Prostate Organogenesis

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The prostate is a male exocrine gland that secretes components of the seminal fluid. In men, prostate tumors are one of the most prevalent cancers. Studies on the development of the prostate have given a better understanding of the processes and genes that are important in the formation of this organ and have provided insights into the mechanisms of prostate tumorigenesis. These developmental studies have provided evidence that some of the genes and signaling pathways involved in development are reactivated or deregulated during prostate cancer. The prostate goes through a number of different stages during organogenesis, which include organ specification, epithelial budding, branching morphogenesis, canalization, and cytodifferentiation. During development, these processes are tightly regulated, many of which are controlled by the male hormone androgens. The majority of prostate tumors remain hormone regulated, and antiandrogen therapy is a first-line therapy, highlighting the important link between prostate organogenesis and cancer. In this review, we describe some of the data on genes that have important roles during prostate development that also have strong evidence linking them to prostate cancer.

The prostate is a male accessory exocrine gland found exclusively in mammals that functions to produce components of the seminal fluid. Induction and development of the prostate and homeostasis of the adult gland are controlled by androgens, male hormones derived from the testis. Androgens act by binding to the androgen receptor (AR), a hormone-transcription factor found in both the prostate epithelia and stroma, and activate AR by translocating it from the cytoplasm to the nucleus. During prostate development, hormonal activity is integrated with other signaling pathways to regulate transcriptional programs that drive the formation of a fully functional organ. Embryological tissue grafting and transplantation experiments and the analysis of mice carrying genetic mutations

have been instrumental in the dissection of the genes and signaling pathways involved in prostate organogenesis. These studies have shown that, together, molecular and cellular pathways tightly regulate a series of processes that occur to give rise to the adult gland. Many of the same genes and cellular processes, such as proliferation and migration that are important during organ development, are reactivated or deregulated during cancer (Abate-Shen and Shen 2000). A better understanding of the biology of how these pathways orchestrate prostate development can give clues to the mechanisms that are involved in the initiation and progression of prostate cancer, which has increased significance given the high prevalence of this disease in men. In this review, we highlight some of the pathways that are im-

Editors: Michael M. Shen and Mark A. Rubin

Additional Perspectives on Prostate Cancer available at www.perspectivesinmedicine.org

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Cite this article as *Cold Spring Harb Perspect Med* 2018;8:a030353

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portant in the development of the prostate that also have robust evidence connecting them to prostate cancer.

The prostate begins to develop late in embryogenesis and finishes maturation during puberty. The prostate is a branched ductal gland that develops from the urogenital sinus (UGS) formed from the ventral part of the cloaca at

the junction between the bladder and urethra (Fig. 1). The human prostate is acorn-shaped and has three distinct zones—the peripheral, transition, and central zones—which are surrounded by a fibromuscular stroma (Fig. 1A) (McNeal 1981). The ducts do not have a lobular pattern but radiate out to cover most of the urethra. The peripheral zone (the largest zone) is

Figure 1. Schematic diagram showing the structures of the male neonatal urogenital sinus (UGS) that develops into the adult human prostate and adult mouse prostate. (A) The adult human prostate is acorn-shaped and composed of three zones: transition zone, central zone, and peripheral zone. (B) In both the human and mouse prostate, the developing UGS epithelium buds out into the surrounding mesenchyme. (C) The mouse prostate has four prostatic lobes named after their position relative to the bladder: anterior prostate, lateral prostate, dorsal prostate, and ventral prostate. (Panel A is based on data in McNeal 1969. Panel B is reprinted from Bhatia-Gaur et al. 1999, courtesy of Creative Commons License, Attribution—NonCommercial 4.0 International License. Panel C is reprinted from Cunha et al. 1987; adapted, with permission, from Oxford University Press © 2008.)

the site of the majority of prostate cancer, whereas benign prostatic hyperplasia ([BPH] a non-malignant disease) is found in the transition zone. In contrast to the human structure, the mouse and rat prostate consists of four bilateral lobes, named anterior (AP), dorsal (DP), lateral (LP), and ventral (VP) after their orientation with respect to the urethra (Figs. 1C and 2). These display ductal branching organization, histology, and secretory production specific to each lobe (Sugimura et al. 1986b; Hayashi et al. 1991). In the adult human and rodent, the prostate epithelial ductal cells are composed of columnar luminal secretory cells that line the lumen, underneath these are flat basal cells, and dispersed throughout the gland are rare neuroendocrine cells (Fig. 3) (Hayward et al. 1996a; Wang et al. 2001; van Leenders et al. 2003; Hudson 2004; Shappell et al. 2004). The luminal cells express the cytokeratins 8 and 18 and are AR-positive, whereas the basal cells express cytokeratins 5 and 14 and the transcription factor p63. Neuroendocrine cells are marked by chromogranin A and synaptophysin. A basement membrane and a stromal compartment, consisting of smooth muscle cells, fibroblasts, and myofibroblasts, surround the epithelial compartment (Hayward et al. 1996).

