Sall1-dependent signals affect Wnt signaling and ureter tip fate to initiate kidney development

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SUMMARY
Development of the metanephric kidney depends on precise control of branching of the ureteric bud. Branching events represent terminal bifurcations that are thought to depend on unique patterns of gene expression in the tip compared with the stalk and are influenced by mesenchymal signals. The metanephric mesenchyme-derived signals that control gene expression at the ureteric bud tip are not well understood. In mouse Sall1 mutants, the ureteric bud grows out and invades the metanephric mesenchyme, but it fails to initiate branching despite tit-specific expression of Ret and Wnt11. The stalk-specific marker Wnt9b and the β-catenin downstream target Axin2 are ectopically expressed in the mutant ureteric bud tips, suggesting that upregulated canonical Wnt signaling disrupts ureter branching in this mutant. In support of this hypothesis, ureter arrest is rescued by lowering β-catenin levels in the Sall1 mutant and is phenocopied by ectopic expression of a stabilized β-catenin in the ureteric bud. Furthermore, transgenic overexpression of Wnt9b in the ureteric bud causes reduced branching in multiple founder lines. These studies indicate that Sall1-dependent signals from the metanephric mesenchyme are required to modulate ureteric bud tip Wnt patterning in order to initiate branching.

KEY WORDS: Sall1, Spalt, Ureter, Wnt9b, Kidney development, Ureteric bud, Metanephric mesenchyme, β-catenin, Wnt, Tip fate, Mouse

INTRODUCTION
A crucial step in kidney development is the interaction between the outgrown ureteric bud (ub) and the metanephric mesenchyme (mm). Reciprocal signaling triggers the ub to initiate its first T-branch and induces the mm to condense and proliferate. If these tissues fail to interact properly, renal agenesis or severe hypoplasia will occur. Despite the importance of this interaction, defining the precise molecular events that regulate these processes has been particularly difficult because the mm undergoes rapid apoptosis if it is not properly invaded (Donovan et al., 1999). Here, we describe a mouse model in which the initial invasion of the ureter has been captured and can be studied to identify factors that are crucial for this process.

The gene mutated in this mouse model is the transcription factor Sall1 – a multi-zinc-finger transcriptional regulator that is expressed in the mm (Nishinakamura et al., 2001). It is mutated in the autosomal dominant syndrome Townes Brocks (TBS) which is a multi-zinc-finger transcriptional regulator that is Sall1 – a multi-zinc-finger transcriptional regulator that is expressed in the mm. In this report, we use a Sall1 mouse model that mimics TBS (Sall1-ΔZn) and demonstrate that these mice form blind ureters with an overlying undifferentiated cap of mm (Kiefer et al., 2003). The ub grows out and invades the mm, but does not form the first T-branch. The unbranched ub and mm remain associated throughout development, thereby allowing the precise dissection of the initial events in ub morphogenesis that depend on Sall1. We show that Sall1 function in the mm triggers downregulation of the stalk-specific marker Wnt9b and the canonical Wnt downstream gene Axin2 in the outgrown ub. Early ub branching is rescued by a reduction in β-catenin dose supporting the conclusion that ureter arrest is due to altered Wnt signaling in Sall1-ΔZn mutants. This theory is further strengthened by findings that ub branching is reduced in Wnt9b transgenic lines and in a stabilized β-catenin mutant. These results demonstrate that downregulation of Wnt9b and canonical Wnt signaling in the ureter tip is required to initiate ub branching.

