The BMP Family Member Gdf7 Is Required for Seminal Vesicle Growth, Branching Morphogenesis, and Cytodifferentiation

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Epithelial–mesenchymal interactions play an important role in the development of many different organs and tissues. The secretory glands of the male reproductive system, including the prostate and seminal vesicles, are derived from epithelial precursors. Signals from the underlying mesenchyme are required for normal growth, branching, and differentiation of the seminal vesicle epithelium. Here, we show that a member of the BMP family, Gdf7, is required for normal seminal vesicle development. Expression and tissue recombination experiments suggest that Gdf7 is a mesenchymal signal that acts in a paracrine fashion to control the differentiation of the seminal vesicle epithelium. © 2001 Academic Press

INTRODUCTION

Epithelial–mesenchymal interactions are required for normal development of many different organs and tissues (Johnson and Tabin, 1997; Thesleff and Sharpe, 1997). Previous studies have shown that epithelial–mesenchymal interactions are necessary for the development of the male accessory sex organs, which are a group of glands that produce secretory products necessary for male reproductive function (Cunha et al., 1992). These glands include the seminal vesicles, which are derived from the Wolffian duct, and the prostate, which is derived from the urogenital sinus.

Early in development, the Wolffian duct is a simple epithelial tube surrounded by mesenchyme. At day 17 of mouse embryonic development, a portion of this tube grows and curves to form the seminal vesicle precursor. Shortly after birth, the epithelium of the seminal vesicle precursor undergoes dramatic growth and branching (Lung and Cunha, 1981; Shima et al., 1990). By day 15 after birth, the epithelium of the gland begins to elaborate a characteristic set of secretory proteins. In adult males, seminal vesicle secretions are mixed with sperm at ejaculation. The major secretory proteins of the seminal vesicles comprise the bulk of the copulatory plug that forms after mating (Bradshaw and Wolfe, 1977; Fawell et al., 1987).

The Wolffian duct is also the precursor for several other male reproductive structures in addition to the seminal vesicles, including the epididymis, vas deferens, and ampullary gland (Lipschutz et al., 1999). All of these Wolffian duct derivatives can be distinguished by their tissue-specific morphology, histodifferentiation, and tissue-specific secretory proteins (Brooks and Higgins, 1980; Fawell et al., 1986; Taragnat et al., 1988). It is thought that inductive influences from surrounding mesenchyme act to specify the characteristic patterns of epithelial morphogenesis and differentiation along the length of the Wolffian duct. For example, tissue recombination experiments have shown that seminal vesicle mesenchyme is capable of...
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eliciting seminal vesicle differentiation from epithelium that would normally form epididymis, vas deferens, or ureter (Higgins et al., 1989b; Lipschutz et al., 1996) in the absence of mesenchyme, seminal vesicle epithelial growth, branching, and differentiation are inhibited (Cunha, 1992; Higgins et al., 1989a). Thus, signals from seminal vesicle mesenchyme can instructively program Wolffian-derived epithelium to adopt seminal vesicle morphology and cytodifferentiation.

Two members of the fibroblast growth factor (FGF) family, FGF7 and FGF10, previously have been implicated as important mesenchymal signals in seminal vesicle development. FGF7 is produced by seminal vesicle mesenchyme and can stimulate seminal vesicle growth and branching in vitro (Alarid et al., 1999). FGF10 is expressed in the mesenchyme of the developing seminal vesicle (Thomson and Cunha, 1999), and FGF10 knockout mice lack seminal vesicles (A. Donjacour and G.C., unpublished observations).

The transforming growth factor β (TGFβ)/Bone morphogenetic protein (BMP) superfamily has also previously been implicated in epithelial-mesenchymal interactions during the formation of many different organs and tissues (Hogan, 1996). Members of the BMP family are expressed in mesenchyme of the developing gut, tooth, and kidney and can influence the development of the associated epithelium (Godin et al., 1999; Immergluck et al., 1990; Panganiban et al., 1990; Reuter et al., 1990; Thesleff and Sharpe, 1997). TGFβ1 is expressed in the developing prostatic mesenchyme (Timme et al., 1994), and growth of the developing prostate can be inhibited by exogenous TGFβ1 in organ culture (Itoh et al., 1998). Here, we report a combination of genetic, expression, and tissue recombination experiments that identify a highly conserved BMP family member called Growth/differentiation factor 7 (Gdf7) (Storm et al., 1994; Davidson et al., 1999) as an essential mesenchymal signal required for normal growth, branching, and functional differentiation of seminal vesicle epithelium.

