy/Cy rats, we compared relative SamCystin protein levels, normalized to GAPDH, by immunoblot analysis (Fig. 2). SamCystin protein levels were reduced in Cy/+ and Cy/Cy kidneys at both 3 and 7 days of age, compared with +/+ kidneys (set to 100%; Fig. 2B). On the other hand, SamCystin levels were elevated 27 ± 4.5 and 68 ± 8.5% in 21-day-old Cy/+ and Cy/Cy kidneys, respectively, compared with kidneys of age-matched +/+ rats. In normal kidneys, SamCystin protein levels decreased 50 and 60% at 45 and 84 days of age, respectively, compared with 7 days. By contrast, the expression of SamCystin in Cy/+ kidneys increased from 7 to 84 days and was elevated compared with +/+ kidneys at these time points (Fig. 2, A and B).

**SamCystin distribution in +/+, Cy/+ and Cy/Cy kidneys.** To determine the cellular localization of SamCystin, sections of 21-day-old +/+ kidneys were stained with a polyclonal antibody to SamCystin (Fig. 3). A secondary anti-rabbit antibody conjugated to Alexa 568 was used to detect SamCystin (red color). *Phaseolus vulgaris* lectin (PHA-E; Fig. 3, B-D, green) and an antibody to calbindin-D28K (Fig. 3, F-H, green) were used to define proximal tubules and distal convoluted tubules, respectively. We found that SamCystin was expressed at the luminal surface (Fig. 3, C and D) of tubules staining positive for PHA-E, indicating that SamCystin was expressed at the brush border of proximal tubules. By contrast, SamCystin was not detected in the calbindin-D28K-positive distal tubules (Fig. 3, G and H).

To compare SamCystin renal distribution in +/+, Cy/+ and Cy/Cy rats, sections of 7-day-old kidneys were stained for SamCystin (red), PHA-E (green), and DAPI (blue; Fig. 4). There was distinct staining for SamCystin within the luminal brush border of proximal tubules and costaining with the proximal tubule marker in the merged image (Fig. 4Ac). By contrast, there was a marked loss of SamCystin expression at the brush border and a diffuse distribution of SamCystin in the perinuclear and basolateral aspects of cyst epithelial cells of Cy/+ and Cy/Cy kidneys (Fig. 4, Ad-j), indicating that SamCystin was mislocalized in the cyst-lining cells. Immunofluorescence of SamCystin in kidney sections from 21-day-old Cy/+ and Cy/Cy rats (Fig. 4B) and 84-day-old Cy/+ rats (Fig. 4C) also showed a diffuse cytosolic staining pattern for SamCystin in the cystic epithelial cells. On the other hand, there was distinct staining for SamCystin within the luminal brush...
border of normal proximal tubules of Cy/+ kidneys, similar to the expression in normal kidneys (arrows, Fig. 4B).

Immunohistochemical staining of 84-day-old Cy/+ kidneys with an antibody to proliferating cell nuclear antigen (PCNA), a cell proliferation marker, revealed that cysts with elevated SamCystin expression had an increased number of proliferating cells (Fig. 5). Taken together, these data are consistent with the hypothesis that the R823W mutation results in the failure of SamCystin to localize specifically to the brush border of the cyst epithelial cells in Cy rats, altering signaling pathways involved in the regulation of cell proliferation.