MATERIALS AND METHODS
Mouse strains
Sall1-ΔZn mice have been described elsewhere (Kiefer et al., 2003). Double heterozygous Sall1-ΔZn,Hoxb7GFP were mated to Sall1-ΔZn to create EGFP-labeled mutant Wolffian ducts and ureters (Srinivas et al., 1999). Sprouty1 (Spry1) mice were a gift from J. Licht (Basson et al., 2005). Reduction of β-catenin activity was achieved by using a β-catenin floxed allele (Ccnb1fl/+), Jackson Laboratories) that had been first crossed to a β-actin-cre transgenic and then to Sall1-ΔZn and Axin2-ΔZn mice. These were mated to males. These were mated to females to analyze Sall1-ΔZn embryos. The presence of β-actin-cre or Hoxb7GFP transgenes had no effect on the phenotypes observed. Activation of β-catenin was achieved by crossing Hoxb7creEGFP transgenic mice (Zhao et al., 2004) to a β-catenin exon 3 floxed allele (Ccnb1ex3flox/flox, Jackson Laboratories) (Harada et al., 1999). Conditional deletion of this exon produces a stabilized β-catenin protein in both ureter and Wolffian duct. These studies were performed under the auspices of St Louis University and St Louis VA Medical Center animal care guidelines. Noon on the day of the vaginal plug detection was considered to be E0.5.

Overexpression of Wnt9b in the ub
Transgenic mice were created to conditionally overexpress Wnt9b (ACTWnt9b). A C-terminally epitope-tagged Wnt9b (Wnt9b-FLU) was inserted into the EcoRI site of a previously described transgenic construct [a gift from Yiping Chen, Tulane University (Chen et al., 2004)]. In this construct, expression of Wnt9b-FLU is prevented by an upstream loxed

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stop cassette (Novak et al., 2000). Upon exposure to cre recombinase, the stop cassette is removed and transgenic expression is driven by the chicken β-actin promoter. Ten independent founder lines were established by the Washington University Mouse Genetics core. Five of these lines were crossed to Hoxb7creEGFP transgenic mice to overexpress Wnt9b in the ub lineage. Kidneys were photographed using a Leica DVC300FX camera and a M165FC fluorescence-enabled microscope. Photographs of all littersmates were scored by at least two individuals blind to the genotype and examined for reduced kidney size and abnormal morphology. Statistical significance was determined by chi square analysis. The ub branch tips were also counted and are reported with standard error and the statistical difference determined by t-test. Expression of Wnt9b-FLU in E13.5 embryonic kidneys was confirmed by western blotting of kidney lysates probed with anti-FLU antibody (12CA5, Roche, Indianapolis, IN, USA) and detected with anti-mouse HRP (Cell Signaling Technology, Danvers, MA, USA) as described previously (Kiefer et al., 2002).

**Histology, immunohistochemistry and immunofluorescence**

Urogenital regions were dissected at E11.0-18.5. Tissues were fixed, sectioned and stained with Hematoxylin and Eosin as described previously (Kiefer et al., 2003). Corresponding unstained slides were used for immunohistochemistry with anti-uroplakin III (1:10, Fitzgerald Industries, Concord, MA, USA) and anti-smooth muscle actin (1:100, Millipore, Billerica, MA, USA) as described previously (Mahoney et al., 2006). Immunofluorescence was performed on 10 μm frozen sections using rabbit anti-Pax2 antibody (1:2000, Covance, Princeton, NJ, USA) and rat anti-laminin B2 chain antibody (1:2000, Chemicon, Temecula, CA, USA). Antibody reactivity was detected using Alexa 488-labeled anti-rabbit (1:400, Invitrogen, Carlsbad, CA, USA) and Cy3-labeled anti-rat (1:2000, Jackson ImmunoResearch, West Grove, PA, USA), and mounted in Mowiol (Polysciences, Warrington, PA, USA) as previously described (Kiefer et al., 2002). Sections were incubated without primary antibody to control for non-specific staining of secondary antibodies.

**In situ hybridization**

Whole-mount in situ hybridization was performed using digoxigenin-labeled antisense riboprobes for Ret (nucleotides 818-1222), Pax2 (563-1248), Six2 (136-656), Cited1 (1-612), Wnt11 (61-674), Wnt9b (486-1076) and Sall1 (421-848) (Kiefer et al., 2008). In situ hybridization was performed as described previously (Little et al., 2007) on 20 μm frozen sections using a digoxigenin-labeled Gdnf riboprobe (nucleotides 122-781). After incubation with digoxigenin-alkaline phosphatase antibody (1:2500), signal was visualized using the alkaline phosphatase substrate BM purple (Roche, Indianapolis, IN, USA).

