A mouse model of Townes-Brocks syndrome expressing a truncated mutant Sall1 protein is protected from acute kidney injury

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Hirsch S, El-Achkar T, Robbins L, Basta J, Heitmeier M, Nishinakamura R, Rauchman M. A mouse model of Townes-Brocks syndrome expressing a truncated mutant Sall1 protein is protected from acute kidney injury. Am J Physiol Renal Physiol 309: F852–F863, 2015. First published August 26, 2015; doi:10.1152/ajprenal.00222.2015.—It has been postulated that developmental pathways are reutilized during repair and regeneration after injury, but functional analysis of many genes required for kidney formation has not been performed in the adult organ. Mutations in SALL1 cause Townes-Brocks syndrome (TBS) and nonsyndromic congenital anomalies of the kidney and urinary tract, both of which lead to childhood kidney failure. Sall1 is a transcriptional regulator that is expressed in renal progenitor cells and developing nephrons in the embryo. However, its role in the adult kidney has not been investigated. Using a mouse model of TBS (Sall1TBS), we investigated the role of Sall1 in response to acute kidney injury. Our studies revealed that Sall1 is expressed in terminally differentiated renal epithelia, including the S3 segment of the proximal tubule, in the mature kidney. Sall1TBS mice exhibited significant protection from ischemia-reperfusion injury and aristolochic acid-induced nephrotoxicity. This protection from acute injury is seen despite the presence of slowly progressive chronic kidney disease in Sall1TBS mice. Mice containing null alleles of Sall1 are not protected from acute kidney injury, indicating that expression of a truncated mutant protein from the Sall1TBS allele, while causative of congenital anomalies, protects the adult kidney from injury. Our studies further revealed that basal levels of the preconditioning factor heme oxygenase-1 are elevated in Sall1TBS kidneys, suggesting a mechanism for the relative resistance to injury in this model. Together, these studies establish a functional role for Sall1 in the response of the adult kidney to acute injury.

acute kidney injury; Sall1; Townes-Brocks syndrome; renal hypoplasia; nephrotoxicity

EVERY YEAR, ~17 MILLION HOSPITALIZED Americans are treated for acute kidney injury (AKI) (6), with an associated mortality rate as high as 52–80% (3, 7). Patients that survive AKI have significant associated morbidity and an increase in 30-day mortality after hospital discharge (4, 6, 36, 50). In the longer term, any incidence of clinically detectable AKI increases the risk for progressive chronic kidney disease (CKD) (6). Despite the high incidence and complications of AKI, treatment options remain largely supportive due to a lack of understanding of the mechanisms governing repair and recovery from AKI.

Sall1 is a transcriptional regulator that is essential for kidney development (33). Truncating (nonsense) mutations in SALL1 cause Townes-Brock syndrome (TBS; OMIM 107480), an autosomal dominant congenital disorder that is associated with defects in multiple organs, including the kidney (21). Missense mutations in SALL1 contribute to a relatively high percentage of nonsyndromic congenital anomalies of the kidney and urinary tract, a common cause of childhood kidney failure (16, 48). These findings highlight the importance of SALL1 in human kidney development and disease.

Studies in mouse mutants have demonstrated that Sall1 is required to control the balance between self-renewal and differentiation of nephron progenitors during formation of the kidney (2, 18). It has been postulated that developmental pathways are reutilized in regeneration of adult tissues. However, the role of tissue-restricted transcription factors such as Sall1 in normal cell turnover and in repair/regeneration after injury of differentiated renal epithelia is not well understood. In this study, we investigated the role of Sall1 in the adult kidney in a basal state and in response to acute injury. Our data reveal that Sall1 is expressed in renal tubular epithelia and plays an important role in the protective response to ischemic and toxic insults to the kidney.

METHODS

Mouse strains. Sall1TBS, Sall1GFP, Sall1H9252, and β-actin-Cre:ER mouse strains have been previously described (14, 19, 42, 53). The genomic structure of these three Sall1 mutant alleles and their protein products are shown in Fig. 1. Sall1TBS and Sall1GFP mice used in this study are heterozygous mutants. Homozygous mutants of both these strains die in the early postnatal period due to kidney failure as a result of severe renal hypoplasia or agenesis. Sall1TBS mice produce a truncated Sall1 N-terminal protein which mimics that found in TBS; Sall1GFP is a null allele that produces green fluorescent protein (GFP) under the control of the Sall1 genomic locus. Sall1H9252 mice were generated by crossing homozygous Sall1H9252 mice to β-actin-Cre:ER, Sall1H9252 mice, yielding Sall1H9252 β-actin-Cre:ER+/- mice. Lactating mothers were administered 5 mg/kg tamoxifen in corn oil by intraperitoneal injection at postnatal day 1 and 2 to activate Cre. Cre-mediated deletion resulted in homozygous postnatal deletion of Sall1 exon 1, thereby creating a null allele; we refer to this allele as Sall1H9252 mice. Deletion of Sall1 was confirmed by quantitative (q) PCR and immunostaining.