In the mouse, about 1 day before birth at embryonic day 17.5 (E17.5), prostate morphogenesis begins as endodermal-derived UGS epithelia (UGE) cells grow into the surrounding mesenchyme to form buds (Figs. 2 and 3) (Marker et al. 2003). In humans, this occurs at week 10 to 12 of gestation (Hamilton et al. 1959). Solid undifferentiated epithelial buds occur at specific sites in the UGS, which later determine the structure and position of the lobes within the adult gland (Fig. 2). The buds elongate into the mesenchyme, followed by branching morphogenesis and ductal canalization and then cytodifferentiation to give rise to mature secretory epithelial structures (Fig. 3). Concomitantly with these processes, the mesenchyme differentiates into various cell types, including periurethral smooth muscle cells and interfascicular fibroblastic cells (Hayward et al. 1996b).

PROSTATE INITIATION AND IDENTITY

The male androgenic steroid hormone testosterone secreted by the testis is converted to the more potent dihydrotestosterone (DHT) by the 5α -reductase enzyme found in prostate cells; DHT then binds to AR and promotes prostate development. The essential role of AR in prostate

Figure 2. Prostatic buds of the developing mouse urogenital sinus (UGS). (A,B) Schematic diagram of the fetal mouse UGS, with the epithelial prostatic buds highlighted in red (A) and the surrounding periprostatic mesenchyme highlighted in red (B). (C) Whole-mount in situ hybridization of *Sox9* on P0 neonatal mouse UGS. Purple stain marks expression of *Sox9*. Black line marks the plane of the section shown in D. (D) Transverse section of a UGS stained for p63 by immunohistochemistry and counterstained with haematoxylin. BI, bladder; AP, anterior prostate; VP, ventral prostate; DLP, dorsolateral prostate; SV, seminal vesicle; Ur, urethra; DD, ductus deferens. (Images A and B are from the GUDMAP database [www.gudmap.org/Schematics]. Originally designed by Kylie Georgas, University of Queensland.)

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Figure 3. Morphological processes that occur during prostate organogenesis and associated genes and signaling pathways. (A) Prostate cell-fate specification and organ initiation. Red box denotes an area where urogenital sinus (UGS) cells have been specified to become prostate. (B) The UGS epithelium buds out into the surrounding mesenchyme. (C) Branching morphogenesis of the prostate epithelia and canalization to produce lumen. (D) Differentiation of the epithelial cells into luminal cells, basal cells, and rare neuroendocrine (NE) cells and mesenchyme into smooth muscle cells. Yellow is the epithelium and light blue is the mesenchyme.

initiation has been most clearly shown in mice and humans with loss-of-function mutations in AR, where a prostate gland fails to form (Brown et al. 1988; Lubahn et al. 1989; Charest et al. 1991; Gaspar et al. 1991; He et al. 1991). Androgens are also sufficient for prostate development, as an ectopic organ can develop in females exposed to testosterone (Takeda et al. 1986). Not only do androgens control the early stages of prostate organ initiation, but they also have a role in growth during puberty, cellular differentiation, and secretory function. The adult prostate gland remains exquisitely sensitive to withdrawal of circulating androgens and undergoes tissue atrophy, which can regenerate with the administration of androgens. This principle of androgen sensitivity was first used by Huggins and Hodges 75 years ago as a method to treat metastatic prostate cancer patients (Huggins and Hodges 1941). They saw significant responses to surgical castration, demonstrating that these tumors were dependent on androgens.

Today, androgen-deprivation therapy is still the first line of therapy to treat prostate cancer patients in the clinic (see Centenera et al. 2017; Dai et al. 2017). Although these tumors initially respond to antiandrogens, drugs that block the activity of androgens, they frequently become resistant, continue to grow, and become castrate-resistant prostate cancer (CRPC), which is often still dependent on AR signaling. This continued dependency on AR can be because of various alternative mechanisms, such as amplification of the AR gene and/or resurgence of mutant versions of AR that respond to non-androgenic ligands, examples of how developmental pathways can be coopted and deregulated in cancer. These clinical characteristics of AR dependency have highlighted the central role for androgens in prostate cancer.

Elegant studies by Cunha and colleagues uncovered the pivotal role of AR expressed in the mesenchyme in controlling prostate developmental initiation and growth. Tissue-recombination