MATERIALS AND METHODS

Vector Construction and Homologous Recombination

Genomic DNA clones containing the Gdf7 locus were isolated from a mouse 129SvJ genomic phage library (Stratagene). A 1.1-kb fragment containing the mature signaling domain of the Gdf7 gene was replaced by a positive selectable neomycin resistance cassette introduced during the targeting (Fig. 1) using the primer set 5′-CCATATTCAAGCTGGCCTCAGG-3′ and 5′-ACCGCCTCCAACTAAAAGG-3′, and one set that amplifies a 310-bp fragment from the neomycin resistance cassette introduced during the targeting (5′-TGAGAGGCTATCCGCTATGAC-3′ and 5′-TACCTGCTCGCAAGCAAGG-3′). PCR was performed using 35 cycles of 94°C for 30 s, 63°C for 1 min, and 72°C for 30 s (Fig. 1).

In Situ Hybridization

Antisense riboprobes were generated for Gdf7, Bmpr1A, and Bmpr1B by in vitro transcription as previously described (Storm and Kingsley, 1996). The probe for Gdf7 was generated by subcloning a 290-bp PCR fragment amplified from the 3′UTR of the gene using the primer set 5′-GCCACGTACATCTGCTCAG-3′ and 5′-ACCGCCTCCAACTAAAAGG-3′ and cloned into the PCRII vector (Invitrogen). This subclone (Gdf7s) was then linearized with SpeI and transcribed with T7 RNA polymerase. The Bmpr1A probe was generated by linearizing plasmid pTFR-EC (a 796-bp fragment of the Bmpr1A gene cloned into pBlueScript II; a gift from Yugi Mishina) with Xhol and transcribing with T7 RNA polymerase (Mishina et al., 1995). The Bmpr1B probe was generated from an IMAGE cDNA clone (accession number AA350367; Research Genetics) (Lennon et al., 1996) by linearizing with Xhol and transcribing with T3 RNA polymerase. Embryonic male urogenital tissue was frozen in OCT compound, 12-micron cryosections were collected, and in situ hybridization was carried out as previously described (Storm and Kingsley, 1996). Sense strand riboprobes were used as hybridization controls.

Histology

Tissue was fixed overnight in 4% paraformaldehyde in PBS and embedded in paraffin by standard procedures. Then 7-micron sections were collected, dewaxed in xylene, and stained with hematoxylin and eosin.
Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed/paraffin-embedded tissues. Sections (6 μm) were cut, deparaffinized in histoclear (National Diagnostics), and rehydrated through decreasing grades of alcohol into PBS. Antigen retrieval was performed by using antigen unmasking solution (Vector Laboratories) for staining with antibodies against androgen receptor (Affinity Bioreagents, Inc.), cytokeratin 8 (gift of E. B. Lane, University of Dundee, UK), and cytokeratin 14 (Biogenex, Inc.). Antigen retrieval was not performed for staining with antibodies against smooth muscle actin (Sigma) or mouse seminal vesicle secretory proteins (Fawell et al., 1987). Prior to staining, sections were treated for 10 min with 3% H2O2 and blocked with blocking solution (ImmunoVision). For primary antibody staining, antibody dilutions were as follows: androgen receptor (1:50), cytokeratin 8 (undiluted hybridoma-conditioned medium), and cytokeratin 14 (1:40), smooth muscle actin (1:500), and mouse seminal vesicle secretory proteins (1:5000). Secondary antibodies were anti-mouse biotin conjugated or anti-rabbit biotin conjugated (Sigma) used at dilution 1:200 in PBS containing 1% Sheep Serum. Immunostain was developed by using the vectastain peroxidase system (Vector Laboratories, Burlingame, CA), and sections were counter-stained with hematoxylin.

