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Noggin is required for normal lobe patterning and ductal budding in the mouse prostate

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Abstract

Mesenchymal expression of the BMP antagonist NOGGIN during prostate development plays a critical role in pre-natal ventral prostate development and opposes BMP4-mediated inhibition of cell proliferation during postnatal ductal development. Morphologic examination of newborn *Noggin*$^{-/-}$ male fetuses revealed genitourinary anomalies including cryptorchidism, incomplete separation of the hindgut from the urogenital sinus (UGS), absence of the ventral mesenchymal pad, and a complete loss of ventral prostate (VP) budding. Examination of lobe-specific marker expression in the E14 *Noggin*$^{-/-}$ UGS rescued by transplantation under the renal capsule of a male nude mouse confirmed a complete loss of VP determination. More modest effects were observed in the other lobes, including decreased number of ductal buds in the dorsal and lateral prostates of newborn *Noggin*$^{-/-}$ males. BMP4 and BMP7 have been shown to inhibit ductal budding and outgrowth by negatively regulating epithelial cell proliferation. We show here that NOGGIN can neutralize budding inhibition by BMP4 and rescues branching morphogenesis of BMP4-exposed UGS in organ culture and show that the effects of BMP4 and NOGGIN activities converge on P63^{+} epithelial cells located at nascent duct tips. Together, these studies show that the BMP–NOGGIN axis regulates patterning of the ventral prostate, regulates ductal budding, and controls proliferation of P63^{+} epithelial cells in the nascent ducts of developing mouse prostate.
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Keywords: Urogenital sinus; Development; Noggin; Bmp; Prostate

Introduction

The mouse prostate is a male accessory sex organ comprised of three distinct lobes: The coagulating gland (CG, also known as the anterior prostate), dorsolateral prostate (DLP), and ventral prostate (VP). The prostate develops from the urogenital sinus (UGS), a hindgut derivative of endodermal origin (Staack et al., 2003). The first morphological sign of prostate development is outgrowth of UGS epithelium into the surrounding UGS mesenchyme at sites which correspond to the origin of the three adult prostate lobes (Cunha et al., 1987). This process, which generates the main ducts of the adult prostate lobes, is initiated at embryonic day (E)16 in response to androgen stimulation and depends upon signaling interactions between UGS epithelial and mesenchymal layers. A variety of growth and signaling factors play important roles in prostate ductal budding and differentiation. These factors include sonic and indian hedgehog (SHH and IHH), fibroblast growth factor 10 (FGF10), bone morphogenetic proteins (BMP) 4 and 7, transforming growth factor β, notch1, nk3 homeobox 1, and forkhead box a1. Some of these factors promote epithelial proliferation and prostatic bud initiation, elongation, and branching morphogenesis (Almahbobi et al., 2005; Berman et al., 2004; Doles et al., 2006; Donjacour et al., 2003; Freestone et al., 2003; Gao et al., 2005; Huang et al., 2005; Lamm et al., 2002; Pu et al., 2004; Signoretti et al., 2005; Signoretti et al., 2000; Thomson, 2001; Thomson and

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Cunha, 1999; Tomlinson et al., 2004; Wang et al., 2006, 2004). Others inhibit epithelial cell proliferation and restrict prostate budding and branching (Grishina et al., 2005; Lamm et al., 2001). These signaling factors likely exert concerted and complementary actions to initiate bud initiation and outgrowth. However, significant uncertainty persists about the mechanisms that regulate the different signaling pathways, how growth regulation is tied to androgen dependence of prostatic budding, and how positive and negative growth signals are choreographed to produce focal growth at the tip of emerging buds.

Prior to the initiation of prostatic budding, uniform *Bmp4* mRNA expression in UGS mesenchyme mirrors expression of *Shh* in the UGS epithelium. At the onset of ductal budding, *Bmp4* expression is diminished at the tips of buds while *Shh* expression localizes to nascent buds (Lamm et al., 2002, 2001). *Bmp4* expression subsequently diminishes throughout UGS mesenchyme except for tight rings of expression surrounding emerging buds. We postulated that down-regulation of BMP4 activity at sites of bud formation provides for localized de-repression of epithelial proliferation that produces outgrowth of the bud; however, the mechanisms regulating BMP4 expression or activity were unclear. More recent studies have shown *Bmp7* is expressed in both the mesenchyme and epithelium of the developing prostate and, like *Bmp4*, appears to inhibit epithelial proliferation, ductal budding and branching (Grishina et al., 2005). The effects of BMP4 and BMP7 on epithelial proliferation are likely to be a direct effect of the BMP ligands; however, the genes encoding the type I BMP receptors *Bmpr1a* and *Bmpr1b* are expressed in the mesenchyme as well as in epithelium, making paracrine mechanisms also possible. Also like *Bmp4*, *Bmp7* expression decreases in the mesenchyme adjacent to the duct tip. NOGGIN, a secreted factor which binds the BMP ligand in extracellular regions and prevents it from signaling through its receptor (Zimmerman et al., 1996), is critical for normal organogenesis as evidenced by defects in neural tube, somite, and skeletal development and lethality of *Noggin*$^{-/-}$ mutants (McMahon et al., 1998; Smith, 1999). A critical role of NOGGIN in opposing and balancing BMP4 activity is demonstrated by a partial rescue of the *Noggin*$^{-/-}$ phenotype by *Bmp4* haploinsufficiency (Wijgerde et al., 2005). We report here that the BMP–antagonist *Noggin* is expressed in mouse UGS mesenchyme and is required for patterning ventral prostate development. In addition, we show that NOGGIN neutralizes inhibition of epithelial proliferation by BMP4 and postulate that the opposing actions of BMP4 and NOGGIN specify sites of ductal budding and regulate bud outgrowth.