Effect of VP on renal SamCystin distribution in Cy/+ rats. Treatment of Cy/+ rats with VP, a Ca\(^{2+}\) channel blocker, increases kidney weight/body weight and cystic area and results in a decline in renal function (15). There is also a corresponding increase in renal activity of the B-Raf/MEK/ERK signaling pathway and the number of proliferating epithelial cells in cystic Cy/+ kidneys, consistent with the hypothesis that Ca\(^{2+}\) restriction accelerates PKD progression. By contrast, VP has no effect on the function or morphology of +/- kidneys. To determine whether an acceleration of PKD progression with VP correlates with changes in expression and/or distribution of SamCystin, 20 mg/kg VP were given to Cy/+ and +/- rats by gavage twice daily from 5 to 12 wk of age. VP had no effect on SamCystin localization (Fig. 6, A-C) or protein expression (Fig. 7) in normal kidneys. By contrast, VP caused a further elevation in SamCystin levels in Cy/+ rat kidneys (187.8 ± 7.7 vs. 221.2 ± 11.3%; \(P < 0.01\); Fig. 7). The intensity of SamCystin staining appeared to be elevated in the cyst-lining epithelial cells of the 84-day-old Cy/+ rats treated with VP, similar to its intensity and staining pattern in kidneys of 180-day-old untreated Cy/+ rats (Fig. 6F). Quantification of cells with SamCystin staining near the basolateral membrane indicated that VP treatment increased the number of cells with aberrant SamCystin expression from 1.7 ± 0.1 to 6.5 ± 0.1% (\(P < 0.005\)), indicating that the Ca\(^{2+}\) channel blocker caused further mislocation of mutant SamCystin. We suggest that accelerated cyst progression due to intracellular Ca\(^{2+}\) restriction is correlated with increased mutant SamCystin expression and its translocation into the cytosolic and basolateral aspects of cyst epithelial cells of Cy/+ kidneys.

**DISCUSSION**

In the Cy rat model of dominantly inherited cystic disease, a missense mutation in Ank6 (formally called Pkd1) causes an arginine (CGG) at position 823 to be replaced with a tryptophan (TGG) within the SAM of SamCystin (3), a protein of unknown function. SAM is an evolutionary conserved protein module of ~70 amino acids present in many proteins (>60) involved in protein-protein (e.g., scaffolding proteins) and protein-nucleic acid (e.g., transcriptional and translational regulators) interactions (10, 12, 23). SAM domains are thought to specifically bind other SAM domains to form homo-oligomers and hetero-oligomers and can bind to non-SAM domain-containing proteins by interacting with other functional domains including ankyrin repeats, KH (K homology) domains, and SH2 domains to form large protein complexes (21, 23).

While the function of SamCystin is unknown, the SAM module of SamCystin shares extensive similarities to the SAM domain of the mouse homolog of Drosophila Bicaudal C (Bicc1). Bicaudal C contains three KH domains and a COOH-terminal SAM domain and is expressed in developing embryos, consistent with a role in RNA-binding and/or protein-protein interactions during embryogenesis (4). In Xenopus laevis, Bicc1 plays a critical role in embryonic patterning; and mutations in Bicc1 cause dilated pronephric tubules and ducts, a PKD-like phenotype (27). Mutations in Bicc1 are responsible for PKD in jcpk and bpk mouse models. These data indicate that Bicaudal C, possibly through its SAM domain, is necessary for establishment and maintenance of normal tubule architecture (4).
SamCystin and Bicc1 are not expressed on primary cilia or basal bodies of kidney cells but instead colocalize within the cytoplasm (23). The two proteins were shown to interact via the SAM domain of SamCystin in IMCD cells overexpressing epitope-tagged recombinant proteins (23). This interaction was not affected by the Cy mutation or truncation of the ANK repeats, but it was dependent on the presence of the SAM domain. SamCystin proteins can also self associate and it appears that both functional domains of SamCystin are required for this interaction. Interestingly, mutated SamCystin

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**Fig. 4.** Cellular distribution of SamCystin in +/+ , Cy/+ , and Cy/Cy kidneys. 

**A:** SamCystin (red) localized to brush border of normal proximal tubules stained for PHA-E lectin (green) in 7-day-old +/+ and Cy/+ kidneys. By contrast, there was diffuse SamCystin staining in the cytosol and the basolateral aspect of cells in proximal tubule cysts of Cy/+ and Cy/Cy kidneys. Nuclei were stained with DAPI (blue) to indicate individual cells in the merged images. 