experiments were used to reveal the importance of epithelial–mesenchymal interactions required for normal prostate development. In these studies, UGE and UGS mesenchyme (UGM) tissue were dissected and trypsinized, recombined, and grafted under a host male mouse renal capsule for 4 to 8 weeks. If separated, UGE and UGM tissue from wild-type mice or rats are recombined and grafted; normal prostate morphological structures develop that express all of the molecular markers of normal adult prostates, including differentiation markers and functional secretory proteins. UGE or UGM grafted alone in a host male mouse fail to grow, showing that both tissue components are needed for prostate growth (Cunha 1972). Similar recombination grafting experiments using tissue from seminal vesicle (SV) or skin showed that SV mesenchyme that is androgen regulated can replace urogenital mesenchyme and allow normal prostate development, whereas skin mesenchyme that does not express AR is not able to do so (Cunha 1972). These early embryological experiments showed the critical role of the mesenchyme in controlling prostate development and suggested an important role of hormones in this process. Confirmation of the role of AR in the mesenchyme came from studies using *Testicular feminization (Tfm)* mice. These mutant mice are insensitive to androgens because of a frameshift mutation resulting in a nonfunctional AR, which causes no prostate to develop in these animals (He et al. 1991). To study the impact of AR loss in epithelia or mesenchyme, recombinants were grafted consisting of each tissue type from either *Tfm* mutant or wild-type mice (Fig. 4). When grafted in male hosts, UGS tissue recombinants comprising *Tfm* mesenchyme and wild-type epithelia or *Tfm* epithelia do not form prostate tissue and vagina-like differentiation occurs. (Cunha and Lung 1978; Donjacour and Cunha 1993). In contrast, *Tfm* UGE and wild-type UGM recombinants form prostate structures. These data established that AR activity in the mesenchyme acts in a paracrine fashion and is required for development of the prostatic epithelium. Further tissue-grafting experiments in male host mice have shown that when UGM is recom-

bined with either embryonic or adult bladder epithelia, prostatic differentiation is induced and a fully formed secretory organ develops (Cunha et al. 1980, 1983; Neubauer et al. 1983). In this experiment, the normally AR-negative bladder epithelia is induced to express this hormone receptor and is directed to a prostate identity and gland formation. This ability of the UGM to direct prostate development of non-UGE shows an instructive role of the mesenchyme during glandular development. In contrast to its role during development, during prostate cancer progression, stromal AR expression is frequently reduced compared to normal or benign glands where it is thought to inhibit the growth of epithelial cells through paracrine factors (Singh et al. 2014).

Transcription complexes involved in prostate identity include members of the HOX gene family, which are crucial for the development of many tissues during embryogenesis as regulators of organ body patterning (Krumlauf 1994). Recent data has emerged linking them to prostate cancer, including a number of HOX genes that are overexpressed in this disease (Waltregny et al. 2002; Miller et al. 2003; Hamid et al. 2015; Wang et al. 2015). There has been particular interest in *HOXB13*, as germline mutations in this gene have been shown to be a risk factor for human prostate cancer; it is highly expressed in some CRPCs and it can promote prostate cell proliferation (Kim et al. 2010; Ewing et al. 2012; Xu et al. 2013).

Hox genes are expressed in a colinear fashion along the anterior to posterior axis of the embryo resulting in the temporal and spatial expression of Hox genes throughout different tissues (Mallo and Alonso 2013), with the prostate expressing the 5' posterior Hox genes. The analysis of a number of posterior Hox genes in the rat prostate has shown that each lobe has a specific pattern of Hox genes, suggesting an individual Hox expression signature that regulates their development and function (Huang et al. 2007). Organ culture experiments and castration of rats showed that these Hox genes are differentially regulated by androgens, which may be important for prostate cell differentiation and function (Huang et al. 2007). *Hoxa13*

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Figure 4. Tissue recombination experiments demonstrating the essential role of androgen receptor (AR) during prostate development. Prostate glands form that produce secretory products when wild-type (WT) urogenital sinus (UGS) mesenchyme (UGM) and WT UGS epithelia (UGE) are combined and grafted in a male host animal. When *Testicular feminization* (*Tfm*), AR-mutant, embryonic UGM is combined with either WT UGE or *Tfm* UGE, no prostate structure forms and vagina-like differentiation occurs, demonstrating the essential role of mesenchymal AR in the early stages of prostate organogenesis acting in a paracrine fashion to promote epithelial bud growth. UGE from *Tfm* mutant embryos combined with WT UGM forms prostate structures with no secretory function. (From Cunha 2008; adapted, with permission, from Elsevier © 2008.)

and *Hoxd13* are both expressed in the mesenchyme and epithelia in the prostate in a similar domain and are partially redundant. Mice that have a deletion in the *Hoxa13* or *Hoxd13* gene have mild defects in prostate duct growth and in branching (Podlasek et al. 1997, 1999). Mice that are *Hoxa13* and *Hoxd13* double mutants have a severe prostate phenotype (Warot et al. 1997). In *Hoxa13*^{-/-}; *Hoxd13*^{-/-} mice, there is no separation of the cloaca and as a result no UGS forms. Animals that are *Hoxa13*^{+/-}; *Hoxd13*^{-/-} form a UGS but there is a disruption in prostate development with no AP lobe formation. *Hoxb13* is expressed in the prostate epithelia, with highest levels in the VP and least in AP (Sreenath et al. 1999; Economides and Capecchi 2003; Huang et al. 2007). *Hoxb13*-mutant mice have a normal number of ductal tips but defects in the VP

morphogenesis with epithelial cells that do not differentiate into columnar cells and lack secretory proteins (Economides and Capecchi 2003).

