Am J Clin Exp Urol 2018;6(4):154-163
www.ajceu.us /ISSN:2330-1910/AJCEU0082654

Original Article

WNT2 is necessary for normal prostate gland cyto-differentiation and modulates prostate growth in an FGF10 dependent manner

Ikenna C Madueke, Wen-Yang Hu, Liwei Huang, Gail S Prins

Department of Urology, College of Medicine, University of Illinois at Chicago, Chicago, IL 60612, USA

Received July 16, 2018; Accepted August 12, 2018; Epub August 20, 2018; Published August 30, 2018

Abstract: Wnt proteins are highly conserved secreted morphogens that function in organ development across species. This study investigates the role(s) of Wnt2 during prostate gland development. *Wnt2* mRNA ontogeny in the rat ventral prostate rapidly declines in expression from peak value at post-natal day (pnd) 1 to nadir levels sustained through adulthood. *Wnt2* mRNA is expressed in prostate mesenchymal cells and Wnt2 protein localizes to both mesenchymal and epithelial cells. Sustained expression of Wnt2 by adenoviral expression during rat postnatal prostate gland development resulted in significant reduction in gland size confirming its necessary decline to permit normal development. Wnt2 overexpression in a murine embryonic urogenital sinus mesenchyme cell line, UGSM2 revealed Wnt2 modulated several growth factors including significant down-regulation of Fgf10, an essential stimulator of normal prostate gland branching morphogenesis. Growth inhibitory effects of Wnt2 were reversed by exogenous Fgf10 addition to developing rat ventral prostates. Renal grafts of *Wnt2*$^{-/-}$ male urogenital sinus revealed that *Wnt2*$^{-/-}$ grafts had a disruption in normal lateral polarity, disruption in cell to cell adhesion, and a reduction in the differentiated luminal cell marker, cytokeratin 8/18. Our results demonstrate that the growth inhibiting effects of sustained Wnt2 during prostate development are mediated, in part, by reduction in Fgf10 expression by mesenchymal cells and Wnt2 plays a role in normal prostate luminal cell differentiation and cell to cell integrity. These findings add to the body of work that highlights the unique roles of individual Wnts during prostate development and suggest that their deregulation may be implicated in prostate pathology.

Keywords: Wnt2, prostate gland, Fgf10, development

Introduction

Prostate gland development is an androgen-dependent process and the complex interactions necessary for normal growth involves multiple mesenchymal-epithelial interactions mediated by several signaling pathways [1-3]. The rodent prostate is an exocrine gland derived from the endodermal urogenital sinus (UGS). Budding of the prostate initiates late in fetal life when epithelial buds from the UGS penetrate into the surrounding urogenital mesenchyme in response to rising levels of testicular androgens [4]. At birth, the rodent prostate is rudimentary and branching morphogenesis followed by cytological and functional differentiation occur during the immediate postnatal period [4]. Similarly to other branched structures, the prostate gland utilizes common and organ-specific morphoregulatory genes to pattern the gland and dictate cell differentiation. Some of these genes include secreted paracrine factors including a member of the fibroblast growth factor (Fgf) family, *Fgf10*. Fgf10 is expressed by prostate mesenchymal cells and has previously been identified as a critical morphogen involved in prostate branching morphogenesis [5, 6]. Despite some evidence of regulation of *Fgf10* by various steroids during prostate development, the process is not completely understood and leaves the possibility of regulation by other morphogens critical to prostate gland development. In other branched structures including the lung and kidney, multiple *Wnt* genes have been shown to play critical roles in gland growth and differentiation [7-10]. The literature, however, is lacking extensive insight into any specific role(s) of *Wnt* genes during prostate gland development.

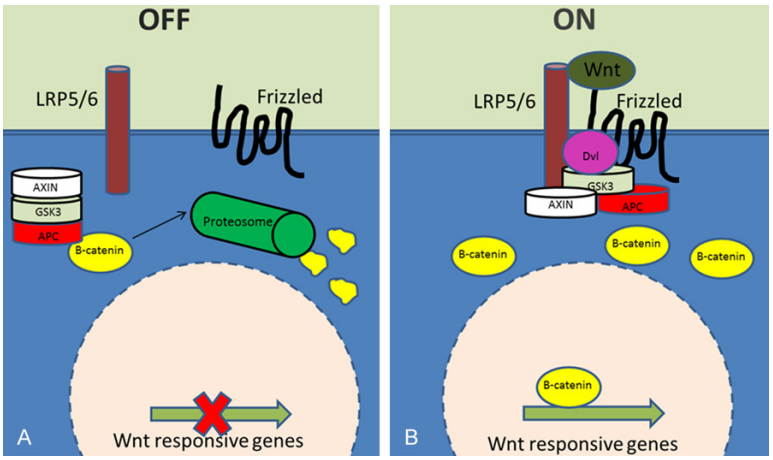

Figure 1. Overview of Canonical Wnt Signaling. A. In the absence of Wnt ligand, cytoplasmic β-catenin forms a complex with Axin, APC and GSK3 where it is then phosphorylated and targeted by proteasome for degradation. Wnt target genes are then consequently repressed in the nucleus. B. In the presence of Wnt ligand, Frizzled and LRP5/6 form a receptor complex, allowing Dvl recruitment which in turn disrupts the destruction complex thus allowing accumulation and translocation of β-catenin to the nucleus where it activates Wnt responsive genes.