AKI. All animal studies were approved by the Veterans Affairs St. Louis Health Care System Institutional Animal Care and Use Committee (IACUC) and conducted in conformity with the Guiding Principles for Research Involving Animals and Human Beings. Renal ischemia-reperfusion injury (IRI) was performed as previously described (11). Eight-week-old male Sall1 mutant mice and wild-type

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littermates were anesthetized with 2–5% inhaled isoflurane and placed on a homeothermic table to maintain core body temperature at 37°C. The renal pedicles were exposed by midline incision, and renal ischemia was induced bilaterally with microvascular clamps for 30 (severe IRI) or 22 min (moderate IRI). Following ischemia, microvascular clamps were removed, and the change in kidney color was noted to confirm ischemia and reperfusion. Incisions were closed with two layers of sutures, and the mice were administered a 1-1 injection of normal saline by intraperitoneal injection, and 0.1 mg/kg buprenorphine, subcutaneously. Sham-operated controls were subjected to an identical procedure without induction of ischemia.

For nephrotoxic injury, 10- to 15-day-old male mice received a single intraperitoneal injection of 5 mg/kg aristolochic acid (AA) in PBS. Vehicle control mice were injected with an equivalent volume of PBS.

Kidney function. Whole blood was obtained by maxillary vein puncture before (day 0, baseline) and after intervention [24 h (day 1) for IRI and 5 days for AA; 10 min to separate the cellular components from the serum. Serum creatinine was measured by HPLC/tandem mass spectrometry performed by the Yale mouse metabolic phenotyping center where indicated or by capillary electrophoresis at the University of Texas Southwestern George M. O’Brien Kidney Research Core. Urine was collected from 10-mo-old mice at a single time point. Urinary albumin and creatinine were measured using a commercial ELISA (Exocell) kit.

Histology. At time of death, mice were perfused with PBS followed by 4% paraformaldehyde (PFA). Right kidneys were collected and fixed with 4% PFA overnight, then dehydrated and embedded in paraffin. Paraffin-embedded sections (Washington University, St. Louis Developmental Biology Histology Core) were stained by periodic acid-Schiff (PAS).

Ten ×400 magnification images of the outer medulla (IRI) or cortex (AA) were captured for each animal. Images were coded to facilitate blinded histology scoring. Injury was then assessed by quantifying the number of surviving and necrotic proximal tubules in each micrograph (10).

Immunofluorescence and Western blot analysis. Eight-week-old male ICR mice were euthanized and perfused fixed with 4% PFA. Bisected kidneys were then fixed in 4% PFA overnight, dehydrated, and paraffin embedded (Washington University Developmental Biology Histology Core). Four-micrometer sections were rehydrated and subjected to antigen retrieval in 10 mM sodium citrate, pH 6.0, in a rice cooker near 100°C for 1 h. Sall1 was detected independently using antibodies to two different Sall1 epitopes [a mouse monoclonal, (1:200, Vector) or biotinylated Dolichos biflorus agglutinin (DBA; 1:220, Vector). Antibody reactivity was detected using ImmPRESS HRP polymer detection reagent followed by Tyramide-Alexa 488 (1:200, Invitrogen). LTL and DBA were detected using streptavidin-TxRed (1:200, Vector). Slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted in Mowiol (Polysciences). To control for nonspecific antibody binding, sections were incubated without primary antibody.

Kidneys isolated from 8- to 9-wk-old male wild-type and Sall1 TREY+- mice were mechanically dissociated in 5 ml of 0.5% trypsin, 0.5% collagenase in PBS for 45 min at 37°C. Cells were passed through a 40-µm filter and pelleted by centrifugation. Cell pellets were resuspended in hypotonic buffer (20 mM Tris, pH 7.4; 10 mM NaCl; 3 mM MgCl2) and incubated on ice for 15 min, after which 0.5% NP-40 detergent was added. The lysate was centrifuged, and the pellet was resuspended in complete cell extraction buffer (100 mM Tris, pH 7.4, 100 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 0.1% SDS), incubated on ice for 30 min with vortexing every 10 min, then centrifuged for 30 min at 14,000 g. Fifteen micrograms of lysate was separated in a 10% SDS-PAGE gel, and heme oxygenase-1 (HO-1)
was detected by Western blotting (ab52947, Abcam). Western blot quantification was performed by densitometry using ImageJ software.