Several signaling pathways, some of which drive epithelial-mesenchyme interactions, are involved in the initial stages of prostate development. The WNT/β-catenin pathway is important in many diverse roles during embryogenesis, and deregulation of this pathway leads to cancers in a number of tissues (Clevers and Nusse 2012). WNTs are a family of secreted glycolipoproteins that function in the canonical pathway to regulate the levels of available transcriptional β-catenin. When no WNT ligand is present, β-catenin is phosphorylated and sent for ubiquitin degradation by a deconstruction complex consisting of Axin1, adenomatous polyposis coli (APC), GSK3β, and CK1α (Mac-

Donald et al. 2009). WNT ligands bind to members of the Frizzled cell-surface receptors, resulting in the stabilization of β -catenin and its translocation to the nucleus. Recent next-generation exome-sequencing studies have uncovered a cohort of advanced metastatic prostate cancer patients with mutations in the WNT/ β -catenin pathway, suggesting it could be involved in the pathogenesis of this disease (Grasso et al. 2012; Robinson et al. 2015). In addition, expression of the ligand WNT16 is induced in the tumor microenvironment in response to prostate cancer treatment (Sun et al. 2012).

Multiple WNT ligands are expressed in UGE and mesenchyme during mouse prostate development (Zhang et al. 2006; Mehta et al. 2011). WNT activity in the UGS has been studied using a sensitive WNT reporter transgene, TCF/Lef:H2B-GFP, where six β -catenin DNA-binding sites drive the expression of green fluorescent protein (GFP) (Kruithof-de Julio et al. 2013). Between E15.5 and E17.5, GFP was detected in UGM and decreased postnatal, and from E17.5, GFP was observed in a subpopulation of prostate ductal tip cells, which also express *Nkx3.1*, an early prostate epithelial marker. In addition, the expression of two WNT/ β -catenin target genes, *Axin2* and *Lef1*, are found in UGS basal epithelial precursors and in the prostatic bud tips (Wang et al. 2008a; Mehta et al. 2011, 2013; Wu et al. 2011; Simons et al. 2012; Francis et al. 2013). These studies suggest that WNT is active in both UGS cellular compartments but that WNT activity is first found in the mesenchyme followed by the prostatic epithelial buds.

Several gene deletion models and drug compound experiments have shown that the WNT/ β -catenin pathway is required for normal prostate bud development and *Nkx3.1* expression. Deletion of β -catenin (gene name *Ctnnb1*) using the *Shh:Cre* expressed in UGS epithelium results in early defects in prostate specification with impaired cloacal division, and subsequently the UGS is much smaller, with loss of cytokeratin 14 expressing basal cells and lack of prostate bud formation (Mehta et al. 2013). Deletion of β -catenin at later stages of development using an inducible *Shh:Cre* mouse or the *Nkx3.1:Cre*

showed a decrease in prostatic bud number and reduced growth, with loss of the prostate epithelial markers *Nkx3.1* and *Fgfr2* (Francis et al. 2013; Mehta et al. 2013). These β -catenin mutants have a decrease in the number of LEF1 and p63 expressing basal progenitor cells and loss of cytokeratin 14 expression. Consistent with these results, tamoxifen-inducible Cre deletion of β -catenin in the UGE and UGM resulted in a failure in prostatic bud formation and loss of *Nkx3.1* and *Foxa2* expression (Simons et al. 2012). Prostate explants grown in the presence of WNT pathway inhibitors displayed a decrease in *Nkx3.1* expression, fewer proliferating cells, and reduced bud tips (Wang et al. 2008a; Simons et al. 2012; Kruithof-de Julio et al. 2013). Together, these studies indicate that β -catenin is required for proper UGS formation, commitment to prostatic cell fate, prostatic bud growth, and regulates the p63 expressing cell population. In *Nkx3.1* null neonatal prostates, there is a reduction in TCF/Lef:H2B-GFP transgene expression, which has led to the proposal that *Nkx3.1* together with WNT signaling act in a feedforward loop to regulate prostate duct branching and differentiation (Kruithof-de Julio et al. 2013).

Expression of a stabilized form of β -catenin (deletion of exon 3 that regulates protein degradation), which is functionally similar to activating mutations in β -catenin found in cancer, within the UGE resulted in discrete structures that failed to form normal prostatic buds and did not express the prostate epithelial markers *Nkx3.1* and *Sox9*, but continued to express basal cell markers and had increased expression of several bone morphogenetic protein (BMP) family members (Francis et al. 2013; Mehta et al. 2013). BMPs have been shown to inhibit prostate branching and growth, and inhibiting this pathway increased the growth of prostate buds in mice with stabilized β -catenin. This data has led to the proposal that in the wild-type prostate epithelium β -catenin regulates the level of BMP expression to control the spacing and number of prostate buds (Mehta et al. 2013). The mutant structures resulting from stabilized β -catenin also express the squamous cell marker CK10, even in the absence of androgens, suggesting

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that high levels of β -catenin expressed in prostate epithelial cells results in an androgen-independent transdifferentiation to squamous cells. Squamous cell metaplasia is also a common feature of β -catenin overexpression in the adult prostate and mammary epithelium (Miyoshi et al. 2002a,b; Bieri et al. 2003; Teuliere et al. 2005; Francis et al. 2013). Squamous identity is not a general feature of human prostate cancer, suggesting that other oncogenic events, such as the loss of PTEN, cooperate with activated β -catenin signaling to drive tumorigenesis (Francis et al. 2013).