Wnt genes are highly conserved secreted glycoproteins and play important roles pertaining to tissue patterning, cell fate and proliferation in both embryonic and adult contexts [11]. To date, there are 19 discovered vertebrate *Wnts* and they are broadly categorized into one of two groups based on their downstream effector. All *Wnts* function by using the cell surface receptor *frizzled (Fzd)*, however, the canonical *Wnts* also recruit a co-receptor low-density lipoprotein receptor-related-protein (LRP5/6) and stabilize cytoplasmic β-catenin leading to its nuclear translocation and gene activation alongside TCF/LEF transcription factors (**Figure 1**) [12-14]. The non-canonical *Wnts* do not recruit LRP5/6 or stabilize β-catenin and use less characterized alternate pathways including Ca^{++}/PKC and RhoA/JNK [15, 16].

Wnt signaling in the prostate is evidenced by work involving *Wnt* signaling antagonists. Culture of prostate explants in the presence of Sfrps or DKKs resulted in increased growth of the gland suggesting a growth inhibitory effect for *Wnts* in the developing prostate [17, 18]. In addition, a screen of the developing rat prostate showed marked expression of *Wnt2*, *Wnt2b*, *Wnt7b*, *Wnt10b*, *Wnt4*, *Wnt5a* and *Wnt11* [19]. In this study, we examined in detail the role of *Wnt2* in prostate gland development.

Wnt2 is a canonical Wnt ligand involved in growth of multiple structures during vertebrate development. Targeted deletion of *Wnt2*$^{-/-}$ in mice resulted in embryonic and perinatal lethality attributed to significant lung hypoplasia [10]. These findings implicate *Wnt2* in the development of organs necessary for life as well as highlight its role in the outgrowth and patterning of another endodermally derived and branched structure, the lung. Rats and mice were used to identify the spatio-temporal expression pattern of *Wnt2* during prostate development, to examine its regulation of prostate expressed growth factors, ductal outgrowth, and cell differentiation. Our results reveal high levels of *Wnt2* expression in the prostate mesenchyme at post-natal day (pnd) 1 with rapid decline through pnd 10 when the bulk of branching morphogenesis occurs. We provide evidence that *Wnt2* is involved in inhibiting prostate gland growth through down-regulation of *Fgf10*. Furthermore, our findings suggest an essential role for *Wnt2* in lateral cell to cell adhesion, cell polarity, and epithelial luminal cell differentiation.

Materials and methods

Animals

All animals were handled according to the principles and procedures of the Guiding Principles for the Care and Use of Animal Research and the experiments were approved by the Institutional Animal Care Committee. Timed pregnant female Sprague-Dawley rats from Harlan (Indianapolis, IN) were monitored for delivery and the day of birth was designated as day 0. The rats were sacrificed by decapitation on postnatal day (pnd) 1, 3, 6, 10, 30 or 90, the urogenital sinus (UGS)-prostate complexes were removed and the separate prostate lobes were micro dissected at 4°C and

Table 1. Sequences for genotyping *Wnt2* embryo

Genotyping primers		
WT PAIR	WT Forward primer	TGAGTCTCACCACTAGCCGCA
	WT Reverse primer	TGAGTCTCACCACTAGCCGCA
KO PAIR	KO Forward primer	TGAGTCTCACCACTAGCCGCA
	KO Reverse primer	TCTCAATGGCTAAGGCGTCG

Table 2. Sequences of primers used for real time PCR

Gene	Forward Primer	Reverse Primer
Rpl19	GGAAGCCTGTGACTGTCCAT	GGCAGTACCCTTCCTCTTCC
Wnt2	TCCGAAGTAGCCGGGAAT	GATCGCAGGAACAGGACTTTAAT

either frozen in liquid nitrogen or fixed for histology.

Wnt2$^{-/-}$ null mutant mice on a SV129 background were generated as previously described [10]. Heterozygote breeders were housed together and the day of vaginal plug was considered gestation day 0.5. Pregnant mothers were anesthetized at gestation day 18.5 (e18.5) and the embryos removed by Cesarean section. The UGS with rudimentary prostates were dissected from male pups and immediately used for renal graft studies. The *Wnt2*$^{-/-}$ embryos were identified visually by a reduced size phenotype and confirmed by PCR while the *Wnt2*$^{+/-}$ and *Wnt2*$^{+/+}$ embryos were distinguished solely by PCR as described below. *Wnt2*$^{-/-}$ did not reveal a Mendelian distribution secondary to significant embryonic resorption by e18.5.

Genotyping

To isolate DNA, a tail snip of each embryo was incubated for two hours at 60°C in 100 µL of PCR digestion buffer (1X PCR buffer (Qiagen), 0.45% NP40 (Sigma), 0.45% Tween 20 (Sigma), 1 mg/mL Proteinase K (Fermentas)). Proteinase K was denatured by boiling at 100°C for 15 minutes followed by 5 minute cooling on ice. 5 µL of digestion sample was used in 25 µL PCR reaction (1X PCR buffer (Qiagen), 1X Q mixture (Qiagen), 0.25 mM DNTP, 10 µM forward and reverse primers, 1.25 U *Taq* DNA polymerase (Qiagen)). PCR was performed on an iCycler (BioRad). Cycling conditions were 95°C for 3 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Primer sequences are listed in **Table 1**. PCR products were separated on 1.5% agarose gel and visualized with ethidium bromide.