**Proliferation and apoptosis.** Proliferation was measured by the incorporation of bromodeoxyuridine (BrDU) into proliferating nuclei. Three hours before death, mice were administered 100 mg/kg BrDU by intraperitoneal injection. BrDU incorporation was visualized by indirect immunofluorescence. Frozen sections were heated to 100°C in 10 mM sodium citrate, pH 6.0, before incubation with a monoclonal antibody to BrDU (MAB3424, Millipore). Apoptosis was measured by terminal transferase-dUTP-nick-end labeling (TUNEL) assay per the manufacturer’s instructions (Millipore).

For each animal, the number of BrDU- or TUNEL-positive cells per high-powered field was counted in 10 ×400 magnification images of the outer medulla. Data were expressed as the average number of positive tubule cells per high-powered field.

**Quantitative real-time PCR.** Total RNA was isolated from injured kidneys, and cDNA was synthesized as described (2). Sequence-specific primers were designed using Primer Express 3.0 (Applied Biosystems) and NCBI Primer-BLAST software (52). Each experiment was performed with three technical replicates and a minimum of three biological replicates. Error bars represent SE for the biological replicates. Relative expression was calculated using the ΔΔCt method (29) with normalization of all samples to GAPDH.

The following primer pairs were used for qPCR analysis: Gapdh (5’-TGTTACGGGCACAGTGATCTGA-3’ and 5’-TTGTGAGTGAATGGCGAGG-3’); Kim-1 (5’-CATTTCACCCCTCTGTC-3’ and 5’-ACAAGCAGAAGATGGGCGCT-3’); Ngal (5’-TGGTGAGGGACAGCAG-3’); Adonal (5’-CAAGATCCCTCTCCGTACA-3’ and 5’-GGCTGGTTTCCACCTCCTCACTCA-3’); Cox2 (Ptgs2; 5’-TGGATTACCGGAAAGGCCTCTTCT-3’ and 5’-CAGGACACACACCTCGTTGACAC-3’); Hspa1a (5’-GACCCCAAAAGATGAAGGGCT-3’); Hmox-1 (5’-CXCL1-3’ and 5’-AGAATTGAGCGCAGCAC-3’). WT TBS cKO

**Fig. 2. Sall1TBS (TBS) mice develop chronic kidney disease.** A and B: kidney weight, adjusted for body weight, is significantly reduced in Sall1TBS mice at 2 mo of age. C: Km-l and Ngal mRNA expression in 2-mo-old kidneys was measured by qRT-PCR. Expression of these biomarkers of kidney injury was elevated in Sall1TBS mice compared with wild-type (WT) controls. D: graph showing average values for serum creatinine (sCr) for 2-mo-old Sall1 mutant mice and WT littermates. GFP-Sall1TBS heterozygous mice, a model of haploinsufficiency (n = 18); TBS-Sall1TBS heterozygous mice, a dominant allele which is a model of 18); TBS-Sall1TBS homozygous mice in which Sall1 was deleted postnaturally to generate Sall1cKO, a model of complete Sall1 loss of function (n = 14). The degree of sCr elevation correlates with the expected functional reduction in Sall1 gene dose. E: sCr was measured in WT and Sall1TBS mice at 6 and 18 mo of age. The sCr of Sall1TBS mice was elevated compared with WT littermates at both time points. *P < 0.05, **P < 0.01, ***P < 0.001.
II6 (5'-CTCTGGAAGAGACTCCATCC-3' and 5'-TGAAGTCTCTCCTCCGGACT-3'); Tnfa (5'-GATCGGTCCCCAAGGGATG-3' and 5'-TGGTTTGTGATGTAGGGTC-3'); and Ifng (5'-GGAGGAACTGGCAAAAGATG-3' and 5'-GTTGCTGATGGCCTGATTG-3').

Statistical analysis. All statistical analyses were performed using a paired 2-tailed t-test, with significance determined at the P < 0.05 level.