Noncanonical WNT signaling has also been implicated in prostate development and disease. WNT5a, a ligand that activates β -catenin-independent WNT pathways, has been detected in advanced prostate cancer samples, can promote aggressive disease characteristics, and ectopic expression of this ligand in a prostate cancer cell line can provide resistance to the antiandrogen enzalutamide (Yamamoto et al. 2010; Miyamoto et al. 2015). In the rat prostate, Wnt5a is expressed in the distal periductal mesenchyme, extracellular matrix, and epithelial cells of the distal ducts, and testosterone can repress Wnt5a expression during early development (Huang et al. 2009). Wnt5a null mouse prostates and rat prostates cultured with Wnt5a protein have shown that this WNT can modulate bud growth, branching morphogenesis, cellular polarity, and lumen formation (Allgeier et al. 2008; Huang et al. 2009).

The transcription factor p63 is expressed in the developing prostate epithelial buds, is required for the normal development of the prostate, and is thought to mark a stem/progenitor cell population. $p63^{-/-}$ mice die at birth and newborn mutant animals do not form prostate glands (Signoretti et al. 2000). Transplantation experiments of $p63^{-/-}$ UGS tissue into a host male mouse can rescue prostate development, which lack basal cells but contain luminal cells and some cells that display mucinous cell characteristics and markers of intestinal differentiation (Kurita et al. 2004; Signoretti et al. 2005). These experiments suggest that p63 in the UGS is required for basal cell differentiation but that luminal differentiation can still take place.

However, cell lineage studies using chimeric $p63^{-/-}$ mice and mice with Cre knocked into the p63 locus have shown that the prostatic buds and the adult luminal, basal, and neuroendocrine cells are formed from p63-positive cells (Signoretti et al. 2005; Pignon et al. 2013). Together, these studies indicate that during prostate development, the p63-expressing cells of the buds contain a stem/progenitor cell population that can give rise to all of the epithelial cell types of the adult prostate and that there may be other progenitor cells that can differentiate into luminal cells.

BUDDING AND BRANCHING MORPHOGENESIS

Gene deletion and organ culture experiments have identified several members of the fibroblast growth factor (FGF) family, signaling through mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathways, as critical for early prostate bud growth and branching (Thomson and Cunha 1999; Donjacour et al. 2003; Kuslak and Marker 2007; Lin et al. 2007; Pu et al. 2007). FGFs are secreted glycoproteins that regulate proliferation, differentiation, survival, and migration in a wide variety of tissues during development and in the pathogenesis of cancer (Turner and Grose 2010). Several FGF ligands and receptors are overexpressed in prostate cancer compared to benign samples and are associated with a higher grade of disease (Leung et al. 1996; Tanaka et al. 1998; Dorkin et al. 1999; Sahadevan et al. 2007; Murphy et al. 2010; Feng et al. 2013; Wan et al. 2014). Moreover, mouse models that overexpress FGF ligands or receptors develop prostate cancer (Wang et al. 2004; Acevedo et al. 2007; Elo et al. 2010).

Fgf10 and *Fgf7* are expressed in the mesenchyme of the UGS and are thought to act in a paracrine manner with their cognate receptor *Fgfr2* expressed in the epithelia (Huang et al. 2005). Although testosterone can regulate the expression of FGF7 and FGF10 in stromal cells in vitro, in vivo evidence suggests that these genes are not androgen regulated (Thomson and Cunha 1999). Both ligands promote epithe-

lial, but not mesenchymal, proliferation (Lu et al. 1999; Thomson and Cunha 1999). In neonatal rat prostate organ cultures, FGF7 and FGF10 can substitute for androgens, providing epithelial growth and ductal branching morphogenesis (Sugimura et al. 1996; Thomson and Cunha 1999), but FGF10 is not sufficient to initiate prostate budding in the absence of androgens (Donjacour et al. 2003). *Fgf7*-deficient mice do not display any apparent prostatic phenotype, which may be because of redundancy with *Fgf10*. Mice with deletion of *Fgf10* have rudimentary prostatic buds with a decrease in the number of proliferating cells, which also fail to develop further (Donjacour et al. 2003). This shows that, whereas *Fgf10* is required for early prostate development, it is not required for bud induction.