Adenovirus propagation

Ad-*Wnt2*-GFP and Ad-GFP were kindly provided by Tong-Chuan He (University of Chicago) and viral stock was propagated in HEK293 cells. Cells were harvested 72 hours after infection, pelleted, resuspended in PBS, and lysed by three freeze-thaw cycles using dry ice-chilled methanol and 37°C water bath. Cell debris was removed by centrifugation at 2000 *g* and concentrated virus aliquoted and stored at -80°C. Viral titer is represented as Multiplicity Of Infection (MOI).

Prostate organ culture

To examine the effect of *Wnt2* on normal rat prostate development, rudimentary ventral prostate lobes (VP) were removed on pnd 0 (within 4 hr of birth) and paired, contralateral lobes from a single pup (n = 6) were cultured for 6 days in either basal culture medium (BOCM) with 50 MOI Ad-*Wnt2*-GFP or 50 MOI Ad-GFP as controls. The BOCM consisted of DMEM/F-12 (Invitrogen/GIBCO, Carlsbad, CA), 50 µg/ml Gentamycin, 1 × insulin-transferrin-selenium and 10 nM testosterone and was replaced every 48 hr. Tissues were floated on Millicell-CM filters (Millipore Corp., Bedford, MA) in 2 mL medium in BD Falcon 12-well plates (BD Biosciences, San Jose, CA) inside a closed culture chamber at 37°C with ports for introduction and exhaust of humidified 5% CO_2 (Carl Zeiss MicroImaging, Inc., Thornwood, NY). The culture chamber was placed within a heated incubator attached to a Zeiss Axiovert 200 inverted microscope with an automated X-Y-Z stage and Axiocam HRm digital camera. Movement of the automated stage to photograph each cultured prostate in sequence and image acquisition were controlled by AxioVision 4.7 and Zeiss Imaging 25A software. Digital photographs of each sample were captured at 30 minute intervals.

Renal grafting

The UGS-prostate complexes from e18.5 *Wnt2*$^{-/-}$ and wild-type mice were grafted under the renal capsule of 7-8 week old male nude

Figure 2. Ontogeny of *Wnt2* mRNA expression in the rat ventral prostate lobes as quantified by real-time RT-PCR. *Wnt2* expression in the VP was high at birth and rapidly declined through pnd 10. The expression level increased at pnd 30 and fell to nadir levels maintained through adulthood.

mice as described (http://mammary.nih.gov/tools/mousework/Cunha001/index.html). Wild-type and mutant tissues were grafted to contralateral kidneys of recipient mice which were supplemented with subcutaneous 0.5 cm silastic capsules filled with crystalline testosterone to maintain adequate testosterone in these mildly hypogonadal males. After 30 days, the kidneys were removed and grafts were cut out en masse and fixed in methacarn. A small piece of graft tissue was taken before fixation and used for genotyping.

Immunohistochemistry (IHC)

Proteins of interest were localized by IHC in fixed, dehydrated, and paraffin-embedded tissues. Tissues were fixed in methacarn overnight followed by another 24 hours in 70% ethanol. Fixed tissues were then dehydrated in gradient ethanol 70-100% and paraffin embedded. Paraffin-embedded tissue sections were heat treated in a Decloaker pressure cooker (Biocare Medical, Walnut Creek, CA) in Tris. EDTA pH 9.0. Sections were next blocked with SuperBlock PBS (Thermo-Fisher Scientific, Waltham, MA) and incubated overnight at 4°C with rabbit anti-Wnt2 antibody (1:200, Santa Cruz), guinea pig anti-CK8/18 (1:800, American Research Products 03-GP11, Belmont, MA), rabbit anti-E cadherin (1:200, Santa Cruz), rabbit anti-Ki67 (1:200, Abcam). The sections were reacted with biotinlyated anti-IgG (Vector Laboratories, Inc., Burlingame, CA) and detected with avidin-biotin peroxidase (ABC-Elite, Vector Laboratories) using diaminobenzidine tetrachloride (DAB) as chromagen. For controls, normal rabbit, or guinea pig IgG was substituted for primary antibody. The sections were counterstained with Gill's #3 hematoxylin (1:4).

Whole mount in situ hybridization (wmISH)

The rat *Wnt*2 plasmid was kindly provided by Dr. Terry Yamaguchi at the National Institutes of Health. The plasmid was linearized by Hind III and XbaI after which a 600 bp anti-sense digoxigenin-labeled RNA probe was prepared by *in vitro* transcription with T7 RNA polymerase (Roche). WmISH was performed as previously described using 0.2 µg/ml digoxigenin-labeled RNA probe [6]. Following overnight incubation with anti-digoxigenin alkaline phosphatase-conjugated antiserum (Roche, Indianapolis, IN), the samples were color reacted with NBT and BCIP (Roche). Pnd 1, 3, 6 prostatic complexes were processed together within each assay to permit direct comparisons of signal intensity for temporal analysis. To identify cellular localization of gene expression, wmISH-stained tissues were then cross-sectioned at 10 µm.

Culture of cell lines

UGSM2 cells were obtained from Dr. Wade Bushman (University of Wisconsin, Madison) and cultured in DMEM/F12 media (Gibco) supplemented with 10% FBS, 1% penicillin/streptomycin, 1% insulin transferrin selenium (ITS) (Gibco), and 10^{-8} M dihydrotestosterone (DHT). All cells were cultured at 37°C, 5% CO_2, and passaged at sub-confluence.

Real-time PCR

Total RNA was extracted using RNeasy Kit (Qiagen, Valencia, CA) with on-column DNase I digestion. 1 µg of RNA was reverse transcribed with RNase H+ MMLV reverse transcriptase at 42°C for 30 minutes using iScript™ cDNA synthesis kit (Bio-Rad). A blend of oligo (dT) and random primers were used for reverse transcription.