Polyacrylamide Gel Electrophoresis and Western Blotting

Protein samples were prepared by collecting 100-μl volumes of both mutant and wild-type seminal vesicle (tissue + secretion) and boiling them in 200 μl of 10% SDS for 5 min. These samples were then spun in a microfuge and 12 μl of the supernatant was either mixed directly or diluted 1:10 and mixed with 8 μl of loading buffer (125 mM Tris, pH 6.8, 20% glycerol, 4.1% SDS, 2% β-mercaptoethanol, and .001% bromophenol blue). Samples were boiled for 5 min and run on a 15% polyacrylamide gel by using the system of Laemmli (1970). Gels were then either immunoblotted or directly stained with Coomassie blue to visualize the proteins (0.25% Coomassie blue, 45% methanol, 10% acetic acid). Gels were transferred electrophoretically to nitrocellulose membrane by using a BioRad transblot apparatus (transfer buffer contained 25
FIG. 2. Seminal vesicle defects in Gdf7 mutant mice. Dissected organs of 12-wk-old adult wild-type (A) and mutant mice (B) show a significant reduction in the size of the seminal vesicles (SV) in the Gdf7 mutant. Seminal vesicles cut in longitudinal section from mutant mice show absence of normal epithelial folds (G vs. H, arrows), and accumulation of large numbers of small, dark staining cells beneath abnormally large epithelial cells (I vs. J, arrowhead). In contrast, the gross and histological appearance of the testes (T), epididymis (E), and vas deferens (VD) are similar in wild-type (A, C, E) and mutant (B, D, F). P, prostate; B, bladder; small arrows, spermatozoa. Scale bars: 2 mm (A, B), 50 microns (C–F), 500 microns (G, H), 35 microns (I, J).
FIG. 3. Immunohistochemical analysis of Gdf7−/− and Gdf7+/− (littermate control) seminal vesicles. Gdf7−/− (B, D, F, H) and control (A, C, E, G) adult seminal vesicle tissue from 10-wk-old mice was cut in cross section and analyzed by immunohistochemistry with four different marker antisera. Anti-smooth muscle actin antisera showed similar staining in control (A) and Gdf7−/− seminal vesicles (B), demonstrating that the smooth muscle is unaffected in the Gdf7 mutant. Androgen receptor and cytokeratin 8 are normally expressed in the seminal vesicle epithelium (C, E) and are present in Gdf7−/− epithelium, but at reduced levels (D, F). Expression of the basal cell marker cytokeratin 14 is seen in rare, scattered cells in control seminal vesicles (G), and in a much larger number of cells in the Gdf7−/− mutant (H). Scale bars: 50 microns (A–H).
Germline transmission was obtained for three independent homologous recombination events. A total of 7.5% of selected into embryonic stem cells, and the resulting colonies were targeted clones. Gdf7 mutants were generated by intercrossing animals heterozygous for the targeted allele. As expected, homozygous mice were completely missing sequences from the Gdf7 mature region (Fig. 1). Mutant animals were present at normal Mendelian ratios (37 +/-; 80 +/--; 34 --/--; P = 0.8459) and most showed no overt phenotype. A small percentage of the Gdf7 animals died by 8 wk of age and their skulls showed indications of hydrocephaly as previously described (Lee et al., 1998). Expression of Gdf7 was seen in the choroid plexus, which is responsible for the production of cerebrospinal fluid. Because of the variability and reduced penetration of the hydrocephalus phenotype, it was not further studied.

**Organ Culture**

Neonatal mouse seminal vesicles were cultured as previously described (Shima et al., 1990). The seminal vesicles, prostate, and a small section of urethra were dissected together from day-0 pups. The combined tissues were then cultured on nucleopore filters supported by metal screens in 24-well tissue culture dishes containing 1 ml Dulbecco's Modified Eagle's Media (DMEM) high glucose and Ham's F-12 medium (1:1 vol:vol ratio) supplemented with insulin (10 \( \mu \)g/ml), transferrin (10 \( \mu \)g/ml), l-glutamine (2 mM), Dihydrotestosterone (DHT), gentamycin (50 \( \mu \)g/ml), and fungizone (0.25 \( \mu \)g/ml). DHT (Sigma) was dissolved in ethanol and used at a final concentration of 10\(^{-8}\) M. Media inside the wells were replaced daily and the tissues were grown at 37°C in 5% CO\(_2\).

**Tissue Grafting and Recombination**

Seminal vesicles were dissected from neonatal Sprague–Dawley rats, from 10-wk-old Gdf7+/− mice, and from 10-wk-old Gdf7−/− mice (littermate controls). Rat seminal vesicle mesenchyme was separated from the seminal vesicle epithelium by using trypsin digestion and mechanical separation as previously described (Higgins et al., 1989b). Dissected tissues and tissue recombinants were grafted under the renal capsule of athymic male hosts for 4 wk as previously described (Higgins et al., 1989b).