Deletion of *Fgfr2* using a conditional allele and the *Nkx3.1:Cre* driver results in prostates that lacked anterior and ventral lobes (Lin et al. 2007). In these mutant mice, prostate buds form in all lobes but the anterior and ventral lobes fail to progress beyond this stage. Analysis of *Fgfr2;Nkx3.1:Cre* mutant dorsal lobes at 6 weeks revealed thinner epithelial ducts with reduced cell proliferation and defects in ductal branching. FGF receptor (FGFR) substrate 2 α (FRS2 α) is a key adapter protein that is phosphorylated by activated FGFRs and recruits other factors to activate the MAPK and PI3K pathways (Turner and Grose 2010). Recent clinical evidence suggests that FRS2 α -mediated signaling promotes prostate cancer angiogenesis and predicts poor clinical outcome (Liu et al. 2016). During prostate development, FRS2 α is expressed throughout the prostate epithelial buds, becoming restricted to the bud tips at 2 weeks of age and diminishing in the adult (Zhang et al. 2008). Although *FRS2 α ;Nkx3.1:Cre* mice had no prostate budding phenotype, these mutants had smaller organs with fewer ductal branches and a reduction in ductal tips associated with a decrease in the number of proliferating cells. *Fgfr2* mutant prostates had reduced levels of phospho-FRS2 α , phospho-ERK and phospho-AKT, whereas *FRS2 α* mutant prostates only had altered phospho-ERK. Organ culture experiments with MAPK and PI3K in-

hibitors confirmed that both of these pathways are important for prostate ductal branching, and that during prostate development *FRS2 α* mediates *Fgfr2* signaling through the MAPK pathway (Zhang et al. 2008).

The PI3K/mTOR (mammalian target of rapamycin) signaling pathway has been implicated in prostate branching and bud invasion into the surrounding mesenchyme. Inhibition of the PI3K/mTOR pathway in UGS organ culture experiments resulted in prostate cultures with a decrease in bud number and smaller buds that had reduced cell motility (Ghosh et al. 2011). Through the use of specific inhibitors to members of this pathway, it was proposed that a balance of PI3K activity and mTOR signaling are required for epithelial prostate bud migration (Ghosh et al. 2011).

Treatment of UGS ex vivo organ cultures with drug compounds inhibiting the FGF pathway before prostate development prevents bud development, whereas treatment after this stage has little effect on growth but affects branching morphogenesis (Huang et al. 2005; Kuslak and Marker 2007; Thomsen et al. 2008b). Taken together, these genetic and in vitro drug experiments show that FGF signaling in the prostate is responsible for multiple stages of development with a requirement during early budding and later branching morphogenesis.

Members of the transforming growth factor β (TGF- β) family, including the BMPs, are a group of secreted growth factors that have diverse cellular functions throughout embryological development (Wu and Hill 2009). Current evidence suggests that these proteins have a complex role during prostate cancer, with some studies indicating this pathway can act as a barrier to disease progression and others showing a tumor-promoting role (Tu et al. 2003; Morrissey et al. 2010; Ding et al. 2011; Li et al. 2012; Fournier et al. 2015). This difference in phenotypic outcome is likely because of a context-dependent activity of this pathway in prostate cancer.

During prostate development, TGF- β /BMP signaling has been shown to play a role in regulating ductal budding and branching. In the mouse and rat, several components of the TGF- β signaling pathway are expressed in the

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developing prostate epithelia and mesenchyme (Timme et al. 1994; Itoh et al. 1998; Raghoebar et al. 1999), and ex vivo organ culture studies have shown that TGF- β 1 represses prostate growth and branching (Tanji et al. 1994; Itoh et al. 1998; Tomlinson et al. 2004).

In the rodent, *Bmp4* is expressed in the UGM before prostate development and subsequently becomes restricted to cells surrounding the buds, whereas its receptor *Bmpr1* is expressed in both the UGM and epithelia, indicating that this pathway is active in both cell compartments (Lamm et al. 2001; Omori et al. 2014). Prostates from *Bmp4* heterozygous knockout animals have an increase in ductal branching, whereas ex vivo prostate cultures grown with exogenous Bmp4 protein inhibited epithelial cell proliferation and bud number (Lamm et al. 2001). Based on these data, and evidence showing *Bmp4* is regulated by signaling molecules such as androgens, *Fgf10*, and *Sonic hedgehog*, it has been proposed that *Bmp4* has an important role in integrating signals to prevent prostatic budding and branching along the ducts (Lamm et al. 2001; Pu et al. 2004; Huang et al. 2005; Prins and Putz 2008). Another member of this family, *Bmp7*, is expressed in the UGM before prostate budding and is later found in the epithelia along with its growth factor receptors. *Bmp7* null prostates have an increase in distal tips and branch points, whereas prostates cultured with recombinant Bmp7 show a decrease in ductal branching. Components of the Notch signaling pathway, which are expressed in the developing prostatic buds and are thought to promote prostate growth, are expanded in *Bmp7* null prostates (Grishina et al. 2005). This suggests that Bmp7 acts, at least in part, through repression of Notch1 signaling in the epithelial ducts during branching morphogenesis (Grishina et al. 2005).

The secreted BMP antagonist Noggin is expressed at high levels in the mouse UGM from E14 before prostate initiation, and then becomes restricted to the mesenchyme surrounding the developing buds (Cook et al. 2007). *Noggin* null mice have several defects in the urogenital tract, including incomplete separation of the UGS from the hindgut. Analysis of

Noggin mutant prostates and renal graft rescued mutant prostate tissue showed a loss of ventral mesenchyme and VP buds, with a decrease in ductal buds in the remaining lobes. Consistent with a model of BMP/Noggin signaling axis controlling sites of budding and branching, BMP4 protein inhibited proliferation of p63-expressing cells in the distal tips of prostate buds, which could be reversed with the addition of Noggin (Cook et al. 2007).

