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Beta-catenin (CTNNB1) induces *Bmp* expression in urogenital sinus epithelium and participates in prostatic bud initiation and patterning

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ABSTRACT

Fetal prostate development is initiated by androgens and patterned by androgen dependent and independent signals. How these signals integrate to control epithelial cell differentiation and prostatic bud patterning is not fully understood. To test the role of beta-catenin (*Ctnnb1*) in this process, we used a genetic approach to conditionally delete or stabilize *Ctnnb1* in urogenital sinus (UGS) epithelium from which the prostate derives. Two opposing mechanisms of action were revealed. By deleting *Ctnnb1*, we found it is required for separation of UGS from cloaca, emergence or maintenance of differentiated UGS basal epithelium and formation of prostatic buds. By genetically inducing a patchy subset of UGS epithelial cells to express excess CTNNB1, we found its excess abundance increases *Bmp* expression and leads to a global impairment of prostatic bud formation. Addition of NOGGIN partially restores prostatic budding in UGS explants with excess *Ctnnb1*. These results indicate a requirement for *Ctnnb1* in UGS basal epithelial cell differentiation, prostatic bud initiation and bud spacing and suggest some of these actions are mediated in part through activation of BMP signaling.

Introduction

Mouse prostate development initiates from a subcompartment of the lower urogenital tract known as the definitive urogenital sinus (UGS). The UGS must complete a series of developmental events to initiate a normal prostate. One requirement is appropriate UGS epithelial differentiation. The mouse UGS forms from cloacal endoderm at around mouse embryonic day (E)13. Primitive UGS epithelium then differentiates into a multilayered transitional epithelium consisting of basal, intermediate and superficial layers (Abler et al., 2011a). It is generally believed formation of histologically normal prostatic ducts in mice requires basal epithelium, as evidenced by the abnormal prostate ductal phenotype in mice deficient in the basal epithelial cell transcription factor transformation related protein 63 (*Trp63*) (Kurita et al., 2004; Signoretti et al., 2005; Signoretti et al., 2000). However, there is conflicting evidence about whether basal epithelium is required for the earliest stage of prostate ductal development, prostatic bud formation. Kurita et al. (2004) observed in the formation of epithelial evaginations that histologically resembled prostatic buds in mice lacking basal epithelium. However, Signoretti et al. (2000, 2005) reported that prostatic buds do not form in the absence of basal epithelium. It is further unknown how primitive UGS epithelium, marked by TRP63 protein undergoes maturation to form a mature keratin 14 (KRT14)-positive UGS basal epithelium.

Prostatic development also requires appropriate androgen receptor (AR) activation. Testicular androgens activate AR signaling in UGS mesenchyme, which induces prostatic bud formation in UGS epithelium (Cunha et al., 1987; Lasnitzki and Mizuno, 1980). Mouse prostatic buds emerge as solid basal epithelial outgrowths and later arborize and differentiate into a pseudostratified prostate ductal epithelium consisting of neuroendocrine, basal and luminal epithelial cells. Mouse and rat prostatic buds are patterned along two asymmetric axes (dorsoventral, craniocaudal) and one symmetrical axis (mediolateral) to give rise to a ductal network organized into ventral, anterior, dorsal and lateral prostate lobes (Cunha et al., 1987; Timms et al., 1994). In turn, UGS epithelium reciprocally interacts with UGS stroma to pattern

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stromal architecture (Cunha et al., 1992; Hayward et al., 1998). How prostatic buds are patterned is not fully known.