**RESULTS**

**Generation of Gdf7-Deficient Mice**

A 1.1-kb fragment containing the entire mature signaling region of the Gdf7 gene was deleted by using positive selection in embryonic stem cells (Fig. 1A) (Joyner, 1993). Previous studies have shown that the activity of BMP molecules resides in the mature region (Massague, 1987). In addition, previous studies of other BMP family members have shown that mutations that disrupt only the mature signaling region of the molecule produce the same phenotypes as mutations that completely disrupt the gene (Marker et al., 1997; Storm et al., 1994). The knockout construct should thus generate a null allele of Gdf7. The targeting construct was linearized and transfected into embryonic stem cells, and the resulting colonies were screened by Southern blot hybridization to identify homologous recombination events. A total of 7.5% of selected colonies (9 of 120) showed the restriction fragments expected for integration of homologous recombination events. Germline transmission was obtained for three independent
Gdf7−/− seminal vesicles, suggesting that smooth muscle has formed correctly from the seminal vesicle mesenchyme of Gdf7 mutants (Figs. 3A and 3B). Immunostaining with anti-androgen receptor antisera showed the expected staining in control epithelial cells, but reduced staining in Gdf7−/− (Figs. 3C and 3D). Immunostaining with anti-cytokeratin 8, another marker for luminal cells, also showed the expected staining in the epithelium of control seminal vesicles, and was reduced in the epithelium of Gdf7−/− seminal vesicles (Figs. 3E and 3F). Immunostaining with anti-cytokeratin 14 antisera was used to identify the number and location of basal cells (Peehl et al., 1994). In control seminal vesicles, little staining is seen, reflecting the low number of basal cells normally scattered throughout the epithelium (Fig. 3G). In the Gdf7 mutant, a dramatic increase in cytokeratin 14-expressing cells was seen (Fig. 3H), suggesting a large increase in the number of basal cells.

Mouse seminal vesicle epithelium normally produces large quantities of six secreted proteins, known as seminal vesicle secreted proteins 1–6 (SVSP 1–6), which comprise the bulk of the copulatory plug and give the seminal vesicle its characteristic white appearance (Bradshaw and Wolfe, 1977; Fawell et al., 1987; Higgins et al., 1989a,b). In seminal vesicle extracts from wild-type mice, all six proteins are readily detected by Coomassie blue staining of SDS-PAGE gels and their presence was confirmed by Western blotting with anti-SVSP antisera (Fig. 4, lanes a, b, e, f). None of the six bands could be detected on a Coomassie blue-stained gel of mutant seminal vesicle proteins. Western blotting with anti-SVSP antisera confirmed a dramatic reduction or complete absence of these secretory products (Fig. 4, lanes c, d, g, and h).

**Gdf7 Is Required for Normal Branching Morphogenesis Early in Seminal Vesicle Development**

Seminal vesicle development occurs mostly after birth when the simple epithelial tube that makes up the early seminal vesicle begins to branch and grow. Previous studies have shown that the growth of the organ and the process of branching morphogenesis can be recapitulated in vitro by using an organ culture system (Cooke et al., 1987; Shima et al., 1990). Wild-type and Gdf7 mutant reproductive systems appeared similar at birth. The seminal vesicle precursors showed no obvious defects in size or morphology (Figs. 5A and 5C). The seminal vesicles, prostate, and a small portion of attached urethra were removed intact from newborn mice, and grown together in organ culture. During 5 days in organ culture, the reproductive tract of wild-type males increased substantially in size, and the seminal vesicles precursors showed extensive branching (Fig. 5B). In contrast, little epithelial branching was seen in the seminal vesicle precursor of the Gdf7 mutant in organ culture, although the gland did expand in size (Fig. 5D). In the same cultures, prostate epithelium showed extensive branching in both wild-type and mutant mice (data not shown).

