The mouse seminal vesicle shape mutation is allelic with *Fgfr2*

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The mouse seminal vesicle shape (svs) mutation is a spontaneous recessive mutation that causes branching morphogenesis defects in the prostate gland and seminal vesicles. Unlike many other mutations that reduce prostatic and/or seminal vesicle branching, the svs mutation dramatically reduces branching without reducing organ growth. Using a positional cloning approach, we identified the svs mutant lesion as a 491 bp insertion in the tenth intron of *Fgfr2* that results in changes in the pattern of *Fgfr2* alternative splicing. An engineered null allele of *Fgfr2* failed to complement the svs mutation proving that a partial loss of FGFR2(IIIb) isoforms causes svs phenotypes. Thus, the svs mutation represents a new type of adult viable *Fgfr2* allele that can be used to elucidate receptor function during normal development and in the adult. In the developing seminal vesicles, sustained activation of ERK1/2 was associated with branching morphogenesis and this was absent in svs mutant seminal vesicles. This defect appears to be the immediate downstream effect of partial loss of FGFR2(IIIb) because activation of FGFR2(IIIb) by FGF10 rapidly induced ERK1/2 activation, and inhibition of ERK1/2 activation blocked seminal vesicle branching morphogenesis. Partial loss of FGFR2(IIIb) was also associated with down-regulation of several branching morphogenesis regulators including *Shh*, *Ptch1*, *Gli1*, *Gli2*, *Bmp4*, and *Bmp7*. Together with previous studies, these data suggest that peak levels of FGFR2(IIIb) signaling are required to induce branching and sustain ERK1/2 activation, whereas reduced levels support ductal outgrowth in the prostate gland and seminal vesicles.

**KEY WORDS:** *Fgfr2*, *Prostate*, *Seminal vesicle*, *Branching morphogenesis*, *Shh*, *Gli1*, *Fgf10*, *Gli2*, *Ptch1*, *Bmp4*, *Bmp7*, *svs*, *Seminal vesicle shape mutation*

**INTRODUCTION**

The mouse seminal vesicle shape (svs) mutation is a spontaneous recessive mutation that arose during the creation of the mouse CXB5 recombinant inbred strain from parental strains Balb/cBy and C57BL/6By. The svs mutation is absent from the other CXB recombinant inbred lines and was first identified because it alters the adult morphology of the seminal vesicles (Shukri et al., 1988). The altered seminal vesicle morphology results from a complete failure of branching morphogenesis during seminal vesicle development (Marker et al., 2003a). In addition, the svs mutation reduces branching morphogenesis in the prostate gland by approximately 40% (Marker et al., 2003a), owing to increased ductal length between the bifurcation events of the elongating buds, resulting in a reduced number of ductal tips with no reduction in organ weight (Marker et al., 2003a).

Branching morphogenesis is a developmental process common to almost all organisms in the animal kingdom (Davies, 2002). In mammals, the kidney, lung and most glands including the pancreas, mammary, prostate and seminal vesicles undergo branching morphogenesis during development. Organogenesis of branched organs has been described in terms of five steps: organ specification, epithelial bud initiation, epithelial duct elongation into the mesenchyme, bifurcation of the ducts leading to complex branching patterns, and cellular differentiation of the newly branched structure (Affolter et al., 2003). During prostate and seminal vesicle development, the svs mutation specifically affects budding/branching morphogenesis because organ specification, ductal elongation and cellular differentiation are normal in both organs (Marker et al., 2003a; Shukri et al., 1988). This contrasts with many other spontaneous and engineered mutations that decrease both organ growth and branching simultaneously in the prostate and/or seminal vesicles. Examples include mutations affecting *Ar*, *Gdf7*, *Ghr*, *Fgf10*, *Hoxa13*, *Hoxd13*, *Igf1* and *Srd5a2* (Donjacour et al., 2003; Marker et al., 2003b; Settle et al., 2001). In cases where both growth and branching are affected, it is difficult to determine if branching morphogenesis defects reflect a direct requirement for the gene in regulating branching or indirect effects of compromised organ growth. Because the svs mutation affects branching and not growth, it provides a unique opportunity to investigate the molecular mechanisms that control branching morphogenesis in the prostate and seminal vesicles.