**Quantitative RT-PCR**

E11.5 wild-type or Sall1−/− mutants were dissected to isolate the ureter tips and mesenchyme from each side of the T-stage or unbranched ureter, respectively. Ten tip regions were pooled and cDNA was synthesized as described previously (Kiefer et al., 2008). Samples that lacked reverse transcriptase were prepared as negative controls. Primer sets were designed to amplify Gdnf, Pax2 and Gpc4 specifically using Primer Bank (http://pga.mgh.harvard.edu/primerbank/). cDNA (10 ng) was used for quantitative real-time PCR using SYBR green master mix (Applied Biosystems, Foster City, CA, USA) and an ABI 7300 quantitative PCR instrument (Kiefer et al., 2008). Relative levels of mRNA expression were calculated according to the ΔΔct method and normalized to the gene Rp19. Transgenic copy number was estimated using primers at the C

**RESULTS**

**Sall1−/− mice exhibit blind ureters that are morphologically normal**

Sall1−/− homozygous mice exhibit bilateral renal agenesis (Kiefer et al., 2003). It has been suggested that this phenotype is due to incomplete ureter outgrowth and to mesenchymal apoptosis in Sall1-null mice (Nishimakura et al., 2001). However, upon closer investigation, we were able to discern outgrown ureters in at least 25 independent E14.5-18.5 Sall1−/− embryos in which there was no evidence of kidney formation (Fig. 1). These blind ureters had grown away from the bladder similar to those in wild-type litters, despite no evidence of a morphologically distinct metanephric mesenchyme. Blind ureters exhibited normal histological architecture that consisted of urothelium (u) and smooth muscle (sm) layers, as revealed by Hematoxylin and Eosin staining. By E16.5, the smooth muscle layer should have differentiated and begun to express smooth muscle actin; this occurred normally in the mutant ureters (Fig. 1E,F). Both wild-type and mutant ureters also properly expressed a marker that is exclusive to the differentiated epithelium of the urinary tract: uroplakin III (Fig. 1G,H). Six1−/− mutants display a very similar phenotype in which renal agenesis is accompanied by growth and differentiation of the ureter (Bush et al., 2006), supporting the conclusion that development of the extra-renal ureter is independent of the proximal branching ub that forms the intra-renal collecting system. However, unlike Six1 (Nie et al., 2010), Sall1 is not required for proper formation of the smooth muscle layer. Sall1 expression is not preserved in Six1−/− mutant mm and it has been suggested that Sall1 is a direct transcriptional target of Six1 regulation (Chai et al., 2006). These findings suggest that Six1 and Sall1 function together in a developmental pathway that regulates initial branching of the ureter.

**Blind ureters arrest before the T-stage and maintain persistent mesenchymal cells**

To verify the identity of the outgrown ureters and to visualize ureter development at multiple stages, we crossed Sall1−/− mice with the Hoxb7EGFP transgenic line (F. Costantini, Columbia University). Visualization of GFP fluorescence verified the existence of blind ureters in Sall1−/− mice, by E16.5 embryos (Fig. 2). These ureters had grown at the proper site on either side of the aorta and were morphologically distinguishable from the surrounding tissue. The distal tips appeared dilated and the lengths were often asymmetric. At E11.5, when the wild-type ub has formed its first branch (T-stage), mutant ubs remained unbranched (Fig. 2C,D). Both wild-type and mutant ub emerged from the distal Wolffian duct and invaded the metanephric mesenchyme; however, the mutant ub failed to form the T-stage. This unbranched ub remained viable and continued to grow in the absence of proper mesenchymal induction.