RESULTS

Sall1TBS mutant mice exhibit CKD. We previously demonstrated that a mouse model of TBS (Sall1TBS) recapitulates the autosomal dominant human phenotypes, with renal hypoplasia apparent in mid- to late gestation embryos (19). Studies in rodent models and human epidemiological studies strongly support the conclusion that a congenital nephron deficit predisposes to CKD (30). Because Sall1TBS mice exhibit congenital renal hypoplasia, we examined these mice at several postnatal time points to determine whether they developed CKD. We found that kidney weights adjusted for body weights were reduced in 2-mo-old Sall1TBS heterozygous mice compared with wild-type littermate controls (Fig. 2, A and B). This was accompanied by a 29% higher serum creatinine (sCr) (P < 0.01, Fig. 1D), and a significant increase in albumin excretion (25.5 ± 2.3 vs. 17.4 ± 1.6 μg/mg Cr, P = 0.011, n = 8) in Sall1TBS heterozygotes. However, because body weight was reduced in the Sall1TBS mice compared with wild-type animals (Fig. 2A), the reduction in estimated GFR in Sall1TBS may be greater than suggested by the sCr. Sall1TBS mice also exhibited twofold increases in Kim1 and Ngal mRNA at 2 mo of age (Fig. 2C), suggesting ongoing chronic injury and repair.

To determine whether CKD was progressive, we examined ageing mice. The sCr in Sall1TBS mice was 52% (P = 0.019, n = 4) higher than wild-type littermates in 6-mo-old animals (Fig. 2E). At 18 mo of age, sCr remained elevated in Sall1TBS mice at a level that was comparable to 6-mo-old mice. Albu-

![Image](https://www.ajprenal.org/fig3.png)

Fig. 3. Sall1 expression in the adult kidney. A: Sall1 mRNA was detected in the adult kidney by qRT-PCR. The level of expression was significantly lower (~12%) than in the embryonic day (E) 13 kidney. B: immunofluorescence shows Sall1 is expressed in the tubular epithelium of the nephron. Sall1 is expressed in the proximal tubule as indicated by Lotus tetragonolobus lectin (LTL) costaining, but is excluded from the Dolichos biflorus agglutinin (DBA)-positive collecting duct. No significant staining is detected in kidneys in which both Sall1 alleles were conditionally deleted in the postnatal period (cKO).

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min excretion was increased in ageing Sall1TBS compared with wild-type controls at 10 mo (15.0 ± 1.8 vs. 10.7 ± 1.5 μg/mg Cr, P < 0.05, n = 9), an increase that was similar to that observed in 2-mo-old mice. Together, these results indicate that Sall1TBS mice have slowly progressive CKD.

Sall1 is expressed in the tubular epithelium of the adult kidney. While the nephron deficit present at birth in Sall1TBS could account for the development of CKD, it is also possible that Sall1 has a role in maintaining renal tubular epithelia after nephron formation has ceased. To address this possibility, we examined Sall1 expression in adult kidneys. At 8 wk of age, Sall1 mRNA was detected by qRT-PCR in the adult kidney, but at levels that are significantly less (~12%) than in the embryonic kidney (Fig. 3A). To define the localization of Sall1 in the kidney, we performed immunofluorescence microscopy (IF). In the cortex and outer medulla, nuclear Sall1 staining was observed in LTL-positive proximal tubules (Fig. 3B). Sall1 was also expressed in LTL-negative tubules, but excluded from DBA-positive collecting ducts. This pattern of expression corresponds with the developmental expression of Sall1 in nephron progenitor cells, which give rise to all tubular segments of the kidney except the collecting ducts (2). The specificity of IF for Sall1 was confirmed in kidneys in which Sall1 was deleted (Fig. 3B). We utilized a Sall1 conditional allele (Sall1fllox) (53) to bypass the requirement of Sall1 for kidney development. We deleted Sall1 in the early postnatal period using a tamoxifen-inducible Cre to generate adult mice with homozygous deficiency of Sall1 (Sall1cKO). In Sall1cKO kidneys, expression of Sall1 was rarely detected in tubular epithelia, validating the staining in wild-type tissues. We thus conclude that Sall1 expression is maintained in tubular epithelia of the mature kidney in a pattern consistent with its embryonic expression. This finding raised the possibility that Sall1 has a role in the mature adult kidney.