Previous work mapped the svs mutation to a 2.7 cM interval on mouse chromosome 7 that included the *Fgfr2* locus (Marker et al., 2003a). *Fgfr2* was initially considered as a candidate for the gene affected by the svs mutation, and the *Fgfr2* open reading frame was sequenced but no changes were identified. This was not surprising because there was an apparent disconnect between phenotypes of known *Fgfr2* mutations and the phenotypes present in svs mutant mice. All previously reported loss-of-function *Fgfr2* mutations in mice caused dysgenesis or agenesis of organs throughout the body that resulted in embryonic or perinatal lethality (Arman et al., 1998; Arman et al., 1999; De Moerlooze et al., 2000; Hajihosseini et al., 2001; Xu et al., 1998), whereas we had identified only branching defects in the urogenital tract of svs mice. Additionally, gain-of-function mutations in *FGFR2* cause several human diseases including Crouzon, Jackson-Weiss, Apert and Pfeiffer syndromes (Hajihosseini et al., 2001; Hertz et al., 2001; Robertson et al., 1998), whereas svs mutant mice do not display any of the phenotypes associated with these diseases. Furthermore, FGF7 and FGF10 were known to be expressed in the mesenchyme of the developing prostate and seminal vesicles and to act via FGFR2(IIIb) expressed

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in the developing epithelium, and recombinant FGF7 or FGF10 stimulated both growth and branching of developing prostates and seminal vesicles in vitro acting at least in part as pro-proliferative signals for the epithelium (Alarid et al., 1994; Sugimura et al., 1996; Thomson and Cunha, 1999). These data suggested that FGFR2 signaling was crucial for prostate and seminal vesicle growth and therefore did not fit well with the lack of growth defects in svs mutant prostates and seminal vesicles. The requirement for FGF10/FGFR2(IIIb) signaling for prostate growth was subsequently confirmed by analysis of FGF10-knockout mice (Donjacour et al., 2003).

In order to understand the molecular basis for the branching morphogenesis defects present in svs mutant mice, we took a positional cloning approach to identify the affected gene. The map position of the svs mutation was narrowed to a 410 kb interval predicted to contain eight genes. All eight genes were investigated but no coding region sequence changes were identified. Investigation of non-coding sequences identified a 491 bp insertion in the tenth intron of Fgfr2 as a candidate svs mutation. This insertion was associated with changes in the pattern of Fgfr2 alternative splicing, and an engineered null allele of Fgfr2 failed to complement the svs mutation proving that a partial loss of FGFR2(IIIb) isoforms causes svs phenotypes. In addition, signaling by FGFR2(IIIb) through ERK1/2 (MAPK3/1 – Mouse Genome Informatics) and expression of several genes that regulate branching were found to be defective in svs mutant mice.

**MATERIALS AND METHODS**

**PCR**

Taq polymerase (New England BioLabs) was used according to the manufacturer’s instructions to type markers D7Mit66, D7Mit285, D7Mit164, D7Mit205, D7Mit103, D7Mit165, D7Mit134, D7Mit135, D7Mit43, D7Mit107 and D7Mit108 (Research Genetics) on progeny from a previously described intraspecific mouse cross (Marker et al., 2003a).

PCR primers to amplify the svs insertion were: outer-svs, 5'-GTGAA-GACTGGAGCTGCCAGT-3' and 5'-AGTCATAATTCTACACCTATGCTG-3'; nested-svs, 5'-AAAGCTACGGTCCTCTTTGG-3' and 5'-GGCACCTGTCACATGACCTTAA-3'.

Full-length Fgfr2 cDNA was amplified by a dT-primed reverse transcription (RT) followed by a primary and then a nested PCR using High Fidelity Taq according to the manufacturer’s guidelines (Invitrogen). Primary primers, 5'-AGCACAAAGACAGTAAACAAACG-3' and 5'-ACACAGGCACAATGCTCCCCAC-3'; nested primers, 5'-CCCTGAGGCAATGGCCTCTGACTATGTGCACCT-3' and 5'-AAGAAGAAAAGCCGGCCCAAAAGAACAACATGTTTAA-3'.

**Southern blotting**

Genomic DNA from svs mutant mice (Jackson Laboratories) and the two parental control strains Balb/cBy and C57BL/6By (Jackson Laboratories) was digested with BamHI, separated on a 0.8% agarose gel, transferred to Hybond-N+ membrane and then probed with a fragment of Fgfr2 comprising bases 1,024 to 2,254 of GenBank NM_201601.