Real-time PCR in SsoAdvanced™ SYBR green master mix (Bio-Rad) was carried out using CFX96 Touch™ Real-time PCR Detection System (Bio-Rad). Cycling conditions were 95°C for 3 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Primer sequences are listed in **Table 2**. Ribosomal

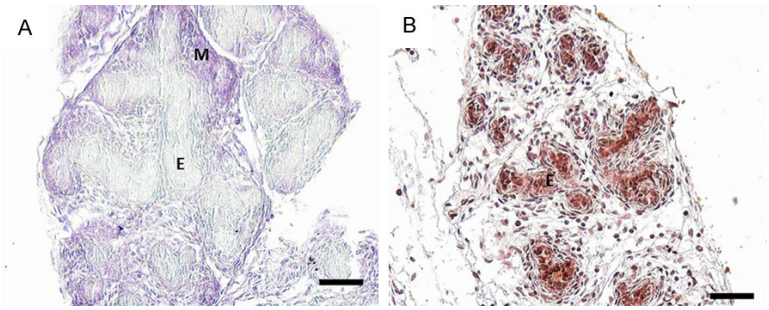

Figure 3. Localization of *Wnt2* transcript (A) and Wnt2 protein (B) in the developing rat prostate gland. *Wnt2* mRNA expression was examined by wmISH and exhibited a periductal pattern typical for mesenchyme-expressed genes. (A) Cross-section of pnd 6 VP from wmISH confirms strong *Wnt2* expression in the mesenchymal (M) cells but not in the epithelial (E) cells. (B) Wnt2 protein was localized by immunohistochemistry in pnd 6 VP to both the mesenchymal and epithelial cells. The tissue sections were counterstained with hematoxylin. Bar = 50 μM.

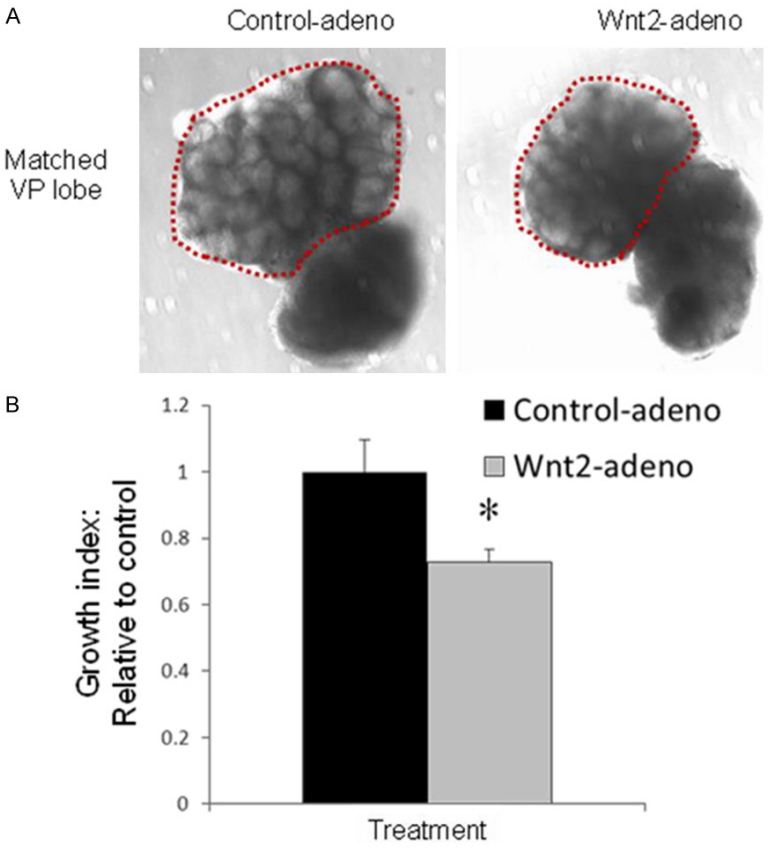

Figure 4. Sustained expression of Wnt2 in the developing rat ventral prostate gland. A. Wnt2 protein was expressed continually in pnd 0 VP explant for 6 days in organ culture using adenovirus containing *Wnt2* expression vector. Red outline denotes glandular portion of gland that is measured for growth. B. Growth index of the VP exposed to sustained Wnt2 protein expression was significantly reduced compared to their contralateral counterpart. N = 3, P < 0.05.

Protein L13 (Rpl13) was quantitated and served as an internal housekeeping gene control for normalization. Optical data obtained by real-time PCR were analyzed with the manufacturer's software (CFX96 Manager™ Software ver. 3.0). Each assay was independently repeated at least 3 times using different cells or tissue.

Statistical analysis

Data were analyzed using Instat ver. 3 (GraphPad Software, Inc., San Diego, CA) using Student's *t* test or ANOVA as appropriate, followed by *post hoc* tests. Values are expressed as mean ± SEM, and $P < 0.05$ were considered significant.

Results

Ontogeny and localization of Wnt2 in the developing rat prostate gland

The temporal and spatial pattern of *Wnt2* expression was followed in the ventral lobe of the developing rat prostate. Expression levels of *Wnt2* mRNA were high at pnd 1 and decreased precipitously to nadir levels by pnd 10 (**Figure 2**). There was a slight but insignificant increase at pnd 30 and nadir levels were maintained through adulthood by pnd 90 (**Figure 2**). During morphogenesis *Wnt2* mRNA localized to the mesenchyme which was confirmed by cross sectional analysis of wmISH pnd 3 rat VP (**Figure 3**). In contrast, *Wnt2* protein localized to both the mesenchyme and epithelial cells documenting its secretion by stromal cells (**Figure 3**).