Materials and methods

Tissue collection

CD-1 timed-pregnant mice (Charles River, Willmington, MA) were euthanized by isoflurane overdose. The day of plug was considered E0. UGS from E14 to E18 and prostate from postnatal day (P) 1, 5, and 10 male mice were dissected in Dulbecco's phosphate-buffered saline (PBS). For RT-PCR, UGS specimens were snap frozen in liquid N_2 and stored at −80 °C. For UGS organ culture, the seminal vesicles and bladder were excised and the UGS was temporarily stored in ice-cold PBS until culture initiation. For scanning electronic microscopy, UGS tissue was either stored in 10% FBS in Hank's Balanced Salt Solution (HBSS) overnight at 4 °C or processed immediately.

RNA isolation and RT-PCR

RNA was isolated from pooled UGS (n=6–9 E14–E18 male fetuses from multiple litters) or individual prostate tissue from P1 to P10 male neonates. Specimens were homogenized in Molecular Grinding Resin™ (Genotech, St. Louis, MO) and tissue homogenates were passed through Qiashredder columns (Qiagen, Valencia, CA). RNA was extracted from tissue homogenates with the Qiagen RNeasy kit (Qiagen). Reverse transcription was carried out for 1 h at 42 °C in 1× First stand buffer containing 500 ng total RNA, 0.5 μM dNTPs, 150 ng random hexamers, 5 mM DTT, and 200 U Superscript II Reverse transcriptase (Invitrogen, Carlsbad, CA). Real-time PCR was performed as described previously (Lin et al., 2003) using the Roche LightCycler (Roche Molecular Biochemicals, Indianapolis, IN). PCR primers used in this study are listed in Table 1.

UGSM-2 cell line

The immortalized UGS mesenchymal clonal cell line (UGSM-2) was generated from a parent mixed cell population derived from the UGS of an E16 male INK4a$^{-/-}$ transgenic mouse embryo (Shaw et al., 2006). For gain of function studies, UGSM-2 cells were transfected with a Noggin/GFP bi-citronic expression vector. *Wild-type* (WT) UGSM-2 cells (8×10^6) or UGSM-2 Noggin overexpressing cells were combined with minced P1 WT prostate tissue, allowed to recombine overnight in organ culture, and grafted under the renal capsule for 3 weeks.

Whole-mount in-situ hybridization

UGS and prostate tissue were fixed in 4% paraformaldehyde and hybridized according to a previously described protocol (Lamm et al., 2001). Briefly, tissues were bleached for 60 min in 6% H_2O_2 and digested with 50 μg/ml proteinase K for 30 min at room temperature. Prehybridization (2 h) and hybridization (overnight) were each performed at 68 °C. After high stringency washes, tissues were incubated at 4 °C overnight with pre-blocked alkaline phosphatase-conjugated anti-digoxigenin. The colorimetric reaction used

Table 1
PCR primers for real-time RT-PCR analysis

GenBank accession no.	Entrez gene ID	Forward primer	Reverse primer
NM008711	Noggin (*Nog*)	ACAGCGCCTGAGCAAGAAG	AGGTGCACAGACTTGGATGG
NM007554	Bone morphogenetic protein 4 (*Bmp4*)	AATGTGACACGGTGGGAAAC	TGGGTGATGCTTGGGACTAC
NM009170	Sonic hedgehog (*Shh*)	GTGGAAGCAGGTTTCGACTG	GGTCCAGGAAGGTGAGGAAG
X06246	Microfibrillar-associated protein 5 (*Sbp*)[a]	AGAGCCCAGAATGTCCTGGG	TTATCACGTGCTCTCCGTCC
U89840	Renin1 (*Ren1*)[a]	ACTCGGTGACTGTGGGTGG	AGGTGGGAACCCCTGTTGTAG
AK137501	Probasin (*Pbsn*)[a]	AAAGAGAAAGTACAGAGACAGG	AATGTACAGCGTATCATGGAC
NM008907	Peptidylprolyl isomerase A (*Ppia*)[a]	TCTCTCCGTAGATGGACCTG	ATCACGGCCGATGACGAGCC

[a] From Lin et al. (2003).