**In-situ hybridization**

Anti-Fgfr2 partial cDNA (39,464-39,833 of GenBank AC157606) was used to synthesize DIG-labeled sense and antisense RNA probes using a DIG RNA Labeling Kit (Roche) according to the manufacturer’s instructions. Fresh tissues were dissected in PBS at 4°C and embedded into OCT. Tissue sections (12 µm) were cut, mounted on Superfrost-plus microscope slides (Fisher), hybridized with the Fgfr2 probe, and visualized as previously described (Thut et al., 2001).

**Organ cultures**

Postnatal day (P) 1 or 5 seminal vesicles were dissected out of CD1 mice (Charles River Laboratories) into basalm medium at 4°C, and cultured in 5% CO2 at 37°C on Millicell-CM Culture Plate Inserts (30 mm, 0.4 µm pore size; Millipore Corp.) in NuncMultidish 24-well plates (Nunc A/C). Cultured medium was changed every other day during the culture period. To monitor branching, pictures were taken of organs on each day of culture.

**Fgfr2 isoform analysis**

Full-length Fgfr2 amplicons and pIRES plasmid (Clontech) were digested with XhoI and NotI (New England BioLabs). pIRES was treated with shrimp alkaline phosphatase (Promega) and purified. Fgfr2 amplicons were ligated into linear pIRES and transformed into Escherichia coli DH5α. Individual clones were characterized by restriction enzyme digestion and sequencing.

**RNA isolation, RT-PCR and real-time PCR**

Seminal vesicles were dissected from P5 svs mutant as well as heterozygous and wild-type littermate mice. Heterozygous and wild-type seminal vesicles were pooled and are referred to as wild type; svs mutant seminal vesicles were pooled and are referred to as mutant. Following dissection, pooled seminal vesicles were crushed in Trizol (Invitrogen) using a pestle and RNA isolated according to the manufacturer’s instructions. Total RNA was amplified using the SMART RNA Amplification Kit (Clontech Laboratories) following the manufacturer’s instructions.

For RT-PCR, 25 ng of poly(A) RNA was first treated with DNase according to the manufacturer’s instructions (Invitrogen). Random primers, MMLV RT (Invitrogen) and other standard reagents were used in the PCR.

Real-time PCR was undertaken using a Lightcycler (Roche). Briefly, the LightCycler FastStart DNA MasterPLUS SYBGreen I Kit (Roche) was used with 5 µl of cDNA from the RT reaction. Primer sequences were as follows:

Shh, 5'-AATGGCCTTGGCCATCTCTGTG-3' and 5'-GCTGCCAGCTCTGTTAGTAGACG-3';

Ptx1, 5'-CTCTGGAGACATTTCACCAAGG-3' and 5'-TGCCGCGAGTTCTTTTGAAGT-3';

Gli1, 5'-GGAAGTTCTCAGTTGAGGCTGA-3' and 5'-CAACTCTGTCTCAGATGGA-3';

Gli2, 5'-CTCTGCTCAATGCGTCAGAC-3' and 5'-GGGTCACCGTGTA-3';

Bmp4, 5'-GGTTACCTAACGAGGATGGATGTG-3' and 5'-TCTTATTTCTCTGCACGCGGT-3';

Bmp7, 5'-AGTGTCCTCAAGCTTGTGAGG-3' and 5'-AGGAGGTTGGCTACGGTG-3'.
Transcript levels were normalized to 18S RNA which was amplified using primer sequences 5’-GCGCTTAGAGTGAATTTCTTG-3’ and 5’-CATTCTTGCAAAATGCTTTCG-3’. An analysis of variance (ANOVA) test was undertaken using StatView 4.1 (Abacus Concepts) to determine statistically significant differences.

**Histology**

Seminal vesicles from 9-month-old Fgfr2<sup>svs/</sup><sup>svs</sup> mice and Fgfr2<sup>2/</sup><sup>svs</sup> mice were dissected into cold PBS and embedded in OCT. Tissue sections (12 μm) were cut using a cryostat, mounted on Superfrost-plus microscope slides, fixed in 100% ethanol at –20°C for 2 minutes, and allowed to dry at room temperature. Slides were stained with Hema-toxylin and Eosin, dehydrated, and mounted using Permount.