TBS mice are protected from IRI. It has been postulated that reactivation of developmental control genes, such as Sall1, in the adult kidney participate in repair and regeneration of tubular epithelium after injury (8, 44). Moreover,
CKD, even at the relatively mild-to-moderate degree found in \textit{Sall1}TBS, is a risk factor for AKI (49). We thus hypothesized that \textit{Sall1}TBS heterozygous mice would have an increased susceptibility to AKI.

To test this idea, we subjected \textit{Sall1}TBS mice to moderate (22 min) and severe (30 min) ischemia followed by reperfusion (IRI). We quantified proximal tubule necrosis in control and mutant mice (Fig. 4, \textit{A} and \textit{B}). While there was morphological evidence of tubular necrosis in \textit{Sall1}TBS mice, this was significantly less than in wild-type littermates (51 vs. 72\%, \(P = 0.016\), Fig. 4\textit{B}). Consistent with the morphological analysis, wild-type mice showed a significant (~3.1-fold) increase in sCr at 24 h after moderate IRI (Fig. 4\textit{C}). In contrast, the sCr of \textit{Sall1}TBS mice did not significantly increase after moderate injury compared with the baseline value before IRI surgery. Induction of \textit{Kim-1} and \textit{Ngal} mRNA expression was significantly (5- to 6-fold) greater in wild-type controls compared with \textit{Sall1}TBS mice, consistent with the sCr and morphological analyses showing reduced injury in \textit{Sall1}TBS heterozygotes. Together, these data demonstrate that \textit{Sall1}TBS mice, a model of human TBS and congenital renal hypoplasia, are protected from IRI.

To further evaluate the ability of the \textit{Sall1}TBS allele to protect the kidney from AKI, we tested the response to severe (30 min) ischemia. Severe IRI resulted in a >10-fold increase in sCr in wild-type control mice and 5/6 deaths at days 5–7 after IRI (Fig. 4, \textit{F} and \textit{G}). In \textit{Sall1}TBS mice, sCr increased to a significantly lesser degree (4.2- vs. 11.8-fold, \(P = 0.011\)), and they exhibited an increased likelihood of survival after severe IRI (1/6 deaths). Thus protection from IRI in \textit{Sall1}TBS mice is dose responsive.

In addition to necrosis, apoptosis contributes to loss of tubular epithelial cells after IRI (26, 35). \textit{Sall1}TBS mice subjected to moderate IRI had a 48\% reduction in TUNEL-positive cells compared with controls (11.5 vs. 22 per HPF, \(P < 0.001\), Fig. 5, \textit{A} and \textit{B}). Surviving epithelial cells proliferate as part of the regenerative process of the kidney, a response that is already evident 24–48 h after IRI. Although sCr did not significantly increase in \textit{Sall1}TBS mice after moderate injury, there was proximal tubule necrosis and biomarker (\textit{Kim-1}, \textit{Ngal}) evidence of injury (Fig. 4, \textit{A}–\textit{E}). Consistent with this observation, we observed increased cell proliferation in injured compared with sham-operated \textit{Sall1}TBS mice. However, the proliferative response was significantly greater in wild-type mice after IRI, as would be predicted due to the more severe injury (48 vs. 38 per HPF, \(P < 0.001\), Fig. 5, \textit{C} and \textit{D}).

\textit{TBS} mice are protected from nephrotoxic injury. In addition to IRI, nephrotoxic injury to renal tubules is a common cause of AKI. To determine whether \textit{Sall1}TBS mice are protected from nephrotoxic injury, we exposed animals to AA, a chem-

![Fig. 5. Apoptosis and cell proliferation in response to IRI. Mice were subjected to moderate (22 min) IRI followed by 24 h of recovery. \textit{A}: cells undergoing apoptosis (red) in WT and \textit{Sall1}TBS (TBS) mice were detected by terminal transferase-dUTP-nick-end labeling (TUNEL) staining. Green represents autofluorescence of renal tubules. \textit{B}: quantitation of TUNEL-positive cells (\(n = 3\)). HPF, high-powered field. \textit{C}: immunostaining for bromodeoxyuridine incorporation (BrdU; red) to detect proliferating cells after IRI in WT and \textit{Sall1}TBS (TBS) kidneys. \textit{D}: quantitation of BrdU-positive cells (\(n = 3\)). ***\(P < 0.001\).](http://ajprenal.physiology.org/)
ical that induces severe AKI in mice and humans (51). Similar
to moderate IRI, sCr was not significantly increased in Sall1TBS
mice after AA exposure, while in wild-type mice Scr increased
4.5-fold (Fig. 6A). Morphological analysis showed less tubular
necrosis in Sall1TBS compared with wild-type mice (13 vs.
57%, P < 0.001, Fig. 6, B and C), indicating protection from
nephrotoxic injury.