Ret was properly expressed in the mutant ub and became restricted to the ureret tip by E12.5 (Fig. 3C,D). Analysis of Pax2 expression revealed a small cluster of mesenchyme surrounding the
and confirms the proper differentiation of mutant ureters. and anti-uroplakin III appears similar in wild-type and mutant sections, similar to pretubular aggregates in the persistent mesenchymal cells and, with this observation, there was no evidence of formation of Cited stage because a gene expressed specifically in cap mesenchyme, (Fig. 3G,H). These cells did not progress beyond the progenitor mesenchyme contained a persistent cap of mesenchyme (Fig. 3E,F). The mutant ureter tip, suggesting that blind ureter formation is associated with a persistent cap of mesenchyme (Fig. 3E,F). The mutant mesenchyme contained Six2-positive metanephric progenitor cells (Fig. 3G,H). These cells did not progress beyond the progenitor stage because a gene expressed specifically in cap mesenchyme, Cited1, was absent (Fig. 3I,J) (Mugford et al., 2009). Consistent with this observation, there was no evidence of formation of pretubular aggregates in the persistent mesenchymal cells and, similar to Sall1-null mice (Nishinakamura et al., 2001), expression of several other mesenchymal markers, including Eyal and Wnt4, were reduced compared with wild-type controls (data not shown). Together, these data show that uninduced metanephric mesenchyme persists atop mature blind ureters and demonstrate that Sall1 expression is required for initial T-branch formation.

**Uregulation of GDNF signaling is not sufficient to rescue branching**

Our data suggest that Sall1-dependent signals from the mm to the ub are required to initiate branching. It has been proposed that a failure to maintain Gdnf expression probably accounts for renal agenesis or for severe hypoplasia in Sall1 mutants (Schedl, 2007). The requirement for proper Gdnf expression in the critical window of initial ub invasion has been shown through analysis of nephrectin (Npnt) and integrin α8 (Itga8) mutant mice (Linton et al., 2007). In these mutants, Gdnf expression appears to be normal at the time of ub outgrowth, but is dramatically reduced at E11.5 when the ub invades the mesenchyme. A genetic reduction of Spry1 gene expression rescued the kidney defect in Itga8 mutants confirming that decreased GDNF-Ret signaling was responsible for renal agenesis in these mice. Therefore, we assayed expression of Gdnf in Sall1-ΔZn mutant mesenchyme at E11.5 and found that its expression was significantly reduced when measured using in situ hybridization and quantitative real-time PCR (Fig. 3N, 0.22±0.05 fold change). Mesenchymal Pax2 expression appeared similar to wild-type at the same time point (Fig. 3K,L).

To test the hypothesis that Gdnf deficiency accounts for ub arrest in the Sall1-ΔZn mutant, we next performed metanephric organ culture with exogenous GDNF and with increased GDNF signaling achieved by reducing the dose of the Spry1 tyrosine kinase inhibitor in vivo. For organ culture, E11.5 Hoxb7GFP wild-type and homozygous Sall1-ΔZnΔZn metanephric regions were cultured with beads coated with bovine serum albumin (BSA) or recombinant GDNF protein. Recapitulating the in vivo phenotypes, mutant explants failed to branch, whereas wild-type explants underwent several rounds of branching when incubated with BSA-coated beads (Fig. 3O,P). As previously described, exogenous GDNF caused increased branching and altered patterning of the wild-type ub, confirming that GDNF was active in our culture system (Fig. 3Q). Addition of GDNF-coated beads resulted in only a single branch with abnormal morphology in eight out of 11 mutants (Fig. 3R). Interestingly, mutant ureteric buds did not swell as dramatically as wild-type tissues when treated with exogenous GDNF, suggesting that ampulla formation is abnormal. A similar reduction in Gdnf expression at E11.5 was observed for Grem1-deficient embryos, which arrest prior to ub outgrowth. However, in this mutant, exogenous GDNF induced ub branching of Grem1-/- metanephroi in organ culture that was comparable with that seen in the wild type (Michos et al., 2007). Thus, the failure of GDNF to rescue the Sall1-ΔZnΔZn explants is best explained by an inability of the Sall1-ΔZn mutant to respond to this branch-promoting factor.
We next tested whether the deficiency in Gdnf could be overcome by reducing Spry1 dose in vivo. Spry1 normally functions to attenuate GDNF activation of the Ret receptor, and loss of Spry1 in Spry1-null embryos produces multiple ectopic buds because of increased Ret signaling (Basson et al., 2005; Basson et al., 2006). Therefore, we postulated that increasing Ret signaling in the presence of low levels of GDNF would allow mutant ureteric buds to branch. In contrast to the rescue observed for Itga8-null mice, heterozygosity of a Spry1+/- allele (J. Licht, Northwestern University) did not alter the frequency of renal agenesis and blind ureter formation for Sall1-ΔZn mutants in any of the Spry1+/−:Sall1-ΔZn mutants examined (n=0/5). Together, these results suggest that although low levels of Gdnf might contribute to ureter arrest in the Sall1-ΔZn mutant, reduced signaling through this pathway alone cannot account for this phenotype.