Deletion of the BMP receptor *Bmpr1* in the developing prostate epithelium using the inducible *Shh;CreERT2* resulted in the formation of a prostate gland with defects in epithelial cell differentiation, including less epithelial infolding, reduced expression of the secretory protein probasin, and loss of *Nkx3.1* expression (Omori et al. 2014).

Sox9 is a transcription factor that is a member of the SOX gene family and is required for prostate development (Thomsen et al. 2008a; Wang et al. 2008b; Huang et al. 2012). Sox genes have important roles in the development of a number of embryonic tissues, including Sertoli cells of the testis, chondrocytes, the neural crest, and the pancreas (Kamachi and Kondoh 2013). Sox9 is frequently one of the first genes to be expressed in these tissues and is required for the initial step in cell differentiation and organ development (Pritchett et al. 2011). In human prostate tumor samples, SOX9 expression is associated with more aggressive disease, and in prostate cancer cell line models SOX9 increases growth and invasion (Wang et al. 2007, 2008b; Thomsen et al. 2010; Cai et al. 2013). Sox9 overexpression can also promote cancer in mouse models and can cooperate with loss of the tumor suppressor *Pten* to drive tumor formation (Thomsen et al. 2010; Cai et al. 2013; Wang et al. 2013).

Expression of Sox9 is found at the early stages of prostate development when the buds first appear in the UGS, with strongest expression at the distal tips (Schaeffer et al. 2008; Thomsen et al. 2008a; Wang et al. 2008b; Huang et al. 2012). As Sox9 is essential in early embryonic mouse development, this gene has been deleted in the UGS using an inducible system and in the developing prostate epithelia using

the *Nkx3.1:Cre* mice, which led to loss of Sox9 just after prostate bud induction (Thomsen et al. 2008a; Huang et al. 2012). Because of lethality at birth, *Sox9;Nkx3.1:Cre* mutant UGS were dissected and grown under the renal capsule of adult male mice or in ex vivo culture. These *Sox9* mutants had significantly smaller ventral prostates, which displayed a decrease in proliferation and reduced expression of prostate epithelial cell genes such as *Nkx3.1*, *Shh*, and *Fgfr2* (Thomsen et al. 2008a). In agreement with this, deletion of *Sox9* before prostate initiation and cultured in vivo or ex vivo resulted in a loss of prostate structure and the luminal cell markers *Nkx3.1* and CK18 (Huang et al. 2012). Deletion of *Sox9* at later stages of development resulted in the disruption of normal prostate morphogenesis (Thomsen et al. 2008a; Huang et al. 2012). These data suggest that *Sox9* regulates an early step in prostate epithelial differentiation, consistent with its role in other tissues.

PROSTATE CYTODIFFERENTIATION

Epithelial AR plays a crucial role in the regulation of the differentiation and secretory profile of the prostate. Grafting experiments using the *Tfm* mice have shown that when mutant UGE is grafted with wild-type UGM, branched prostatic glandular tissue can form with columnar epithelium that proliferates similar to the wild-type gland (Cunha and Lung 1978; Sugimura et al. 1986a; Donjacour and Cunha 1993). Nevertheless, *Tfm* mutant UGE glands form mature prostate tissue at a much lower rate than wild-type UGE and the mutant glands lack secretory function (Fig. 4). These glands comprising mutant epithelial AR and wild-type stroma were equally sensitive to castration of the host mice compared to completely wild-type tissue recombinants, suggesting that epithelial AR is not required for androgen-regulated epithelial apoptosis and this process is controlled by stromal paracrine factors (Kurita et al. 2001).

The UGE is also required to provide signals to fully differentiate the surrounding mesenchyme. When UGM is grafted alone, few cells expressing smooth muscle markers are formed, but when grafted with UGE, prostate, or blad-

der, fully differentiated adult stroma is formed (Cunha et al. 1992). These differentiation signals are species-specific, as rat UGM grafted with human prostatic epithelium forms differentiated smooth muscle organized similarly to human prostate tissue (Hayward et al. 1998).