Expression of Gdf7 and Bmpr1A and -1B in Developing Seminal Vesicles

To better understand the role of Gdf7 during seminal vesicle development, we examined the developmental expression of both Gdf7 and the two BMP receptors, Bmpr1A and Bmpr1B. Gdf7 expression was seen in the seminal vesicle mesenchyme as early as 17 days postcoitum (the earliest stage examined) and as late as 9 days after birth (the latest stage examined; data not shown). At 1 day postpartum, Gdf7 is expressed in the mesenchyme adjacent to the epithelium, but no expression is seen in the epithelium itself (Fig. 6A). By day 7, Gdf7 expression is still seen in the mesenchyme, but it appears to be more concentrated to the region of the mesenchyme directly adjacent to the epithelium and is still absent from the epithelium (Fig. 6D). In contrast, the expression of the two BMP receptors, Bmpr1A and Bmpr1B, is restricted to the epithelium at day 1 and day 7 (Figs. 6B, 6C, 6E, and 6F). The ligand and receptor are thus expressed in adjacent tissues, consistent with a role for Gdf7 in mesenchymal–epithelial interactions.

*Fig. 4.* Gdf7 mutant epithelium fails to produce the normal set of seminal vesicle secreted proteins (SVSP). Protein samples from seminal vesicles of 12-wk-old wild-type (a, b, e, f) or mutant (c, d, g, h) mice were run undiluted (a, c, e, g) or diluted 1:10 (b, d, f, h) on SDS-PAGE gels that were stained with Coomassie blue (a–d) or subjected to Western blotting with anti SVSP antisera (e–h). The six characteristic secreted proteins (sizes indicated) that were clearly visible in wild type (a, b, e, f) are absent in the Gdf7 mutant (c, d, g, h).
Wild-Type Mesenchyme Restores Functional Cytodifferentiation of Mutant Epithelium in Tissue Recombination Experiments

Although Gdf7 RNA expression is limited to mesenchyme cells, many of the effects of the mutation are seen in the overlying epithelium, including defects in branching, decrease expression of epithelial markers (androgen receptor and cytokeratin 8), and lack of expression of seminal vesicle secretory proteins. To test whether these epithelial defects could be restored by wild-type mesenchyme, we conducted tissue recombination experiments between rat and mouse. Neonatal rat seminal vesicle mesenchyme (rSVM), adult Gdf7−/− seminal vesicle, and adult Gdf7+/− (littermate control) seminal vesicle were isolated. Immunohistochemistry with anti-SVSP revealed strong staining in the apical cytoplasm of luminal cells and in the adjacent lumen for the Gdf7+/− seminal vesicles (Fig. 7A). Staining was not observed in Gdf7−/− seminal vesicles (Fig. 7B). Tissue of each type was grafted under the renal capsule of male athymic mouse hosts for 1 month. In addition, recombinant grafts of neonatal rSVM with Gdf7−/− or Gdf7+/− tissue were grafted. After 4 wk, grafts were harvested and analyzed by immunohistochemistry for the presence of mouse SVSP. Grafts of nonrecombined rSVM and Gdf7−/− seminal vesicle were negative for staining, while grafts of nonrecombined Gdf7+/−, recombinant rSVM + Gdf7+/− seminal vesicle, and recombinant rSVM + Gdf7−/− seminal vesicle were strongly positive for staining (Fig. 7C, and data not shown). The SVSP-positive epithelium from the rSVM + Gdf7−/− seminal

FIG. 5. Gdf7 is required for branching morphogenesis during early postnatal development. Wild-type (A, B) and Gdf7 mutant (C, D) reproductive systems were dissected on the day of birth (day 0) and grown in culture on Millipore filters for 5 days. Both wild-type (A) and Gdf7 mutant (C) are similar in size and showed no signs of branching at day 0. After 5 days in culture, the wild-type seminal vesicles showed substantial signs of growth and branching (B), while the Gdf7 mutant seminal vesicles showed a normal amount of growth, but little or no branching (D). P, prostate; SV, seminal vesicle. Scale bars: 100 microns (A–D).
FIG. 6. Expression of Gdf7 and BMP receptors in reciprocal layers of developing seminal vesicles. Antisense RNA in situ hybridization experiments were carried out on day 1 (A–C) and day 7 (D–F). Gdf7 expression was clearly seen in mesenchymal tissue surrounding the epithelium at both stages (A, D). In contrast, Bmpr1B (C, F) and Bmpr1B (B, E) receptor expression is seen in the epithelium. A–C and D–F are near adjacent sections. Scale bars: 100 microns (A–F).
vesicle grafts was confirmed to be of mouse (and thus Gdf7−/−) origin by Hoechst 33258 staining that shows a punctate nuclear pattern for mouse but not rat tissue (D, example arrow). Scale bars: 50 microns (A–C), 25 microns (D).