Although Sall1 is not expressed in the ub, it is expressed in the nephric duct at E9-10, immediately prior to ub outgrowth. Thus, Sall1 expression in the duct might be required to pattern the ub and enable it to form an ampulla properly. To test this possibility, we performed recombination experiments to determine whether Sall1-ΔZn mutant ub is competent to branch when recombined with wild-type mm. As shown in Fig. 3S,T, mutant ubs underwent several rounds of branching morphogenesis (average=4.3 ub with four out of six recombinations exhibiting branching) when recombined with wild-type mesenchyme; this was comparable with that observed in the wild type ↔ wild type recombination (average=4.8 ub tips with four out of six recombinations exhibiting branching). Wild-type recombinations were performed prior to the T-stage to verify that unbranched ubs were capable of branching in organ culture. Together, these results indicate that the Sall1-ΔZn-Δ ub is competent to respond to mesenchymal signals, and suggest that Sall1-dependent factors in the mm, independently of GDNF, must be involved in the regulation of ampulla formation and the initiation of ureteric bud branching.

**Wnt9b is ectopically expressed in mutant ureteric bud tips**

We next postulated that Sall1 might be affecting ureter tip fate. If mutant ub tips are not properly specified, they could adopt a distal ub phenotype and prohibit branching (Caruana et al., 2006; Michael and Davies, 2004; Schmidt-Ott et al., 2005; Shakyra et al., 2005; Sweeney et al., 2008). Tip identity was specified in Sall1-ΔZn mutant ubs because both Ret and Gdnf expression in the mutant mm is properly tip restricted in the blind ureter (Carroll et al., 2005). Therefore, we performed recombination experiments to determine whether Sall1-ΔZn mutant ub is competent to branch when recombined with wild-type mm. As shown in Fig. 3S,T, mutant ubs underwent several rounds of branching morphogenesis (average=4.3 ub with four out of six recombinations exhibiting branching) when recombined with wild-type mesenchyme; this was comparable with that observed in the wild type ↔ wild type recombination (average=4.8 ub tips with four out of six recombinations exhibiting branching). Wild-type recombinations were performed prior to the T-stage to verify that unbranched ubs were capable of branching in organ culture. Together, these results indicate that the Sall1-ΔZn-Δ ub is competent to respond to mesenchymal signals, and suggest that Sall1-dependent factors in the mm, independently of GDNF, must be involved in the regulation of ampulla formation and the initiation of ureteric bud branching.

**Fig. 3. Mutant cap mesenchyme persists and expresses low Gdnf, but this does not account for the blind ureter phenotype.** (A-J) Hoxb7GFP expression (A,B) or whole-mount in situ hybridization (C-J) in wild-type or Sall1-ΔZn homozygous mutant E12.5 mouse tissues. Ret is properly tip restricted in the blind ureter. Pax2 and Six2 expression reveal a cap of metanephric mesenchyme (mm) atop the blind ureter. (A,B) Anti-Pax2 (green) and anti-laminin (red) staining show that Pax2-positive mm cells encircle the Pax2-positive ureteric bud (ub) outlined with anti-laminin. (C,D) In wild-type mm, independently of GDNF, must be involved in the regulation of ampulla formation and the initiation of ureteric bud branching.
**Reduction in β-catenin dosage rescues early branching**