Protection of TBS mice is not accounted for by Sall1 loss of
function. TBS phenotypes result from truncating SALL1 mutations
that lead to expression of a mutant protein with dominant
effects (13, 19, 20). Homozygous loss-of-function mutations in
SALL1 result in severe renal hypoplasia in mice and humans (2,
45). To determine whether Sall1 loss of function is responsible
for protection from insults that cause AKI, we utilized null and
conditional alleles of Sall1. Sall1GFP is a germine null allele in
which GFP is knocked in to the Sall1 genomic locus (42). At
2 mo of age, sCr was elevated in Sall1GFP mice compared with wild-type littersmates, but to a significantly lesser degree than in
age matched Sall1TBS mice (14 vs. 29%, Fig. 2D). We sub-
jected Sall1GFP heterozygous mice to moderate IRI. We found
that sCr and proximal tubule necrosis were not significantly
different in Sall1GFP heterozygous mice compared with wild-
type controls (Fig. 7, A–C), indicating that Sall1 haploinsuf-
ficiency does not account for protection from IRI. Similarly,
these mice were not protected from AA-induced nephrotoxic
injury. The percent tubular necrosis in Sall1GFP mice (60.6.3 ±
8.8, n = 5) was similar to the degree of injury noted in wild-type littersmates (61 ± 8.7, n = 5). Consistent with the
injury data, there was not a significant difference in sCr
between wild-type and Sall1GFP mice 5 days after exposure to
AA (5.1- vs. GFP 4.7-fold compared with pretreatment sCr, 
P = 0.49, n = 5).

The truncated protein expressed in TBS has the capacity to
act as a dominant negative by dimerization with wild-type
Sall1 protein (19, 41). Thus it is possible that >50% reduction
in Sall1 function is required for protection from IRI. To test
this idea, we utilized a Sall1 conditional allele (Sall1homo) to
generate adult mice with homozygous deficiency of Sall1
(Sall1KO, Figs. 1 and 3B). Sall1KO kidneys were significantly
smaller (0.56 ± 0.04 vs. 0.82 ± 0.04 mg kidney/g body wt
ratio, P < 0.001), and at baseline these mice had a significantly
elevated sCr at 2 mo of age compared with wild-type litter-
mates (Fig. 2D). However, similar to Sall1GFP, homozygous
postnatal deletion of Sall1 did not confer protection after
moderate IRI (Fig. 7, D–F). Together, these results indicate
that Sall1 loss of function does not account for protection of
Sall1TBS mice from acute injury to the kidney.

The HO-1 preconditioning pathway is induced in TBS mice.
Because the protection from ischemic and nephrotoxic insults in
Sall1TBS mice was so dramatic and occurred within 24 h
after IRI, we hypothesized that basal gene expression changes
in the uninjured state in this Sall1 allele may mediate protec-
tion. Transcriptional profiling of nephron progenitor cells from
embryonic Sall1 mutant kidneys identified several misregu-
lated genes that have been implicated in ischemic precondition-
ing (2, 15, 27, 31, 46). We reasoned that some of these

Fig. 6. Sall1TBS (TBS) mice are protected from nephrotoxic injury. WT and Sall1TBS (TBS) mice were analyzed 5 days after intraperitoneal injection of
aristolochic acid (AA) or vehicle control (n = 4). A: sCr measurements in WT and Sall1TBS (TBS) mice exposed to AA or vehicle. B: quantitation of proximal
tubule (PT) necrosis after AA exposure. C: PAS staining of kidneys to demonstrate the degree of morphological injury after AA injection in WT and Sall1TBS
(TBS) mice. Day 0 values were obtained just before injection of AA, day 5 values after AA injection. ns. Not significant. ***P < 0.001.
Fig. 7. Sall1 loss of function is not protective in IRI. We subjected Sall1^GFP (GFP) and Sall1^cKO (cKO), 2 different Sall1 mutant alleles, to moderate (22 min) IRI followed by 24 h of recovery. A–C. Sall1^GFP (GFP) heterozygous mice are a model of haploinsufficiency for Sall1. D–F. Sall1^cKO (cKO) mice had both copies of Sall1 deleted in the postnatal period using Sall1^flx mice and an inducible βAct-Cre transgene. A and D: PAS staining of Sall1^GFP (A) and Sall1^cKO (D) kidneys subjected to IRI compared with WT controls. B and E: quantitation of PT necrosis in Sall1^GFP (B) and Sall1^cKO (E) kidneys (n = 4 for each genotype). C and F: sCr measurements in Sall1^GFP (C) and Sall1^cKO (F). Comparisons in B, C, E, and F between WT and Sall1 mutants were not statistically significant. Day 0 values were obtained just before IRI, day 1 at 24 h after IRI.
genes would have altered expression in adult Sall1 mutant kidneys and be potential candidates for preconditioning factors. Among these candidates, Heme oxygenase-1 (Hmox-1) mRNA expression in the uninjured kidney was increased 2.3 ± 0.4-fold in Sall1TBS mice compared with age-matched control littermates (Fig. 8A). We confirmed the upregulation of Hmox-1 protein by Western blotting (Fig. 8B). These data suggest that protection of Sall1TBS mice from acute injury could be due to increased expression of Hmox-1, a preconditioning factor in kidney and other tissues.