Though many questions remain regarding mechanisms of normal prostate development, there is evidence that CTNNB1 is likely to participate in prostatic bud formation. *Ctnnb1* has been identified in almost every structure that undergoes a budding program; its activation is necessary and/or sufficient for specification of hair follicle buds (Gat et al., 1998; Lo Celso et al., 2004), mammary gland buds (Faraldo et al., 2006), feather buds (Noramly et al., 1999; Widelitz et al., 2000) and tooth buds (Liu et al., 2008). Potential CTNNB1-stabilizing WNT ligands are abundant in male mouse UGS during epithelial differentiation, prostatic bud formation and prostatic branching morphogenesis (Mehta et al., 2011; Yu et al., 2009; Zhang et al., 2006). CTNNB1-responsive *Axin2* and lymphoid enhancer binding factor 1 (*Lef1*) mRNAs are present in basal epithelial cell precursors of the definitive UGS (Abler et al., 2012) and later localize to developing prostatic bud tip epithelium (Mehta et al., 2011; Wu et al., 2011). While these data indicate a role for CTNNB1 in prostate development, there is growing controversy over its exact function. Some evidence supports a role for CTNNB1 in promoting prostatic budding and branching (Joesting et al., 2008), especially during bud specification when CTNNB1 is required (Simons et al., 2012). Other evidence supports an inhibitory role, especially during prostatic ductal branching morphogenesis (Wang et al., 2008; Yu et al., 2009).

In this manuscript, we examined the impact of genetically deleting or inducing excess CTNNB1 expression in UGS epithelium. We specifically sought to identify the CTNNB1 role in UGS epithelium because we previously identified a sexually dimorphic and male dominant pattern of CTNNB1-responsive gene expression in this tissue compartment (Mehta et al., 2011). We show that *Ctnnb1* is needed in UGS epithelium for prostatic bud formation and for formation of UGS basal epithelium. In contrast, we found that expression of excess CTNNB1 in a patchy subset of UGS epithelial cells increases BMP ligand expression in the same cells, increases BMP signaling in surrounding cells, and globally inhibits prostatic bud formation. The latter role positions CTNNB1 to participate in the mechanism by which prostatic bud spacing intervals are established. Because it fulfills at least two roles during prostate development, titration of CTNNB1 signaling is crucial for establishing the normal pattern and number of mouse prostatic buds.

Materials and Methods

Mice

Mice were housed as described previously (Mehta et al., 2011). All procedures were approved by the University of Wisconsin Animal Care and Use Committee. Mice carrying the R26R allele (*Gt(ROSA)26-Sor*^{tm1Sor}), *Ctnnb1* exon 2–6 targeted deletion loss-of-function (LOF) allele (*Ctnnb1*^{tm2Kem}) or the *Ctnnb1* exon 3 targeted deletion gain-of-function (GOF) allele (*Ctnnb1*^{tm1Mmt}) were mated to wild type mice or to mice carrying *Shh*^{cre} (*Shh*$^{tm1(EGFP/cre)Cjt}$) or *Shh*^{creERT2} (*Shh*$^{tm2(cre/ERT2)Cjt}$). To induce Shh^{creERT2}, dams were injected with sterile corn oil (2.5 ml/kg *i.p.* maternal dose) containing 10% ethanol, tamoxifen (25 mg/kg maternal dose, Sigma #T56482, St. Louis MO) and progesterone (18.75 mg/kg maternal dose, Watson #NDC0591-3128-79, Corona CA) and dams were euthanized by CO_2 asphyxiation. Embryos of the following genotypes were assessed alone or together with their phenotypically normal paired littermate controls: *Shh*$^{cre/+}$;*R26R*, *Shh*$^{+/+}$;*R26R*, *Shh*$^{creERT2/+}$;*R26R*, *Shh*$^{+/+}$;*R26R*, conditional *Ctnnb1* loss-of-function (cLOF) *Ctnnb1*^{cLOF} (*Shh*$^{cre/+}$;*Ctnnb1*$^{tm2Kem/tm2Kem}$) and its paired control (*Shh*$^{cre/+}$;*Ctnnb1*$^{tm2Kem/+}$), inducible *Ctnnb1* loss-of-function (iLOF) *Ctnnb1*^{iLOF} (*Shh*$^{creERT2/+}$;*Ctnnb1*$^{tm2Kem/tm2Kem}$) and its paired control (*Shh*$^{+/+}$;*Ctnnb1*$^{tm2Kem/tm2Kem}$) *or Ctnnb1*^{iLOF};*R26R* (*Shh*$^{CreERT2/+}$;*Ctnnb1*$^{tm2Kem/tm2Kem}$;*R26R*$^{+}$) and its paired control (*Shh*$^{CreERT2/+}$; *Ctnnb1*$^{tm2Kem/+}$*R26R*$^{+}$), inducible *Ctnnb1* gain-of-function (iGOF) *Ctnnb1*^{iGOF} (*Shh*$^{creERT2/+}$;*Ctnnb1*$^{tm1Mmt/tm1Mmt}$) and its paired control (*Shh*$^{+/+}$; *Ctnnb1*$^{tm1Mmt/tm1Mmt}$) (Brault et al., 2001; Harada et al., 1999; Harfe et al., 2004; Soriano, 1999). *Ctnnb1* ^{tm1Mmt} mice were from Dr. Makoto Mark Taketo, Kyoto University, and all other mice were from The Jackson Laboratory. The morning of copulatory plug identification was considered E0.5. Genotyping primer sequences are listed in Supplementary Material Table S1.