Since Wnt9b and at least two canonical Wnt-responsive genes were upregulated in Sall1-ΔZnαβ μ mutant ureters, we hypothesized that ub branching could be rescued by lowering β-catenin activity. In contrast to reduced Spry1 dose, which had no effect on the Sall1-ΔZnαβ phenotype, lowering the dose of β-catenin rescued branching in 50% of Sall1-ΔZnαβ;Ctnnb1β/+ E12.5-13.5 embryos. Seven out of 14 mutant kidneys branched to the T-stage and initiated at least a second round of branching from each tip, resulting in a statistically significant rescue of the blind ureter phenotype (Fisher’s exact test P<0.005, Fig. 4K,N,O). Sall1-ΔZnαβ;Ctnnb1β/+ littermates (21 out of 22) and the remainder of the Sall1-ΔZnαβ;Ctnnb1β/+ kidneys arrested with blind ureters (Fig. 4J,M,O). Sall1-ΔZnαβ;Ctnnb1β/+ control kidneys branched normally (Fig. 4L), verifying that lowered β-catenin dose alone has no effect on kidney branching. This rescue supports the idea that increased β-catenin signaling is a major component of the Sall1-ΔZnαβ blind ureter phenotype.

**Ectopic Wnt9b or β-catenin activity in the ureter inhibits branching**

Tight control of β-catenin is required during nephrogenesis in both the ureter and mesenchyme (Bridgewater et al., 2008; Carroll et al., 2005; Iglesias et al., 2007; Kuure et al., 2007; Marose et al., 2008; Park et al., 2007). Therefore, we next sought to understand whether increased β-catenin signaling in the ureter tip could be responsible for defective initiation of ub branching. To test this hypothesis, we created a new transgenic mouse to conditionally overexpress Wnt9b (ACTWnt9b) and used an existing conditionally active
As the effects of overexpression of Wnt9b alone were less severe than the Sall1-ΔZn4,3 blind ureter phenotype, we increased Wnt signaling more dramatically in the ub using a stabilized β-catenin allele. A significant number of E11.5-12.5 Hoxb7creEGFP; Ctnnb1ex3Δ/Δ embryos (35 out of 60) exhibited unbranched ureters that were remarkably similar to those seen in Sall1-ΔZn embryos (Fig. 5I). The remaining mutants were able to form the T-branch, but arrested shortly thereafter, and often exhibited abnormal branch patterns (data not shown). Hoxb7creEGFP; Ctnnb1ex3Δ/Δ littersmates branched normally at all stages, confirming that the transgene alone had no deleterious effects. The high frequency of renal agenesis for this cross has recently been reported, but no detailed analysis was provided (Karner et al., 2010). Similarly, inhibition of ureteric bud branching was observed in organ culture when β-catenin signaling was activated using an inhibitor of GSK-3: LiCl (n=8/8, data not shown). Gene marker analysis demonstrated the presence of blind ureters that properly express tip restricted Wnt11 (Fig. 5O,P). Wnt11 expression was expanded in Hoxb7creEGFP; Ctnnb1ex3Δ/Δ kidneys compared with Sall1-ΔZn mutants (compare Fig. 4D with Fig. 5P) and this pattern probably indicates sites that could initiate abnormal branching later in development in these embryos. Wnt9b expression was preserved in ub stalks and was properly downregulated in wild-type and Ctnnb1ex3Δ/Δ mutant ub tips, demonstrating that dominant β-catenin has no effect on Wnt9b gene expression (Fig. 5M,N). Pax2 was expressed in the ureter and the overlying mm in a very similar pattern to that seen in Sall1-ΔZn embryos (compare Fig. 5R with Fig. 3F). Sall1 expression in the mesenchyme was not altered, suggesting that its role in affecting ureter branching occurs upstream of β-catenin signaling. Overall, the strong correlation between the phenotypic consequences of loss of Sall1 in the mm and increased Wnt9b-β-catenin signaling in the ub suggests that increased β-catenin signaling at ub tips is an important mechanism for the Sall1-ΔZn blind ureter phenotype.