**B:** in 21-day-old Cy/+ and Cy/Cy kidneys, SamCystin (red) was diffusely distributed throughout the cystosol and basolateral aspects of cystic epithelial cells. C: as with earlier time points, SamCystin localized to the luminal surface of proximal tubules of kidneys of 84-day-old +/+ rats. Cellular distribution of SamCystin in 84-day-old Cy/+ kidneys was consistent with a loss of specific staining at the brush border. Incubation of the antibody with excess competing peptide eliminated SamCystin staining demonstrating antibody specificity (data not shown).
(R823W) was unable to interact with wild-type SamCystin. Thus, the SAM domain may be necessary for SamCystin to interact with other SAM domain-containing proteins and signaling molecules within a multiprotein complex to regulate the development and maintenance of proximal tubules.

In the current study, we found that SamCystin was expressed in the early postnatal kidney during the final stages of kidney development and that the levels of SamCystin decline between 7 and 45 days in +/+ rats (Fig. 2A). The expression was prominent in proximal tubules (Fig. 4), consistent with a previous report (3), and was localized to the brush border. These data support the hypothesis that SamCystin is involved in the development of the proximal tubule. In contrast to its expression in normal rat kidneys, SamCystin expression in both Cy/+ and Cy/Cy rat kidneys was decreased at 3 and 7 days, but increased 27 and 68%, respectively, at 21 days compared with age-matched +/+ kidneys (Fig. 2). In Cy/+ rats, SamCystin increased even further between 21 and 84 days (Fig. 2). The persistent expression of SamCystin corresponds to the appearance of cysts in the Cy/+ rats and to the extensive cystic growth and kidney enlargement in Cy/Cy rats. Overexpression of SamCystin in the Cy/+ and Cy/Cy rats was accompanied by a loss of specific localization of SamCystin to the brush border in cyst-lining epithelial cells (Figs. 4, 5, and 6), suggesting that the Cy mutation in the SAM domain is associated with improper localization of the protein.

The mechanism by which the Cy mutation and abnormal cellular expression of SamCystin initiate cyst formation in Cy rats remains undefined. On one hand, the Cy mutation may cause the expression of a nonfunctional protein that mislocalizes within the cystosol, and it is the insufficient expression of normal SamCystin due to this haploinsufficiency that initiates cyst formation. SamCystin protein levels are reduced at 3 and 7 days in the Cy/+ kidney, a time of cyst formation. Thus, insufficient expression of normal SamCystin due to the mutation in one allele of Ank6 (Pkdr1) could be the initiating factor for cyst formation. On the other hand, protein levels are elevated in both 21-day-old Cy/Cy and Cy/+ rats, suggesting that elevated expression of mutant SamCystin promotes cyst formation. SamCystin localization in 21-day-old Cy/Cy kidneys, which express only mutant SamCystin, shows the same protein mislocalization as Cy/+ kidneys at 21, 84, and 180 days, suggesting that mutant SamCystin has a dominant negative effect, by preventing distribution of normal SamCystin to the brush border of the proximal tubule. In either case, we speculate that SamCystin interacts with signaling pathways involved in the regulation of cell proliferation and differentiation and...
that expression of the mutant protein disrupts the SamCystin protein complex, leading to cyst formation.

Cy/+ rats have increased renal activity of the B-Raf/MEK/ERK signaling pathway and an increased number of proliferating cells within the kidneys (15). Cysts with higher SamCystin expression appear to have increased number of PCNA-positive cells (Fig. 5). Several studies have demonstrated that cAMP agonists, including arginine vasopressin, stimulate the Raf/MEK/ERK pathway and accelerate the proliferation of cyst epithelial cells (5, 7, 14, 22, 25, 26, 28–32). Aberrant regulation of steady-state intracellular Ca2+ seems to be an important determinant of the mitogenic response to cAMP through the regulation of B-Raf, a target of protein kinase A phosphorylation (30, 32). Treatment of Cy/+ rats with VP, a phenylalkylamine L-type Ca2+ channel blocker, caused a further increase in ERK phosphorylation and cell proliferation and accelerated PKD progression (15). Here, we show that accelerated disease progression in VP-treated Cy/+ rats was associated with a further elevation in the expression and mislocalization of SamCystin in cyst epithelial cells. Thus, the elevation of SamCystin in Cy/+ and Cy/Cy genotypes appears to correlate with increased disease severity.