**RESULTS**

**Identification of a candidate svs mutation in the non-recombinant interval**

We previously conducted an intraspecific backcross to map the location of the svs mutation on mouse chromosome 7 and localized the mutation to a 2.7 cM interval between *Tial1* and *Plekhal* (Marker et al., 2003a). We have now typed nine additional markers on the cross and localized the svs mutation to a 410 kb interval between D7Mit134 and D7Mit43 (Fig. 1A). Annotation of the mouse genome has identified two known and six predicted genes within this interval (Fig. 1B) (Birney et al., 2006; Waterston et al., 2002). We evaluated the candidate genes present between D7Mit134 and D7Mit43 for expression in the developing prostate and seminal vesicles by RT-PCR (data not shown). The predicted open reading frames for all detected candidate transcripts were sequenced in svs mutant mice and parental control strains Balb/cBy and C57BL/6By, but no changes were identified (data not shown). We subsequently initiated an effort to identify structural changes in the non-coding sequences of the candidate interval using Southern blotting. This identified a restriction fragment length polymorphism between svs mutant mice and the parental control strains Balb/cBy and C57BL/6By (Fig. 2A). The location of the polymorphism was predicted based on the mouse genome sequence, and subsequently analyzed by PCR (Waterston et al., 2002). The amplification of a longer fragment from svs mutant mice than from the two parental strains Balb/cBy and C57BL/6By suggested the presence of a novel insertion (Fig. 2B). Sequence analysis identified a 491 bp insertion identical to a murine leukemia virus long terminal repeat in the tenth intron of *Fgfr2* in svs mutant mice (Fig. 2C). This insertion was absent from all other CXB recombinant inbred strains of mice (data not shown), consistent with the possibility that this insertion is responsible for the phenotypes of svs mutant mice.
Partial loss of Fgfr2 causes svs phenotypes

The potential consequences of this insertion for Fgfr2 function were evaluated in several ways. Initially, we conducted western blot analysis using a commercially available antibody directed against the carboxy-terminus of FGFR2 and observed a banding pattern in both wild-type and svs mutant seminal vesicles similar to that previously reported for this antibody in experiments on other mouse tissues (Fig. 3A, upper panel). Previous reports indicate that these bands are specific for FGFR2 because they are eliminated in tissues with an engineered null mutation in FGFR2 (Yan et al., 2005) that strongly recognizes the lower molecular weight form of FGFR2 (Lu et al., 1999). FGF10 is expressed by the developing seminal vesicles, wild-type organs were cultured with recombinant FGF10 protein. FGF10 is expressed by the developing mesenchyme of the prostate and seminal vesicles and can signal through the IIIb isoform of FGFR2 (Lu et al., 1999). FGF10 activates ERK1/2 in control seminal vesicles whereas phosphorylated ERK1/2 could not be detected in svs mutant seminal vesicles (Fig. 6A). To determine if FGF2(IIIb) directly activates ERK1/2 in developing seminal vesicles, wild-type organs were cultured with recombinant FGF10 protein. FGF10 is expressed by the developing mesenchyme of the prostate and seminal vesicles and can signal through the IIIb isoform of FGFR2 (Lu et al., 1999). FGF10 activated ERK1/2 in the wild-type seminal vesicles within 20 minutes, suggesting that this is a direct response of FGF2(IIIb) activation (Fig. 6B). To determine if activated ERK1/2 is a plausible explanation for the loss of branching morphogenesis in the developing seminal vesicles of svs mutants, organ cultures of wild-type seminal vesicles were also conducted in the presence of

Fgfr2 function is regulated by a complex pattern of alternative transcript splicing (Ingersoll et al., 2001). To determine if aberrant alternative splicing of Fgfr2 was occurring in the svs mutant mice, full-length Fgfr2 transcripts were amplified by RT-PCR from svs mutant and control P5 seminal vesicle RNA. A total of 111 control and 96 svs mutant full-length Fgfr2 transcripts were analyzed by restriction enzyme digestion and sequencing. There was a surprisingly complex pattern of alternative splicing present in both control and svs mutant seminal vesicles. Eleven different isoforms were detected in each group, with only three isoforms overlapping between the control and svs mutant groups (Fig. 4A,B). The difference in isoform distribution between svs and control mice was highly statistically significantly (Pearson χ² test, P<0.0001). Strikingly, all of the Fgfr2 transcripts expressed in control seminal vesicles included exon 8IIIb, which confers specificity for the FGF ligands expressed in the mesenchyme of the developing prostate and seminal vesicles (Thomson and Cunha, 1999). By contrast, 10 of 11 Fgfr2 transcripts containing exon 8IIIb observed in wild-type seminal vesicles were reduced in or absent from ss mutant seminal vesicles.