Role of Wnt2 in prostate growth

An organ culture system that permits prostate morphogenesis development *ex vivo* was used

Table 3. Mouse growth factor array results depicting gene expression following 48 hr *Wnt2* overexpression in UGSM2 cells compared to control treated UGSM2 cells

Gene Name	Fold Change
Anti-Mullerian Hormone	-1.97
Brain-derived neurotrophic factor	2.06
Chemokine (C-X-C motif) ligand 12	-2.29
Fibroblast growth factor-2	2.20
Fibroblast growth factor-7	-2.45
Fibroblast growth factor-10	-1.58
Fibroblast growth factor-18	-2.12
Interleukin-7	-1.66
Interleukin-11	2.18
Interleukin-18	-1.61
Kit ligand	-1.90
Nerve growth factor	1.80
Platelet derived growth factor, alpha	1.63
Secreted phosphoprotein 1	4.64
Teratocarcinoma-derived growth factor 1	2.02
Transforming growth factor alpha	2.24

Note: Table depicts only genes significant across arrays (N = 3) with red showing fold decrease and blue showing fold increase. $P < 0.05$.

to examine the role(s) of *Wnt2* during prostate development. Ontogeny of the rat VP showed rapid decrease of *Wnt2* during prostate morphogenesis (**Figure 2**) suggesting that a decrease in *Wnt2* levels might be necessary to permit normal prostate gland growth. To investigate whether this decrease was necessary, contralateral rat VP lobes were cultured in normal organ culture conditions or in the presence of sustained *Wnt2* expression. A convenient method of increasing protein levels in this organ culture system is by adding exogenous protein, however, Wnt2 protein is not commercially available thus rendering this option null. As an alternate approach, Wnt2 protein was sustained by successful infection of the developing gland with GFP-tagged *Wnt2* expressing adenovirus. Empty GFP-tagged adenoviral vectors were used as control. In the empty vector controls, the VP lobe grew to its normal overall size after 6 days. In contrast, contralateral lobes infected with Ad-*Wnt2*-GFP showed a marked reduction in overall gland size after 6 days (**Figure 4**).

To investigate whether the growth suppressive effects of *Wnt2* were mediated through regulation of growth factors within the stromal cells that express *Wnt2*, *Wnt2* message was overexpressed in a mouse E16 mesenchymal cell line, UGSM2, for 48 hours and secretory gene products were assessed by real-time RT-PCR (**Table 3**). Results showed differential regulation of several genes including down-regulation of *Fgf10*. Fgf10 was further investigated because previous work from our laboratory has shown Fgf10 to be a critical morphogen for stimulating normal prostate gland branching and ductal elongation. When newborn rat ventral prostates were cultured in sustained *Wnt2* expression but with exogenous addition of Fgf10, the growth suppressive effects of *Wnt2* were reversed suggesting that Wnt2's inhibitory effects are mediated in part through downregulation of *Fgf10* (**Figure 5**).

Role of Wnt2 in prostate cell differentiation

The role of *Wnt2* on prostate determination and initiation, as well as functional and cyto-differentiation, was examined in *Wnt2*$^{-/-}$ mouse prostates. Although *Wnt2*$^{-/-}$ mice are embryonic and perinatal lethal, the prostates can be rescued by UGS removal prior to birth and engraftment under the renal capsule for 4 weeks. Due to space constraints, this graft system does not permit for proper assessment of branching patterns and ductal elongation. However, the renal graft permits full assessment of prostate identity and cellular cyto-functional differentiation. Loss of prostatic *Wnt2* did not interfere with the ability of the UGS to be determined with a prostate identity or consequently commence bud initiation. This is evidenced by the growth of the *Wnt2*$^{-/-}$ grafts with a prostate phenotype. Furthermore, grafts were positive for the androgen receptor, basal cell marker p63, and the secretory gene product dorsolateral protein which are specific markers for a prostate phenotype (**Figure 6B**). However, *Wnt2*$^{-/-}$ prostate grafts consistently exhibited loss of lateral epithelial cell to cell connections with apparent loss of epithelial surface marker, e-cadherin. *Wnt2*$^{-/-}$ grafts also exhibited defective luminal cell differentiation as evidenced by marked reduction of the differentiated luminal cell marker, CK8/18 (**Figure 6A**).

Discussion

This study establishes an important role for canonical Wnt2 in prostate gland development. Wnt2 is expressed by prostatic mesenchyme

