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Essential roles of *Sall1* in kidney development

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Essential roles of *Sall1* in kidney development. *SALL1* is a mammalian homologue of the *Drosophila* region-specific homeotic gene *spalt* (*sal*) and heterozygous mutations in *SALL1* in humans lead to Townes-Brocks syndrome. We isolated a mouse homologue of *SALL1* (*Sall1*) and found that mice deficient in *Sall1* die in the perinatal period with kidney agenesis. *Sall1* is expressed in the metanephric mesenchyme surrounding ureteric bud and homozygous deletion of *Sall1* results in an incomplete ureteric bud outgrowth. Therefore, *Sall1* is essential for ureteric bud invasion, the initial key step for metanephros development. We also generated mice in which a green fluorescent protein (*GFP*) gene was inserted into the *Sall1* locus and we isolated the *GFP*-positive population from embryonic kidneys of these mice by fluorescence-activated cell sorting (FACS). We then compared gene expression profiles in the *GFP*-positive and -negative population using microarray analysis, followed by in situ hybridization. We detected many genes known to be important for metanephros development, and genes expressed abundantly in the metanephric mesenchyme. We also found groups of genes which are not known to be expressed in the metanephric mesenchyme. Thus a combination of microarray technology and *Sall1-GFP* mice is useful for systematic identification of genes expressed in the developing kidney.

THREE KIDNEYS DURING DEVELOPMENT

The kidney develops in three stages: pronephros, mesonephros, and metanephros. The nephric duct (Wolffian duct) develops in the craniocaudal direction from the intermediate mesoderm and acts upon the surrounding mesenchyme as an inducer of epithelial transformation to nephric tubules. The pronephric and mesonephric tubules and the anterior portion of the Wolffian duct eventually degenerate, and it is the metanephros that becomes the permanent kidney in mammals.

IDENTIFICATION OF *Sall* GENES USING FROG EMBRYOS

The animal cap is a tiny portion of the presumptive ectoderm of *Xenopus* embryos in the blastula stage. In the presence of activin, animal caps differentiate into a variety of tissues. A combination of activin plus retinoic acid induces pronephric tubules efficiently and selectively [1]. We used this animal cap system to identify molecules expressed in pronephros and potentially in mesonephros and metanephros. Thousands of animal caps treated with activin plus retinoic acid were collected at various time points and subjected for a varitety of subtraction procedures. One of the obtained molecules was *Xsal-3*, which is homologous to *Drosophila* region-specific homeotic gene *spalt* (*sal*) and has multiple double-zinc finger motifs characteristic of the *sal* gene family [2]. We also isolated a mouse homologue (*Sall1*) and found it to be expressed in otic vesicles, limb buds, anus, hearts, and kidneys (metanephric mesenchyme) [3].

Sall1 IS ESSENTIAL FOR KIDNEY DEVELOPMENT

When we generated *Sall1* knockout mice, all of homozygous mice died within 24 hours after birth, and kidney agenesis or severe dysgenesis were present (Fig. 1) [3]. About one third had no kidneys or ureters, bilaterally (Fig. 1B). The remaining mice had either unilateral kidney agenesis, or bilateral hypolasia (Fig. 1C). At day 11.5 of gestation, the ureteric bud invades the metanephric mesenchyme and subsequent reciprocal interaction between these two tissues leads to development of a metanephric kidney (Fig. 1D). In *Sall1*-null mice, morphologically distinct metanephric mesenchyme was formed, albeit the size being reduced (Fig. 1E). In contrast, the ureteric bud formed but failed to invade the metanephric mesenchyme. Thus, loss of *Sall1* leads to a failure of ureteric bud invasion into the mesenchyme, the initial key step for metanephros development.