To determine if partial loss of specific Fgfr2 isoforms caused svs mutant phenotypes, a genetic complementation test was conducted using a previously described mutant allele of Fgfr2. This allele has exons 7, 8IIIb, 8IIIc and 9 (7-9) flanked by loxP sites (Fgfr2lox) and encodes normal Fgfr2 transcripts (Yu et al., 2003). However, in the presence of Cre recombinase, exons 7-9 are deleted and a null allele of Fgfr2 is created (Fgfr2Δ). Crosses were conducted to create Fgfr2lox/Δ mice. Seminal vesicles from both genotypes were evaluated for branching morphology at P5 (Fig. 5A-C) and by histology in adults (Fig. 5D,E). The Fgfr2lox allele complemented the svs mutation, but the Fgfr2Δ allele did not, proving that svs phenotypes are due to a partial loss of Fgfr2 function.

Signaling and gene expression change downstream of the svs mutation

Fgfr2 is a receptor tyrosine kinase that can signal through multiple intracellular pathways (Chen et al., 2000; Kim et al., 2003; Nakamura et al., 2001; Newberry et al., 1997; Sakaguchi et al., 1999; Xiao et al., 2004; Yan et al., 1993). In the Drosophila tracheal, the FGFR homolog breathless controls branching morphogenesis through the MEK-ERK pathway (Gabay et al., 1997; Lee et al., 1996; Sutherland et al., 1996). To investigate whether this pathway might be important for the phenotypes of svs mutant mice, the status of ERK1/2 activation was examined in svs mutant and control seminal vesicles. There was an abundance of phosphorylated ERK1/2 in control seminal vesicles whereas phosphorylated ERK1/2 could not be detected in svs mutant seminal vesicles (Fig. 6A). To determine if FGFR2(IIIb) directly activates ERK1/2 in developing seminal vesicles, wild-type organs were cultured with recombinant FGF10 protein. FGF10 is expressed by the developing mesenchyme of the prostate and seminal vesicles and can signal through the IIIb isoform of FGFR2 (Lu et al., 1999). FGF10 activated ERK1/2 in the wild-type seminal vesicles within 20 minutes, suggesting that this is a direct response of FGFR2(IIIb) activation (Fig. 6B). To determine if activated ERK1/2 is a plausible explanation for the loss of branching morphogenesis in the developing seminal vesicles of svs mutants, organ cultures of wild-type seminal vesicles were also conducted in the presence of
UO126, a synthetic inhibitor of MEK1/2 (MAP2K1/2 – Mouse Genome Informatics), the upstream kinases that activate ERK1/2 (Davies et al., 2000). Following 4 days in culture, seminal vesicles cultured with testosterone branched significantly, whereas loss of ERK1/2 activation due to UO126 led to a complete loss of branching morphogenesis (Fig. 6C,D). These effects were unlikely to be due to interference with androgen receptor signaling because the levels of activated ERK1/2 were indistinguishable in organs cultured with or without testosterone (Fig. 6D). These data demonstrated that activation of the MEK1/2-ERK1/2 signaling pathway downstream of FGFR2(IIIb) is crucial for branching morphogenesis in the developing seminal vesicles. These data are also consistent with the possibility that the loss of ERK1/2 activation in svs mutant seminal vesicles is the proximal mechanism responsible for defects in branching morphogenesis.

Previous in vitro studies using recombinant FGF10 to treat prostatic organ cultures have suggested that FGF10 regulates the expression of several genes implicated as regulators of branching morphogenesis in the prostate and/or seminal vesicles (Huang et al., 2005). We used real time RT-PCR to evaluate the effects of partial loss of FGFR2(IIIb) on the expression of branching-regulatory genes (Fig. 7A-G). Although expression of the upstream ligand Fgf10 was unaffected, mRNA levels for several other branching regulators, including Shh, Gli1, Gli2, Ptc1, Bmp4 and Bmp7, were all reduced in svs mutant seminal vesicles.