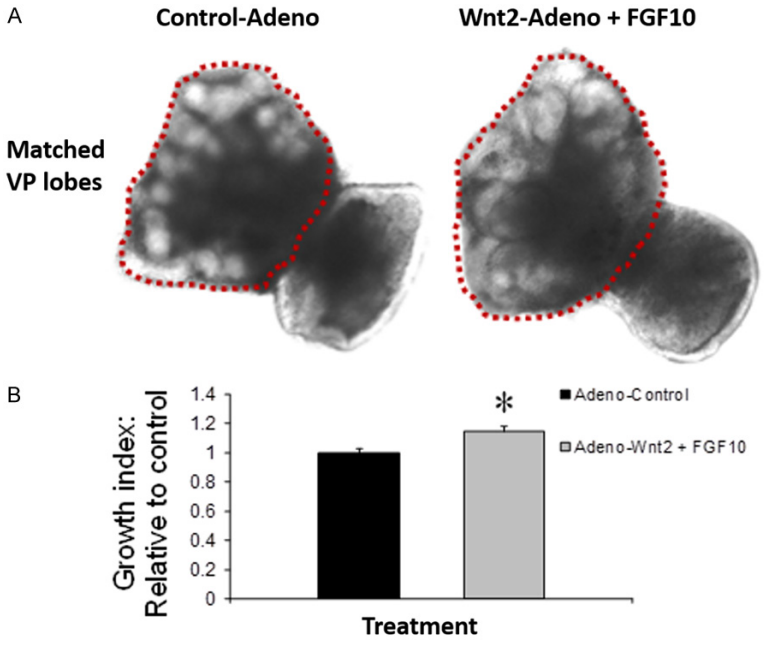

Figure 5. Sustained expression of Wnt2 in the developing rat ventral prostate gland in the presence of FGF10. A. Wnt2 protein was expressed continually in pnd 0 VP explant for 6 days in organ culture using adenovirus containing Wnt2 expression vector but with the addition of 0.5 μg/mL FGF10. Red outline denotes glandular portion of ventral prostate that is measured for growth. B. FGF10 addition to the developing VP in the presence of sustained Wnt2 expression is able to compensate for the reduction in growth index observed with sole Wnt2 expression (**Figure 4**). N = 3, P < 0.05.

which is similar to localization in other developing structures including the fetal lung [8, 10]. Evidence for Wnt2 secretion is documented by immunohistochemistry which localizes Wnt2 protein to the mesenchymal cells, extracellular matrix, and epithelial cells. This implicates Wnt2 as an important morphogen in mesenchymal-to-epithelial interactions that contributes to the morphoregulatory gene network that coordinates normal development of the prostate.

In vivo studies of rat prostates revealed that Wnt2 expression is highest at birth but decreases precipitously to a maintained nadir through adulthood. Concomitant with this drop in Wnt2 expression is rapid proliferation and growth of the prostate gland suggesting a growth inhibitory role for Wnt2. *Ex vivo* cultures of newborn rat prostates confirmed that sustained high Wnt2 expression markedly reduced prostate gland size. Together these findings indicate that Wnt2 has a growth suppressive effect on the prostate and that its rapid decline postnatally is necessary for normal branching and elongation to take place. These results also further corroborate our previous findings where inhibition of canonical *Wnt* signaling by DKK resulted in increased gland growth [19]. Interestingly, *ex vivo* studies of newborn rat prostates cultured in exogenous non-canonical Wnt5a also revealed a growth suppressive effect suggesting inhibition of gland growth by Wnts is not unique to canonical Wnt signaling or Wnt2 alone [20]. Similarly, Wnt5a ontogeny decreases postnatally as observed herein for Wnt2 ontogeny. This implies complexity in Wnt signaling and their downstream pathways in mediating coordinated outgrowth of prostatic ducts during branching morphogenesis.

Despite similarities in *Wnt2* mRNA localization in other structures, tissue specific differences exist as well. Unlike the growth suppressive role discovered for Wnt2 in the prostate, mesenchymal Wnt2 in the lung is growth stimulatory with germline deletion of Wnt2 resulting in significant lung hypoplasia. This underscores the importance of tissue specific morphoregulatory networks and suggests that differences in Wnt2 action across tissues and with other Wnt ligands may be a function of interactions with other morphoregulatory factors.

Mesenchymal-epithelial interactions are integral to the prostate morphoregulatory network [1]. Overexpression of *Wnt2* revealed that *Wnt2* in prostate mesenchymal cells modulates several secreted growth factors including members of the FGF, IGF, IL and BMP families. Included in the FGF family, the present findings show that Wnt2 significantly decreased *Fgf10* expression. This is particularly noteworthy since our laboratory, as well as others, has shown a critical stimulatory role for Fgf10 in prostate ductal branching [5, 6]. In this study, exogenous Fgf10 addition during *ex vivo* cul-