KIDNEY ABNORMALITIES CAUSED BY HUMAN *SALL1* MUTATIONS

Humans and mice have four known *sal*-related genes, respectively (*SALL1-4* for humans and *Sall1-4* for mice). Mutations in *SALL1* have been associated with Townes-Brocks syndrome, an autosomal-dominant disease with features of dysplastic ears, preaxial polydactyly, imperforate anus, and, less commonly, kidney and heart anomalies [4]. Mice deficient in *Sall1* show kidney agenesis or

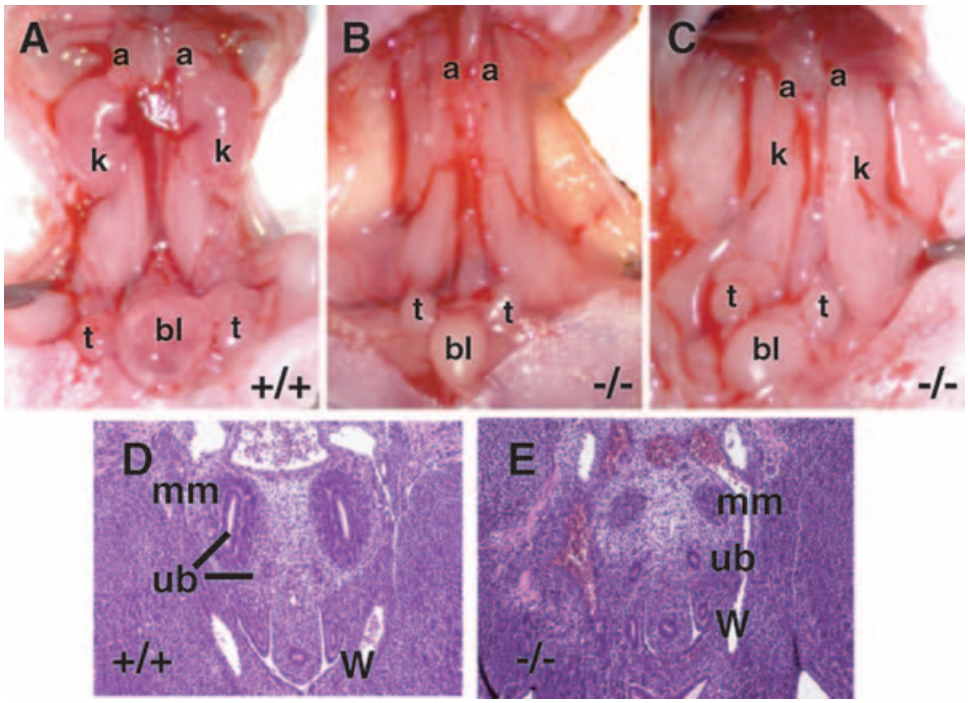

Fig. 1. Kidney phenotypes in *Sall1*-deficient mice. (*A*) Kidneys (k) of wild-type newborn. Urinary bladder (bl) is filled with urine. (*B*) Kidneys of *Sall1*-deficient newborn. Note kidneys are absent and the urinary bladder is not inflated with urine. Other organs, such as adrenal glands (a) and testis (t), are normal. (*C*) Kidneys of another *Sall1*-deficient newborn with severe bilateral kidney hypoplasia. Urine is absent in the bladder. (*D*) Metanephros in wild-type mice at 11.5 days past coitus (dpc). Ureteric bud (ub) branches from Wolffian duct (W) and metanephric mesenchyme (mm) are condensed around the bulging ureteric bud. (*E*) Metanephros in *Sall1*-deficient mice at 11.5 dpc. Metanephric mesenchyme is formed but reduced in size and is not invaded by the ureteric bud.