The ANK motifs of SamCystin may also participate in the binding of SamCystin to other protein complexes. ANK motifs are present in several ion channels including transient receptor potential (TRP) channels and Ca2+-activated Ca2+ release channels.

Fig. 6. Effect of verapamil (VP) on expression and distribution of SamCystin in +/+ and Cy/+ rat kidneys. Rats were treated with vehicle (Control; A, D) or 20 mg/kg VP (B, E) twice daily from 5 to 12 wk of age. Representative kidney sections were stained for SamCystin (brown color) and counterstained with hematoxylin and eosin. Kidney section from 180-day-old untreated +/+ rats (C) and Cy/+ rats (F). SamCystin staining was very weak in +/+ kidneys of 84- and 180-day-old rats (A–C), consistent a reduction in overall SamCystin expression. B: VP had no effect on SamCystin expression in +/+ kidneys. In contrast to the normal kidney, there was persistent expression of SamCystin in the Cy/+ kidney at 84 days of age (D) and VP treatment caused a further increase in SamCystin expression and mislocalization, similar to its staining pattern in kidneys of an 180-day-old Cy/+ rat (F).

Fig. 7. Effect of VP on SamCystin protein levels in the kidneys of +/+ and Cy/+ rats. Western blot analysis shows that there was increased expression of SamCystin in vehicle (CONT)-treated Cy/+ kidneys at 84 days of age compared with age-matched +/+ kidneys and that VP treatment caused a further increase in its expression. By contrast, VP treatment had no effect of SamCystin expression in +/+ kidneys. **P < 0.001, compared with CONT-treated +/+ kidneys. #P < 0.01, compared with CONT-treated Cy/+ kidneys. †P < 0.001, compared with VP-treated +/+ kidneys.
channels in the membrane of the endoplasmic reticulum. The presence of ANK domains in TRP channels suggests that these ion channel proteins functionally interact with other signaling molecules. Polycystin-2 is a member of the TRP superfamily and is thought to function as a Ca\(^{2+}\) release channel (9, 11).

Recently, stromal interaction molecule 1 (STIM1) was identified as an ER-associated Ca\(^{2+}\) sensor that activates a store-operated Ca\(^{2+}\) entry pathway in response to depletion of Ca\(^{2+}\) stores (33). STIM-1 contains a Ca\(^{2+}\)-binding EF-SAM, a protein complex due to point mutation in the SAM domain of SamCystin promotes ERK activation, cell proliferation, and protein complex consisting of an EF-hand motif and a SAM module that is thought to be responsible for sensing changes in local Ca\(^{2+}\) levels (17, 24). It is possible that SamCystin interacts with protein complexes involved in intracellular Ca\(^{2+}\) regulation and that the changes in expression and/or localization of mutant SamCystin in the kidneys of Cy rats disrupt intracellular Ca\(^{2+}\) regulation in renal cells causing initiation of cyst formation. Clearly, additional studies are required to identify the binding partners for SamCystin and how disruption of a protein complex due to point mutation in the SAM domain of SamCystin promotes ERK activation, cell proliferation, and cyst formation.

In conclusion, a point mutation in Anks6 that causes an R823W substitution in the SAM domain of SamCystin leads to aberrant expression and mislocalization of SamCystin in cyst epithelial cells of Cy/+ and Cy/Cy rats. The Cy mutation may disrupt the interaction of SamCystin with other signaling molecules and secondarily increase SamCystin expression as a compensatory response to the loss-of-functional protein. This could then give rise to accumulation of defective SamCystin protein and a dominant-negative response leading to cellular dedifferentiation and cyst formation. Treatment with VP, an L-type Ca\(^{2+}\) channel blocker, which increases the activity of the MEK/ERK signaling pathway and accelerates renal cyst growth, caused a corresponding increase in SamCystin expression within cyst-lining cells. We propose that SamCystin is a molecular scaffold and part of a multiprotein complex involved in the regulation of a cellular function important for the proper control of cell proliferation and/or differentiation. Identification of key molecules involved in protein-protein interactions with SamCystin may elucidate novel cellular processes involved in cyst formation and may have important implications for therapeutic approaches to target renal cyst formation in human PKD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