HO-1 induction often occurs as a result of cellular stress, such as inflammation (31). To determine whether Sall1TBS kidneys could be due to increased expression of Hmox-1 (Fig. 8C). However, Il1b, also which modulate innate immunity, did not exhibit altered expression. Expression of inducible nitric oxide synthase (iNos), typically upregulated by oxidative stress, was also not increased in Sall1TBS kidneys (Fig. 8A). Together, these results suggest that HO-1 induction in Sall1TBS is not likely due to chronic inflammatory or oxidative stress.

DISCUSSION

The data presented here identify an important functional role for Sall1 after the kidney has formed. In the adult kidney, we found that Sall1 expression was maintained in renal tubular epithelia derived from multipotent Sall1-positive nephron progenitors, but not in collecting ducts derived from Sall1-negative ureteric buds. Analysis of Sall1TBS mice from ages 2–18 mo revealed elevated sCr and an increase in albumin excretion and injury biomarkers, providing evidence of CKD. At 2 mo of age, sCr was increased ~30% in Sall1TBS, while in Sall1GFP heterozygotes the increase in sCr (14%) was significantly less (Fig. 2D). Sall1TBS is a dominant allele, which expresses a truncated protein that interferes with wild-type Sall1 function (19, 38), while Sall1GFP is a model of haploin sufficiency (34). Thus the difference between estimated GFR in these two genetic models suggests that a >50% reduction in Sall1 activity is required to produce both significant renal hypoplasia and CKD. Consistent with this idea, when both copies of Sall1 (Sall1cKO) were deleted in the postnatal period (Fig. 3), sCr was increased ~80% at 2 mo of age, compared with the 30% increase in Sall1TBS (Figs. 2D, 7F). Together, these findings suggest a dose-dependent response to the level of wild-type Sall1 protein in the overall function and maintenance of the kidney.

Sall1TBS and Sall1cKO mutants exhibit renal hypoplasia due to impaired nephrogenesis (17, 20). Reduced nephron number at birth is an established risk factor for development of CKD (30). Moreover, the degree of reduced kidney weight adjusted for body weight (15% for Sall1TBS vs. 31% for Sall1cKO) also correlated with the severity of CKD in these two different Sall1 mutant alleles, as assessed by sCr. A significant proportion of patients with TBS due to dominant mutations in SALL1 develop CKD, with progression to end-stage kidney disease (9, 32, 39). Based on these observations, we conclude that congenitally reduced nephron number contributes to reduced glo-
merular filtration in adult Sall1 mutant mice. However, our studies demonstrate a functional role for Sall1 in acute kidney injury, suggesting that in addition to nephrogenesis, Sall1 also has a role in the mature kidney in maintaining the integrity of renal tubular epithelia. In support of this idea is the finding that sCr is modestly increased in Sall1<sup>GFP</sup> mice in which overall kidney size is not different from wild-type littermates (Ref. 2 and Rauchman M and Basta J, unpublished observations). While we cannot exclude a modest reduction in nephron number in Sall1<sup>GFP</sup>, it is likely that the significantly elevated sCr in these mice reflects a role for Sall1 in mature renal tubular epithelia.