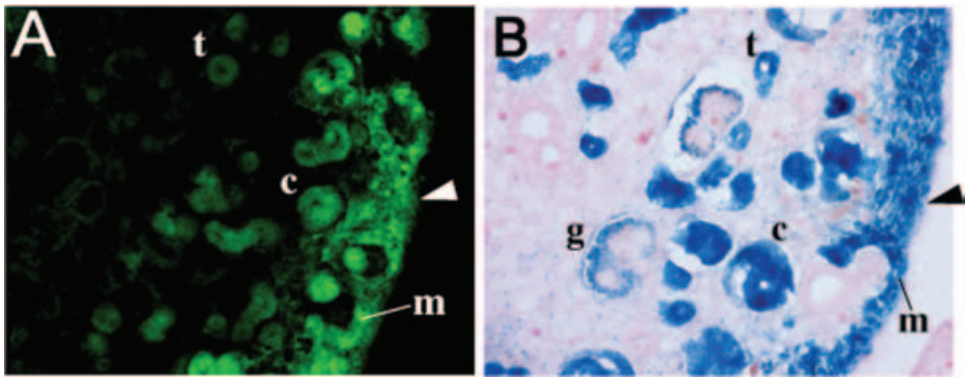

Fig. 2. Generation of *Sall1*-green fluorescence protein *(GFP)* knockin mice. (*A*) *GFP* expression in embryonic kidney of heterozygous *Sall1-GFP* knockin mice. (*B*) 5-bomo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) staining of embryonic kidney in heterozygous *Sall1-LacZ* knockin mice. Arrowhead is the stroma; m is condensed mesenchyme; c is comma-shaped bodies; t is tubles; and g is glomerulus.

severe dysgenesis, but other phenotypes observed in human disease are not apparent, as described above [3]. This discrepancy could be explained by truncated SALL1 proteins by human mutations, possibly functioning in a dominant-negative manner, as mutant mice that produce a truncated Sall1 protein exhibit more severe defects than *Sall1-null* mice, including renal agenesis, exencephaly, limb, and anal deformities [5]. *Sall2*-deficient mice show no apparent phenotypes, and mice lacking both *Sall1* and *Sall2* show kidney phenotypes comparable to those of *Sall1* knockout [6]. *Sall3*-null mice die on the first postnatal day and deficiencies in cranial nerves and abnormalities in the oral structures are present [7]. Mutations of *SALL4* cause an autosomal-dominant disorder Okihiro syndrome, characterized by limb deformity and eye movement deficits, and, less commonly, anorectal and kidney anomalies [8, 9], and we are currently generating *Sall4*-deficient mice. Generation of mice lacking all of the *Sall* genes would be necessary to address developmental roles of *Sall* family.

IDENTIFICATION OF KIDNEY MESENCHYMAL GENES BY A COMBINATION OF MICROARRAY ANALYSIS AND *Sall1-GFP* KNOCKIN MICE

In the embryonic kidney, *Sall1* is expressed abundantly in mesenchyme-derived structures from condensed mesenchyme, S-shaped, comma-shaped bodies, to renal tubules and podocytes (Fig. 2). We generated mice in which *GFP* gene was inserted into the *Sall1* locus and we isolated the *GFP*-positive population from embryonic kidneys of these mice by FACS [10]. The *GFP*-positive population indeed expressed mesenchymal genes, while the negative population expressed genes in the ureteric bud. To systematically search for genes expressed in the mesenchyme-derived cells, we compared gene expression profiles in the *GFP*-positive and *GFP*-negative populations using microarray analysis, followed by in situ hybridization. We detected many genes known to be important for metanephros development, including *Sall1*, *GDNF*, *Raldh2*, *Pax8*, and *FoxD1*, and genes expressed abundantly in the metanephric mesenchyme such as *Unc4.1*, *Six2*, *Osr-2*, and *PDGFc*. We also found groups of genes, including *SSB-4*, *Smarcd3*, μ-*Crystallin*, and *TRB-2*, which are not known to be expressed in the metanephric mesenchyme. Therefore, a combination of microarray technology and *Sall1-GFP* mice is useful for systematic identification of genes expressed in the developing kidney. To find essential genes from this large list, efficient and rapid screening is needed. Recently emerging siRNA technology is one potent method, but generating knockout mice of each candidate gene is necessary for proof.

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