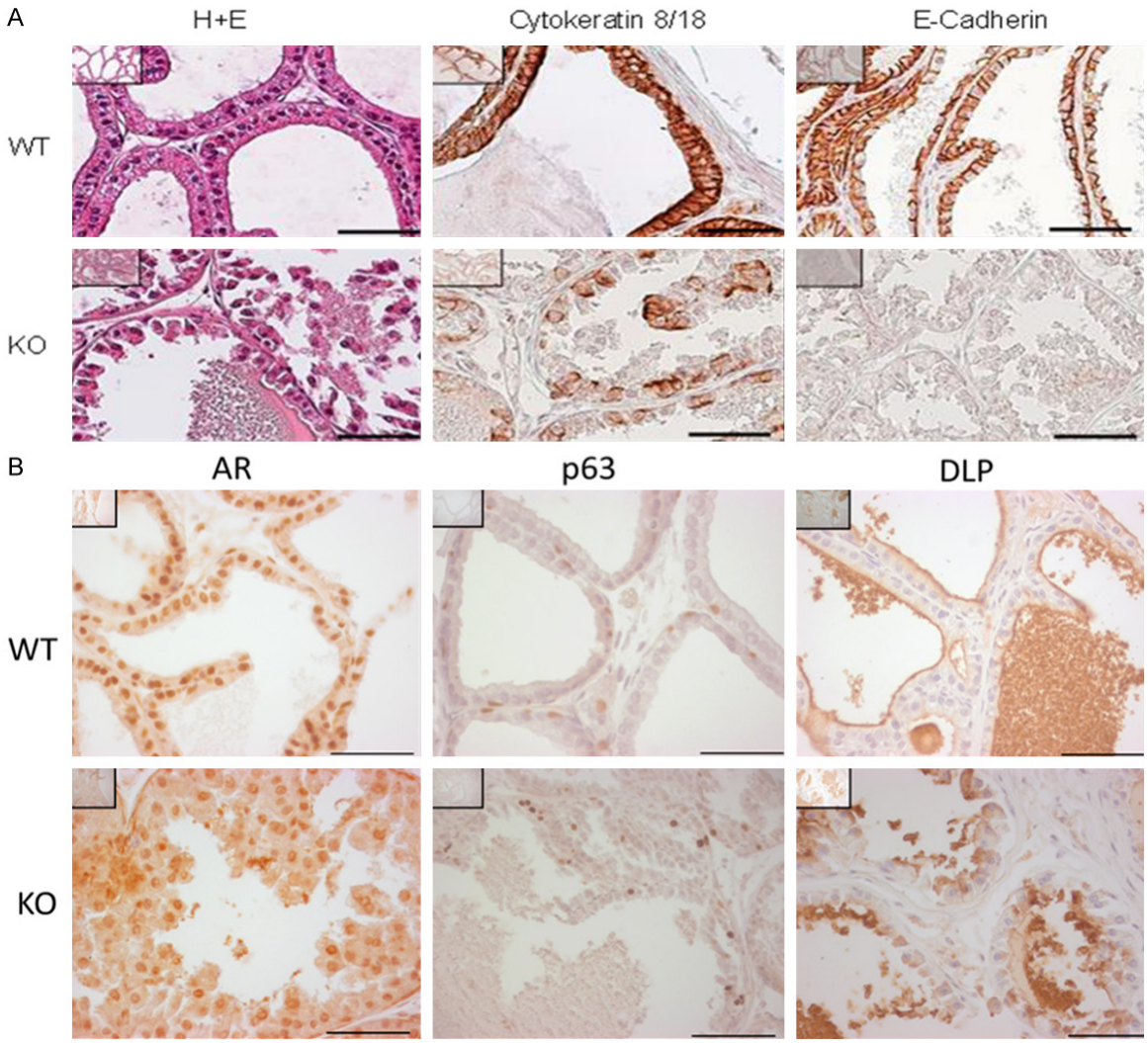

Figure 6. Renal grafts of *Wnt2*$^{+/+}$ (top row) and *Wnt2*$^{-/-}$ knockout (bottom row) mouse prostates. The UGS/prostate complex was removed at e18.5 and grafted under the renal capsule of nude mice for 30 days. A. Tissues were sectioned and stained with H&E or immunostained for CK8/18 (luminal cell marker) and e-cadherin. H&E shows loss of normal lateral cell to cell connections in the mutant and defects in luminal differentiation by reduced expression of CK8/18 and marked reduction in e-cadherin. Scale bar = 50 μM. B. *Wnt2*$^{-/-}$ grafts however express androgen receptor (AR), basal marker p63, and the secretory gene product dorsolateral protein (DLP), which are specific markers of a prostate phenotype. Scale bar = 50 μM (4×).

ture of newborn rat prostates in the presence of sustained Wnt2 reversed the growth suppressive effect of Wnt2 suggesting that the growth suppressive effect of Wnt2 is mediated, in part, through down-regulation of *Fgf10*. Published *Wnt5a* findings from our lab revealed that despite exogenous addition of Wnt5a also having a growth suppressive effect on newborn rat prostate glands, it did not affect Fgf10 levels [20], suggesting that downregulation of *Fgf10* by Wnt2 is a unique mechanism by which Wnt2 suppresses prostate gland growth.

Wnt2 has been implicated in cell lineage specification [21]. In the developing lung, its expression is necessary for specifying lung endoderm [10]. Prostates from null mutant *Wnt2*$^{-/-}$ mice grafted under the renal capsule were able to form prostate tissue expressing AR, p63, and DLP suggesting that Wnt2 is not critical for specifying prostate endoderm. However, the grafts consistently showed defects in epithelial luminal cell architecture with lateral cell to cell connections severely compromised. Furthermore, luminal cell differentiation was also perturbed as the cells lost significant expres-

sion of the luminal differentiation marker CK8/18. The epithelial surface marker E-cadherin was also markedly reduced and could account for the loss in cell connectivity. Although not investigated in these studies, it is worth noting that the selective perturbation of luminal as opposed to basal cell differentiation might be a function of the hierarchy of prostate epithelial cells from a stem cell pool. One camp in this field of study posits that basal and luminal cells arise from a common progenitor while the other believe that they originate from distinct progenitor cell pools [22-24]. If the second is true, then it is plausible that *Wnt2* selectively maintains the luminal progenitor pool and loss of *Wnt2* leads to selective luminal cell differentiation defects while having no effect on basal cell fate.