FIG. 7. Wild-type mesenchyme restores secretion in the Gdf7−/− epithelium. Seminal vesicle tissue was subjected to immunocytochemistry using mouse specific anti-SVSP antisera. Staining was abundant in the apical cytoplasm and in the lumen adjacent to the epithelium in Gdf7+/− (littermate control) seminal vesicles (A), but totally absent from Gdf7−/− mutant seminal vesicles (B). When rat seminal vesicle mesenchyme was grafted with Gdf7−/− mutant epithelium for 4 wk, expression of mouse SVSP staining was restored to normal levels in Gdf7−/− epithelium (C). The secretory epithelium of the graft in (C) was confirmed to be of mouse (and thus Gdf7−/−) origin by Hoechst 33258 staining that shows a punctate nuclear pattern for mouse but not rat tissue (D, example arrow). Scale bars: 50 microns (A–C), 25 microns (D).

DISCUSSION

Epithelial–mesenchymal signals are important in the development of a number of different organs and tissues. In some cases, the molecules involved in these signaling events have been identified. For example, in the developing limb bud, signals from the apical ectodermal ridge, an epithelial structure, are required to signal the underlying mesenchymal progress zone so that limb bud outgrowth can occur. In this case, both BMPs and FGFs are required for the reciprocal signaling between the epithelium and mesenchyme (Johnson and Tabin, 1997). During the development of the mammalian tooth, BMPs and FGFs have also been shown to act as signals in both the mesenchyme and epithelium (Thesleff and Sharpe, 1997).

In many cases, classical embryological experiments have revealed reciprocal signaling between mesenchyme and epithelium, but the signaling molecules responsible have not been identified. The male reproductive system is a good example of a system in which a number of classical experiments demonstrate reciprocal epithelial–mesenchymal interactions (Cunha et al., 1992). FGF7 is expressed in the seminal vesicle mesenchyme and seminal vesicle branching morphogenesis is blocked by anti-KGF monoclonal antibody. FGF7 is also capable of partially substituting for
testosterone to stimulate growth and branching of seminal vesicles grown in vitro (Alarid et al., 1994). FGF10 is also a critical mesenchymal factor in seminal vesicle development. FGF10 is expressed in the mesenchyme of the developing seminal vesicle (Thomson and Cunha, 1999), and FGF10 knockout mice lack seminal vesicles (A. Donjacour and G.C., unpublished observations). These results implicate FGF7 and FGF10 as mesenchymal signals responsible for some aspects of seminal vesicle growth and branching morphogenesis. However, many of the other molecules involved in the epithelial–mesenchymal interactions of the male reproductive system remain to be identified.

Our results demonstrate that Gdf7 is a mesenchymal signal critical for the development of the seminal vesicles. The complementary expression patterns of the Gdf7 ligand and BMP receptors suggest that a major target of Gdf7 signaling is the seminal vesicle epithelium. Although the ligand is normally expressed in the mesenchyme, the epithelium of the Gdf7 mutant shows abnormal growth, abnormal cytodifferentiation, failure of normal branching morphogenesis, and failure to produce the normal complement of secretory proteins. Furthermore, the defect in secretory protein production can be rescued by wild-type mesenchyme, strongly arguing that Gdf7 acts as a paracrine mesenchymal signal that is required for normal seminal vesicle development.