Assay for β-galactosidase activity

LacZ-dependent β-galactosidase activity was assessed as described previously (Cheng et al., 1993) with modifications described by Mehta et al. (2011).

Immunohistochemistry (IHC)

For most IHC analyses, UGS tissues were fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in xylene, infiltrated with paraffin and stained as described previously (Mehta et al., 2011). To preserve color intensity in *LacZ*-stained samples, tissues were cleared in XS-3 xylene substitute (Statlab, McKinney, TX) prior to paraffin infiltration. Antibodies are listed in Supplementary Material Table S2. Labeling of TRP63 and CTNNB1 in the same tissue section required using primary antibodies from the same mouse host. The first primary and secondary antibody were added in sequence, tissues were then blocked with unlabeled monovalent anti-mouse IgG and the second primary and secondary antibody were added in sequence. Immunolabeled tissues were counterstained with 4′,6-diamidino-2-phenylindole, dilactate (DAPI) and mounted in anti-fade media (phosphate-buffered saline containing 80% glycerol and 0.2% *n*-propyl gallate).

In situ hybridization (ISH)

Detailed protocols are available at www.gudmap.org and were described previously (Abler et al., 2011b). Primer sequences for generating PCR-amplified probe templates are listed in Supplementary Material Table S3, except for *Bmp4* (Feng et al., 1995) and *Fgf10* (Bellusci et al., 1997), which were generated from plasmid DNA. Some ISH-stained tissues were also immunolabeled using previously described protocols (Abler et al., 2011b; Keil et al., 2012). The staining pattern for each hybridized riboprobe was assessed in at least three litter-independent mice per genotype. Control and mutant tissues were processed together in the same tubes and as a single experimental unit to allow for qualitative comparisons among biological replicates and between genotypes or treatment groups.

UGS organ culture

Dissected UGSs were placed on 0.4 μm Millicell-CM filters and cultured as described previously (Vezina et al., 2008) in media containing 5α-dihydrotestosterone (DHT, diluted from an ethanol stock solution to a 10 nM final media concentration). The following supplements were added alone or in combination with organ culture media: 4-hydroxytamoxifen (Sigma #H6278, > 70% Z-isomer, dissolved in ethanol and diluted in media to 1 μM 4-hydroxytamoxifen and 0.1% ethanol), or recombinant NOGGIN protein (R&D Systems, dissolved in saline and diluted in media to 1 μg/ml). Media and supplements were changed every 2 days.

Scanning electron microscopy

Scanning electron microscopy was conducted as described previously (Lin et al., 2003) at the University of Wisconsin Biological and