**DISCUSSION**

Here we report that the spontaneous recessive mutation in svs mutant mice is a unique hypomorphic allele of Fgfr2 caused by a 491 bp insertion in intron 10 of the Fgfr2 gene. The insertion sequence is identical to a murine leukemia virus long terminal repeat (Fig. 2). This mutant lesion is consistent with previous studies which have shown that ~10% of spontaneous mutations in mice are due to transposable elements, including many instances of de novo murine leukemia virus insertion (Maksakova et al., 2006). The main effect of this insertion in the developing seminal vesicles is to change the pattern of alternative splicing of the Fgfr2 transcript (Figs 3, 4). During seminal vesicle development, the epithelium expresses Fgfr2 transcripts containing exon 8IIlb, whereas transcripts containing exon 8IIlc are absent. In other tissues, mesenchymal cells express transcripts containing exon 8IIlc. The aberrant alternative splicing in svs mutant mice does not appear to change the primary localization pattern of Fgfr2 transcripts in the seminal vesicles; in situ hybridization using a probe containing sequences found in all isoforms of Fgfr2 exhibited epithelial-specific localization in both wild-type and svs mutants (Fig. 3). However, the presence of low levels of Fgfr2 transcripts containing exon 8IIlc in the mesenchyme cannot be ruled out. In situ hybridization was conducted using probes specific to exon 8IIlb and exon 8IIlc of Fgfr2, but these probes were not sensitive enough to detect Fgfr2 transcripts in svs mutant seminal vesicles (data not shown).

The change in alternative splicing presumably results from the disruption of primary sequence, or from secondary structure elements within the Fgfr2 pre-mRNA that are required for the highly regulated and complex pattern of Fgfr2 alternative splicing previously described (Ingersoll et al., 2001). Alternative usage of exon 8IIlb and 8IIlc is regulated by cis elements present in the intronic sequences between exon 8IIlb and 8IIlc that are recognized by transactivating factors such as FOX-2 (RBM9 – Mouse Genome Informatics) (Baraniak et al., 2006). It is possible that the svs mutation disrupts these cis-acting regulatory sequences thereby blocking the recruitment of important transactivating splicing factors. However, the svs insertion is approximately 2 kb 3' of known Fgfr2 splicing-regulatory elements, including IAS1 (intronic activating sequence), ISAR (intronic splicing activator and repressor), IAS1 (intronic activating sequences 1), IAS2, IAS3, ISE1 (intronic silencing enhancers 1), ISE2 and ISE3 (Baraniak et al., 2006; Baraniak et al., 2003; Hovhannisyan and Carstens, 2005). Thus, the svs mutation may indicate the presence of one or more previously unrecognized intronic regulatory splicing elements in the tenth intron of Fgfr2.