One striking aspect of the altered cytodifferentiation in Gdf7 mutants is a high ratio of cytokeratin 14-positive basal epithelial cells to luminal epithelial cells. In wild-type mice, basal epithelial cells are rare; however, in Gdf7 mutants, a continuous layer of basal cells is found. An increased ratio of cytokeratin 14-positive basal cells to cytokeratin 8-positive luminal cells, and loss of secretion of major seminal vesicle proteins, is also seen in castrated rats (Hayward et al., 1996). However, androgen receptor and cytokeratin 8 expression are greatly reduced in Gdf7 mutants, and maintained in castrated animals. It is possible that the reduced expression of major seminal vesicle secretory proteins is secondary to loss of androgen receptor expression in the seminal vesicle epithelium of the Gdf7 mutant. The lineage relationship between basal and luminal epithelial cells has not been firmly established for the seminal vesicle. It has been proposed that the basal cell layer includes stem cells precursors of the secretory epithelium in the prostate (Bonkhooff and Remberger, 1996). If this is true in the seminal vesicle, the accumulation of basal epithelial cells and low number of luminal epithelial cells in Gdf7 mutants may reflect a normal role for Gdf7 in stimulating basal cells to differentiate into luminal cells. Alternatively, Gdf7 may normally act on a common precursor to basal and luminal cells to direct precursor cells toward a luminal cell fate, or Gdf7 may act directly on basal cells to suppress proliferation. Although these potential mechanisms cannot currently be distinguished, the mutant phenotype suggests that Gdf7 normally acts to control the ratio of basal to luminal epithelial cells in the seminal vesicle.

FGF signaling has recently been shown to control branching morphogenesis of the Drosophila trachea (Metzger and Krasnow, 1999). In that system, ectopic expression of FGF is sufficient to trigger new branching of the developing epithelium. Although Gdf7 is required for normal epithelial growth, we have no evidence that it is also sufficient to stimulate epithelial growth and branching morphogenesis. In preliminary experiments, addition of recombinant GDF7 protein to developing seminal vesicles, either globally in the culture media or by local implantation of GDF7-soaked Affigel Blue beads, did not stimulate additional epithelial growth in organ culture (unpublished data). Members of the BMP family may normally signal as hetero- or homodimers, and it is possible that the recombinant protein experiments have not mimicked the form of GDF7 signal actually present in vivo. Alternatively, Gdf7 may normally be a permissive signal for seminal vesicle growth, or may only act in concert with several other molecules to control the development of seminal vesicle epithelium.

We have observed expression of Gdf7 in the mesenchyme of the other Wolffian duct derivatives, including the vas deferens and the epididymis (unpublished data). No obvious morphological or histological defects were observed in either of these structures in mutant mice. It is possible that the normal growth and differentiation of these structures is maintained by other molecules that can substitute for Gdf7. Two good candidates for such molecules are the closely related BMP family members, Gdf5 and Gdf6. Both molecules show almost 90% amino acid identity with Gdf7 in the mature signaling region, and are likely to share many receptors and signaling activities (Storm et al., 1994). We examined the expression of both Gdf5 and Gdf6, and did not detect significant expression in the Wolffian duct derivatives. Mice carrying mutations in either Gdf5 or Gdf6 also showed no obvious defects in the male reproductive tract (unpublished observations). In contrast, mutations in the BMP receptor 1B have recently been reported to elicit sterility in males (Baur et al., 2000; Yi et al., 2000). Although no detailed phenotypic analysis has been reported, it will be interesting to compare the defects in mice missing Gdf7 with the defects in mice missing one of the likely receptors for GDF signaling.

Previous genetic studies have suggested that members of the Gdf5, Gdf6, and Gdf7 subgroup may function in development of both skeletal and soft tissues. Loss of function mutations in Gdf5 are responsible for the classical skeletal trait brachyptism, and subsequent studies in both mice and humans suggest that Gdf5 plays an important role in development of both joints and cartilage (Grunenberg, 1977; Gruneberg and Lee, 1973; Landauer, 1952; Polinkovsky et al., 1997; Storm et al., 1994; Storm and Kingsley, 1996; Thomas et al., 1997). Ectopic expression of Gdf5, Gdf6, or Gdf7 can also stimulate cartilage and bone formation, and tendon and ligament development in vivo (Francis-West et al., 1999; Hotten et al., 1996; Merino et al., 1999; Storm and Kingsley, 1999; Tsukada et al., 1999; Wolfman et al., 1997). Although skeletal structures appear grossly normal in Gdf7
mutant mice, we are currently testing to see whether more subtle defects may be present in skeletal or tendon structures.

Previous studies suggest that Gdf7 is required for normal development of particular axon subtypes within the spinal cord (Lee et al., 1998) and can stimulate the formation of cerebellar granule cells (Alder et al., 1999). The current studies add an essential role in mesenchymal–epithelial interactions to the growing list of functions for the Gdf5/6/7 subgroup of BMP signaling molecules. Further studies will be required to determine how closely related molecules are able to stimulate a variety of distinct cellular responses in skeletal, neural, and urogenital precursors.

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