In response to acute injury, renal tubular epithelia dedifferentiate, proliferate, and then redifferentiate to regenerate tubular epithelia (12, 25). It has thus been hypothesized that pathways required for kidney development are reutilized for its regeneration. The Wnt and BMP signaling pathways have been shown to mediate the injury and repair responses in the kidney (22, 37, 54). While many transcriptional regulators have been identified which are essential for kidney development, their role in renal epithelial repair and regeneration is not well understood. Our studies show that a Sall1 mutant allele that is a faithful genetic model of TBS (Sall1<sup>TBS</sup>) confers significant protection from acute ischemic and nephrotoxic injury (Figs. 4 and 6). Because we did not find protection with heterozygous or homozygous loss-of-function Sall1 alleles, our studies suggest that the protective mechanism likely depends on a gain-of-function or dominant interference mechanism due to expression of a truncated mutant protein from the Sall1<sup>TBS</sup> allele.

The mutant protein encoded by the Sall1<sup>TBS</sup> allele has the potential to interfere with other Sall family members via heterodimerization (19, 41), suggesting a potential genetic mechanism for the protection from AKI. However, it is unknown whether other Sall proteins (Sall2-4) have a functional role in the adult kidney. Alternatively, the Sall1 mutant truncated protein may exert gain-of-function effects through other pathways that alter the response to injury. Our previous studies have shown that Sall1<sup>TBS</sup> heterozygous mice display significant gene expression changes (Rauchman M and Kiefer S, unpublished observations and Ref. 20). This is often manifested as derepression of Sall1 target gene expression and is thought to be mediated through an interaction between the N terminus of Sall1 and the nucleosome remodeling and decacetylase (NuRD) chromatin remodeling complex (23). We thus examined candidate Sall1 target genes identified in our studies of kidney development (2), which have also been implicated in ischemic preconditioning. These studies revealed that expression of HO-1, a well-defined preconditioning factor, was selectively upregulated in Sall1<sup>TBS</sup> mice before acute injury (Fig. 8). The degree to which we observed increased HO-1 protein expression is consistent with levels associated with protection from ischemic injury (28, 40). HO-1 has been shown to protect the kidney, heart, and central nervous system from acute ischemia (15, 27, 31, 47), indicating the general relevance of this pathway for organ injury and repair. Additional functional studies are needed to define whether elevated HO-1 levels are responsible for the protection from AKI seen in Sall1<sup>TBS</sup> mice.

In clinical studies, preexisting CKD is thought to represent a risk factor for the severity of AKI and for progression of CKD after episodes of acute injury (4, 5, 43). Surprisingly, although Sall1<sup>TBS</sup> mice have CKD, they are dramatically protected from AKI. These findings demonstrate that the link between preexisting CKD, reduced GFR, and AKI is not invariable and may depend on the pathobiology associated with specific causes of CKD. Future studies are needed to determine whether the protection from AKI in Sall1<sup>TBS</sup> mice is also associated with less fibrosis and protection from CKD progression after severe acute insults. In addition to highlighting the complex role of preexisting CKD on AKI severity, our studies also point to the variable rate of CKD progression. In the case of Sall1<sup>TBS</sup>, GFR was reduced by 8 wk of age and renal function declined further in ageing mice at 6 mo. However, we did not observe significant progression of CKD beyond 6 mo when mice were examined up to 1.5 yr of age, within the limitations of using sCr to estimate glomerular filtration rate (GFR). Thus, while Sall1<sup>TBS</sup> mice have CKD which is apparent in early adulthood, homeostatic and repair processes are able to preserve function in ageing mice in the absence of superimposed acute or chronic injury. Similar findings have been observed in clinical epidemiological studies. Examination of CKD progression over >5 years in a large Veterans Affairs cohort noted that while a subset of patients progress rapidly [>4 ml·min<sup>−1</sup>·1.73 m<sup>2</sup>·yr<sup>−1</sup> decline in estimated GFR (eGFR)], a significant proportion of patients do not exhibit a decline in eGFR over the same period of longitudinal follow-up (1). These findings underscore the need for further investigation of genetic and environmental factors that determine the rate of CKD progression, beyond the current clinical paradigm (24).

In summary, we have identified a role for Sall1, a gene essential for kidney development, in the adult kidney. We discovered that Sall1 is expressed in mature adult renal tubular epithelia, and a genetic deficiency of Sall1 leads to CKD. A dominant Sall1 allele that mimics TBS, a human congenital anomaly syndrome, confers dramatic protection from acute ischemic and toxic insults to the kidney. Future studies investigating the mechanism of protection observed in this model could reveal novel therapeutic approaches for AKI.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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