During our analysis of Fgfr2 transcripts present during branching morphogenesis in wild-type organs, we identified 11 distinct splice variants (Fig. 4B). Although the importance of alternative splicing has not been well characterized for many of the alternatively-included Fgfr2 exons, the alternative usage of exons 8IIlb and 8IIlc has been extensively studied and shown to function as a key determinant of FGFR2 ligand specificity (Ingersoll et al., 2001). All of the wild-type Fgfr2 transcripts included exon 8IIlb, which is essential for receptor activation by FGF7 and FGF10, the ligands expressed by the prostate and seminal vesicle mesenchyme. In svs mutant organs, 10 of the 11 wild-type Fgfr2 splice variants were reduced in abundance or absent. The dramatic changes observed in alternative splicing, along with the recessive nature of the svs
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caused embryonic lethality around E10.5, a failure of limb bud immunoglobin-like domain III extracellular ligand-binding domain hypomorphic after embryonic day (E) 4.5 (Arman et al., 1998). A strong null mutation caused peri-implantation embryonic lethality shortly after embryonic day (E) 4.5 (Marker et al., 2003a; Shukri et al., 1988). By contrast, many Fgfr2 loss-of-function mutant alleles result in lethality due to affects on multiple organ systems. Fgfr2 is known to be required for early mammalian development because an Fgfr2-null mutation caused peri-implantation embryonic lethality shortly after embryonic day (E) 4.5 (Arman et al., 1998). A strong hypomorphic Fgfr2 allele that deleted the exons encoding the immunoglobulin-like domain III extracellular ligand-binding domain caused embryonic lethality around E10.5, a failure of limb bud induction, and placental defects (Xu et al., 1998). Fgfr2(IIIb), an isoform-specific knockout of Fgfr2 that eliminated receptor isoforms incorporating exon 8IIIb, caused perinatal lethality with multiple organ defects including a complete failure of growth and branching in the lung (De Moerlooze et al., 2000). Subsequent studies revealed that elimination of Fgfr2(IIIb) also caused growth and branching defects in additional organs including the mammary glands and pancreas (Mailleux et al., 2002; Pulkkinen et al., 2003). Although Fgfr2 has previously been implicated in branching morphogenesis in several organs outside of the male reproductive tract (Marker et al., 2003a; Shukri et al., 1988). By contrast, many Fgfr2 loss-of-function mutant alleles result in lethality due to affects on multiple organ systems. Fgfr2 is known to be required for early mammalian development because an Fgfr2-null mutation caused peri-implantation embryonic lethality shortly after embryonic day (E) 4.5 (Arman et al., 1998). A strong hypomorphic Fgfr2 allele that deleted the exons encoding the immunoglobulin-like domain III extracellular ligand-binding domain caused embryonic lethality around E10.5, a failure of limb bud induction, and placental defects (Xu et al., 1998). Fgfr2(IIIb), an isoform-specific knockout of Fgfr2 that eliminated receptor isoforms incorporating exon 8IIIb, caused perinatal lethality with multiple organ defects including a complete failure of growth and branching in the lung (De Moerlooze et al., 2000). Subsequent studies revealed that elimination of Fgfr2(IIIb) also caused growth and branching defects in additional organs including the mammary glands and pancreas (Mailleux et al., 2002; Pulkkinen et al., 2003). Although Fgfr2 has previously been implicated in branching morphogenesis in several organs outside of the male reproductive tract, branching morphogenesis in the seminal vesicles and prostate seem particularly sensitive to the affects of the svs mutation. For example, we have analyzed whole-mount preparations of svs mammary glands and evaluated the histology of svs lungs (data not shown). These efforts did not reveal dramatic branching morphogenesis defects in svs mutants. However, very subtle affects of the svs mutation on branching in these organs might have been missed.

Initial interest in identifying the gene affected by the svs mutation came from the previously described svs phenotypes, which include a complete failure of branching morphogenesis during seminal vesicle development and dramatically reduced branching morphogenesis in the prostate gland without associated defects in organ growth or differentiation (Marker et al., 2003a). Genes from the fibroblast growth factor family, hepatocyte growth factor family, epidermal growth factor family, transforming growth factor beta superfamily, sonic hedgehog pathway and, more recently, notch signaling have all been implicated as regulators of branching morphogenesis (Davies, 2002; Marker et al., 2003b; Wang et al., 2006). However, it is often unclear what precise role each gene plays in controlling branching morphogenesis. Many of the genes are temporally and spatially regulated during development and are likely to regulate multiple steps during organogenesis. The multiple roles of key regulatory genes make it difficult to establish a precise function for each gene during branching morphogenesis.

It has previously been suggested that, in the prostate and seminal vesicles, signaling by FGF7 and FGF10 through FGFRII(IIIb) is important for epithelial proliferation and duct elongation during branching morphogenesis (Thomson, 2001; Thomson and Cunha, 1999). FGF7 and FGF10 are expressed by the mesenchyme of both the prostate and seminal vesicles during development, and recombinant FGF7 or FGF10 stimulated both growth and branching of developing prostates and seminal vesicles in vitro, acting at least in part as pro-proliferative signals for the epithelium (Alarid et al., 1994; Sugimura et al., 1996; Thomson and Cunha, 1999). The requirement for FGF10 for prostate and seminal vesicle development was confirmed by experiments showing that Fgfol0-null embryos develop only minimal prostatic organ rudiments and that the caudal segments of the Wolffian ducts, which are the precursor structures for the seminal vesicles, degenerate in a majority of Fgfol0-null embryos (Donjacour et al., 2003). Additionally, grafting of embryonic prostates revealed that minimal prostate development occurred from Fgfol0-null prostates. Similarly, grafting the caudal Wolffian ducts from the rare Fgfol0-null embryos in which they did not degenerate, revealed that Fgfol0-null embryos had a limited ability to develop rudimentary seminal vesicles, with only one in eight grafted Wolffian ducts resulting in tissue that resembled immature seminal vesicle (Donjacour et al., 2003).