**DISCUSSION**

Sall1 has been implicated as a regulator of canonical Wnt signaling, but it is not clear whether this is a direct or indirect effect (Bohm et al., 2006; Chihara and Hayashi, 2000; Ma et al., 2006; Onai et al., 2004; Ribeiro et al., 2004; Sato et al., 2004; Uez et al., 2008). As Sall1 is a transcription factor expressed in the mm, it is likely to regulate expression of membrane bound or secreted factors that would signal to the ub to undergo initial branching. Based on our results, Sall1 would be predicted to repress Wnt9b expression and β-catenin signaling in ureteric bud tip cells. As Sall1 acts as a potent transcriptional repressor through binding to a nucleosome remodeling and deacetylase (NuRD) complex, an attractive hypothesis is that Sall1 is necessary to repress a branch inhibitor that is secreted by the mm (Kiefert et al., 2002; Laubert and Rauchman, 2006). Whereas branch inhibitors normally function to restrict ub outgrowth to a single bud at the proper location, ectopic expression of these factors could disrupt branching. An alternative possibility is that there is a deficiency in branch-promoting factors in the Sall1-ΔZn mutant. In addition to GDNF, FGFs and other growth factors are thought to positively regulate ub branching (Costantini, 2006). Further studies are needed to determine which of these factors might regulate initial branch formation by the ub via modulation of Wnt9b/β-catenin activity in ub tip cells.

Although our studies point to an important mechanism by which Sall1-dependent signals from the mm regulate the ub, it is likely that this transcription factor also has cell-autonomous functions in the mm. Progenitors derived from Sall1 mutant mm can be induced

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**Fig. 6. Model for the effect of mesenchymal Sall1 on ureter stalk expression of Wnt9b and canonical Wnt signaling.** When the ureteric bud invades the metanephric mesenchyme (pink), mesenchymal progenitor cells (light gray and blue) coalesce at the ureter tip (brown). Wnt9b and β-catenin signaling (yellow) are downregulated in the ub tip, but continue to be expressed in the ub stalk. Signaling from the mesenchyme causes the ureter tips to branch. Reciprocal signals from the ureter to the mesenchyme induce the mesenchyme to form condensates that differentiate into renal vesicles near the Wnt9b-positive stalk. When Sall1 is not expressed in the mesenchyme (dark gray and blue, below the dotted line), Wnt9b is ectopically expressed in ureter tips and ureter branching arrests before the T-stage.
to differentiate into nephron precursors but form smaller colonies than wild-type cells, suggesting that Sall1 is required for maintenance or proliferation of metanephric progenitors (Osafune et al., 2006). Recently, it has been suggested that Sall1 also has a role in modulating compact adhesion of mm cells adjacent to the ub tip through direct regulation of the kinesin family gene Kif26 (Uchiyama et al., 2010). Identification of Sall1 target genes in the kidney will better elucidate its multiple roles in the mm.

Several key studies have addressed the role of canonical Wnt signaling in the developing kidney using both loss- and gain-of-function approaches (Bridgewater et al., 2008; Carroll et al., 2005; Marose et al., 2008; Park et al., 2007). A general conclusion from these studies is that kidney development is extremely sensitive to signaling in the developing kidney using both loss- and gain-of-function. Identification of Sall1 target genes in the kidney will better elucidate its multiple roles in the mm.

We propose a model in which Wnt9b is restricted to ureter stalks to maintain undifferentiated mesenchymal progenitors and to promote subsequent ureter branches in the cortex (Fig. 6). Wnt9b expression is confined to the ureter stalk to allow induction of mesenchymal differentiation without affecting UB tip branching morphogenesis. In the absence of Sall1, this delicate balance of tip versus stalk Wnt signaling is disrupted, which leads to UB branching arrest. Our studies reveal a requirement for Sall1 for the initial interaction between mm and ub that modulates ureter β-catenin signaling and branching competence.

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Competing interests statement

The authors declare no competing financial interests.

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