In summary, this study establishes a role for Wnt2 during prostate gland development. Wnt2 is involved in coordinate regulation of prostate outgrowth though the growth suppressive effects of Wnt2 are mediated in part by a reduction in Fgf10 as well as altered expression of other morphoregulatory factors. Wnt2 is also necessary for normal cytodifferentiation with loss of *Wnt2* resulting in deficient lateral cell to cell connections and luminal cell differentiation defects. Reduction in E-cadherin with loss of *Wnt2* expression suggests a possible pro-oncogenic role. Furthermore, overexpression of *Wnt2* increases several growth factors that are also pro-oncogenic, highlighting that aberrant *Wnt2* signal may be evident in prostate carcinogenesis but a specific role cannot be easily ascribed. In all, our findings contribute to the expanding repertoire of prostate regulatory mechanisms during normal development which will ultimately aide in understanding of abnormal regulation connected with prostate disease.

Acknowledgements

This study was supported in part by NIH training grant T32DK007739, NIH/NIDDK R01 DK40890, and by NIH/NIEHS R01 ES015584. We also wish to thank Lynn Birch for help with animal breeding and manuscript preparation.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Gail S Prins, Department of Urology, College of Medicine, University of Illinois at Chicago, Chicago, IL 60612, USA. Tel: 312-413-9766; E-mail: gprins@uic.edu

References

[1] Cunha GR. Mesenchymal-epithelial interactions: past, present, and future. Differentiation 2008; 76: 578-586.

[2] Siiteri PK and Wilson JD. Testosterone formation and metabolism during male sexual differentiation in the human embryo. J Clin Endocrinol Metab 1974; 38: 113-125.

[3] Wilson JD and Gloyna RE. The intranuclear metabolism of testosterone in the accessory organs of reproduction. Recent Prog Horm Res 1970; 26: 309-336.

[4] Timms BG, Mohs TJ and Didio LJ. Ductal budding and branching patterns in the developing prostate. J Urol 1994; 151: 1427-1432.

[5] Thomson AA and Cunha GR. Prostatic growth and development are regulated by FGF10. Development 1999; 126: 3693-3701.

[6] Huang L, Pu Y, Alam S, Birch L and Prins GS. The role of Fgf10 signaling in branching morphogenesis and gene expression of the rat prostate gland: lobe-specific suppression by neonatal estrogens. Dev Biol 2005; 278: 396-414.

[7] Mcmahon AP and Bradley A. The Wnt-1 (Int-1) protooncogene is required for development of a large region of the mouse-brain. Cell 1990; 62: 1073-1085.

[8] Monkley SJ, Delaney SJ, Pennisi DJ, Christiansen JH and Wainwright BJ. Targeted disruption of the Wnt2 gene results in placentation defects. Development 1996; 122: 3343-3353.

[9] Takada S, Stark KL, Shea MJ, Vassileva G, Mcmahon JA and Mcmahon AP. Wnt-3a regulates somite and tailbud formation in the mouse embryo. Genes Dev 1994; 8: 174-189.

[10] Goss AM, Tian Y, Tsukiyama T, Cohen ED, Zhou D, Lu MM, Yamaguchi TP and Morrisey EE. Wnt2/2b and beta-catenin signaling are necessary and sufficient to specify lung progenitors in the foregut. Developmental Cell 2009; 17: 290-298.

[11] Logan CY and Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20: 781-810.

[12] MacDonald BT, Tamai K and He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 2009; 17: 9-26.

[13] Tamai K, Semenov M, Kato Y, Spokony R, Liu CM, Katsuyama Y, Hess F, Saint-Jeannet JP and He X. LDL-receptor-related proteins in Wnt signal transduction. Nature 2000; 407: 530-535.

[14] Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destrée O and Clevers H. XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. Cell 1996; 86: 391-399.

[15] Strutt DI, Weber U and Mlodzik M. The role of RhoA in tissue polarity and frizzled signalling. Nature 1997; 387: 292-295.

[16] Wallingford JB, Vogeli KM and Harland RM. Regulation of convergent extension in Xenopus by Wnt5a and frizzled-8 is independent of the canonical Wnt pathway. Int J Dev Biol 2001; 45: 225-227.

[17] Wang BE, Wang XD, Ernst JA, Polakis P and Gao WQ. Regulation of epithelial branching morphogenesis and cancer cell growth of the prostate by wnt signaling. PLoS One 2008; 3.

[18] Joesting MS, Cheever TR, Volzing KG, Yamaguchi TP, Wolf V, Naf D, Rubin JS and Marker PC. Secreted frizzled related protein 1 is a paracrine modulator of epithelial branching morphogenesis, proliferation, and secretory gene expression in the prostate. Dev Biol 2008; 317: 161-173.

[19] Prins GS and Putz O. Molecular signaling pathways that regulate prostate gland development. Differentiation 2008; 76: 641-659.

[20] Huang LW, Pu YB, Hu WY, Birch L, Luccio-Camelo D, Yamaguchi T and Prins GS. The role of Wnt5a in prostate gland development. Dev Biol 2009; 328: 188-199.

[21] Wang H, Gilner JB, Bautch VL, Wang DZ, Wainwright BJ, Kirby SL and Patterson C. Wnt2 coordinates the commitment of mesoderm to hematopoietic, endothelial, and cardiac lineages embryoid bodies. J Biol Chem 2007; 282: 782-791.

[22] Wang ZA, Mitrofanova A, Bergren SK, Abate-Shen C, Cardiff RD, Califano A and Shen MM. Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. Nat Cell Biol 2013; 15: 274-283.

[23] Tokar EJ, Ancrile BB, Cunha GR and Webber MM. Stem/progenitor and intermediate cell types and the origin of human prostate cancer. Differentiation 2005; 73: 463-473.

[24] Wang Y, Hayward S, Cao M, Thayer K and Cunha G. Cell differentiation lineage in the prostate. Differentiation 2001; 68: 270-279.