The fact that prostates and seminal vesicles exhibit no size deficit in svs mutant mice (Marker et al., 2003a), whereas Fgfol0-null embryos exhibit a dramatic loss of growth for both organs, suggests that FGF10 can partially signal through the reduced levels of FGFRII(IIIb) still present in svs mutant organs and that this is sufficient to support organ growth. However, branching morphogenesis fails completely in svs seminal vesicles and is reduced by ~ 40% in svs prostates (Marker et al., 2003a). This
suggests that peak levels of FGF10 signaling through FGFR2 are required to induce branching because partial loss of FGFR2(IIIb) in svs mutant mice blocked branching in the seminal vesicles and reduced branching in the prostate. FGFR2(IIIb) can activate several downstream signaling pathways. This study highlights the importance of the MEK1/2-ERK1/2 signaling pathway in branching morphogenesis. The svs mutant seminal vesicles failed to maintain activation of the MEK1/2-ERK1/2 pathway during branching morphogenesis despite similar overall levels of FGFR2 protein expression in wild-type and mutant seminal vesicles. The loss of ERK1/2 activation is likely to result from the shift in Fgfr2 alternative splicing that decreases the abundance of exon 8(IIIb)-containing isoforms and results in the ectopic expression of exon 8(IIIc) isoforms that are normally not expressed during seminal vesicle development (Fig. 4). FGF7 and FGF10 are thought to be the ligands that activate FGFR2 during seminal vesicle and prostate development (Thomson, 2001; Thomson and Cunha, 1999). Since these ligands cannot signal via exon 8(IIIc)-containing FGFR2 isoforms, the partial loss of exon 8(IIIb)-containing isoforms may be sufficient to explain the loss of ERK1/2 activation in svs mutant seminal vesicles. Previous studies have also shown that different isoforms of FGFR2 can heterodimerize (Tanahashi et al., 1996), suggesting that heterodimers between exon 8(IIIb)-containing and exon 8(IIIc)-containing FGFR2 isoforms in svs organs may further reduce the availability of functional receptors for FGF7 and FGF10. It is also possible that the ectopic expression of 8(IIIc)-containing FGFR2 isoforms in svs mutant mice could cause gain-of-function phenotypes owing to signaling through other known FGFR2 downstream signaling pathways such as p38 MAPK, AKT or PLCγ (Ceridono et al., 2005; Chen et al., 2000; Mehta et al., 2001).
activity before examining transcript levels. For the in vitro organ cultures, the ventral prostate and lateral prostate were treated with recombinant FGF10 for 24 hours, then RNA was extracted and transcript levels determined (Huang et al., 2005). This time frame is short compared with the situation for the seminal vesicles from the svs mutant mice, which experienced decreased FGFR2(IIib) signaling throughout organogenesis. Furthermore, it is likely that branching morphogenesis regulators participate in feedback loops that are reiteratively used during ductal elongation and branching, such that long-term loss of a key gene such as Fgfr2(IIib) would cause a general downregulation of the branching process and its associated gene expression.

**Conclusions**

The mouse svs mutation causes a complete loss of branching morphogenesis in the seminal vesicles and a dramatic reduction of branching in the prostate without changes to organ growth or differentiation. These phenotypes are caused by a 491 bp insertion in the tenth intron of Fgfr2, which is associated with aberrant alternative splicing that alters receptor activity without affecting protein expression levels or transcript localization. The partial loss of IIib-containing transcripts is responsible for svs phenotypes because a null allele of Fgfr2 failed to complement the svs mutation. Furthermore, the reduced FGFR2(IIib) activity caused a loss of sustained ERK1/2 activation and a reduction in the transcript levels of Shh, Ptc1, Gli1, Gli2, Bmp4 and Bmp7, which are important regulators of branching morphogenesis. Thus, the svs mutation provides a unique model to study branching morphogenesis of the prostate and seminal vesicles and FGFR2 function during development and in the adult.

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**References**


svs mutation is allelic with fgfr2