NKX3.1/Nkx3.1 is a member of the NK homeobox gene family of transcription factors that has a strong link to prostate cancer. *NKX3.1* expression is frequently lost in human disease and *Nkx3.1* mutant mice develop prostatic lesions (Bhatia-Gaur et al. 1999; Bowen et al. 2000; Abdulkadir et al. 2002; Kim et al. 2002). *Nkx3.1* is the first known marker of prostate identity and is expressed from E15.5, before budding, in the lateral region of the UGS epithelium (Bhatia-Gaur et al. 1999). This expression increases and becomes prominent at sites of prostate budding and in the distal tips of growing buds (Bhatia-Gaur et al. 1999; Chen et al. 2005; Keil et al. 2012). This initial phase of *Nkx3.1* expression in prostate epithelium is before AR is expressed, but hormone manipulations have shown that at later stages of development *Nkx3.1* is regulated by androgens (Bhatia-Gaur et al. 1999). This dual model of transcriptional regulation is also observed in the adult prostate where *Nkx3.1* is found in luminal cells and some basal cells but also in a few epithelial cells with stem properties in the castrate organ (named castration-resistant *Nkx3.1* expressing resistant or CARN cells) (Wang et al. 2009). *Nkx3.1* null mice have shown that it is not required for the initial stages of prostate development, with mutant animals developing all prostatic lobes that are a similar size to control animals (Bhatia-Gaur et al. 1999). However, these mutant animals have a defect in ductal morphogenesis with a decrease in the amount of branching and in the production of secretory proteins. Consistent with the role of *Nkx3.1* in prostate differentiation, recent gain-of-function experiments using tissue transplantations have shown that *Nkx3.1* can change the fate of SV into prostate tissue, which is mediated through the G9a histone methyltransferase and the UTY histone demethylase (Dutta et al. 2016).

The Forkhead box (Fox) genes are transcription factors that contain a winged-helix struc-

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ture of the DNA-binding domain and have diverse roles in development and disease (Hannenhalli and Kaestner 2009). FoxA proteins have been called pioneer transcription factors as they can bind and remodel chromatin, moving histones and making the DNA available for other transcription factors to bind. FoxA1 and FoxA2 can physically bind to AR, are found bound to DNA near AR regulatory sites, and together with AR control the transcription of androgen-regulated genes (Gao et al. 2003; Yu et al. 2005; Zhao et al. 2016). *FoxA1* mutations or alterations in gene expression can alter the transcriptional output of androgen signaling and promote tumor formation (Yang and Yu 2015).

During prostate development, *FoxA1* is expressed throughout the epithelial cells of the UGS, the growing buds, and the adult mouse, whereas *FoxA2* is expressed in the basal epithelial cells of the UGS and only transiently found in the buds (Gao et al. 2005). As *FoxA1*^{-/-} mice die at birth, renal capsule and tissue recombinants were used to study this gene during prostate development. Prostates from mutant mice just after birth have buds demonstrating that FoxA1 is not required for bud initiation (Gao et al. 2005). *FoxA1* null prostatic tissue recovered from renal grafting was consistently smaller and made up of solid disorganized epithelial cells with no lumen formation. In rescued *FoxA1*^{-/-} mutant prostate tissue, there was elevated expression of developmental genes, such as *Shh* and *Notch1*, an expansion of cells expressing basal markers, and a reduction of luminal markers, suggesting a block in cellular differentiation (Gao et al. 2005). Consistent with this, although there was no change in AR expression in *FoxA1* mutant prostate tissue, secretory activity was absent. This *FoxA1* mutant prostate tissue also displayed stromal defects, with an increased smooth muscle layer, and an increase in stromal molecular factors, including *Fgf10* and *Bmp7*.

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor-suppressor gene that is part of the PI3K signaling pathway and negatively regulates AKT activation (Song et al. 2012). *PTEN* is mutated at a high frequency in a number of cancers, with deletions

or mutations in up to 30% of primary and 63% of metastatic prostate cancer samples (Suzuki et al. 1998; Barbieri et al. 2012; Cancer Genome Atlas Research Network 2015; Robinson et al. 2015). In agreement with this, mice that are *Pten* heterozygous develop prostate intraepithelial neoplasia, whereas mice that have both *Pten* alleles deleted form invasive prostate tumors (Podsypanina et al. 1999; Wang et al. 2003).

Conditional deletion of *Pten* with the *Nkx3.1:Cre* mouse resulted in the formation of prostate glands with buds that grew into the surrounding mesenchyme but that were larger and had defects in epithelial cytodifferentiation (Lokody et al. 2015). At later stages of prostate maturation, at a time point when phosphorylated AKT was detected, there was an increase in luminal epithelial cells that filled the ducts. In the normal developing prostate, the majority of proliferating cells occur at the distal tip of the growing ducts, but in *Pten* mutant prostates, dividing epithelial cells were seen throughout the proximodistal axis of the duct. In addition, in *Pten;Nkx3.1:Cre* prostates, the expression of several androgen-regulated genes was deregulated, including *Nkx3.1* and *Probasin*, and there was an early accumulation of luminal cell markers, suggesting that *Pten* controls the timing of differentiation of prostatic epithelial cells and AR-regulated genes, as has been noted in the adult gland (Wang et al. 2003; Carver et al. 2011; Mulholland et al. 2011; Choi et al. 2012; Lokody et al. 2015).

PROSTATE DEVELOPMENT AND CANCER

It has been proposed that the process of tumor formation recapitulates aspects of the normal development of the organ it is derived from. In many cases, this is observed as signaling pathways important in prostate development are reactivated in cancer (Schaeffer et al. 2008; Pritchard et al. 2009). As organ development occurs in well-defined steps, it is an ideal system to determine the roles of different pathways in various processes both at the molecular and cellular level. The essential role of the AR pathway in its various incarnations in prostate cancer indicate the value of continually studying

androgens during development of this organ. Developmental studies have uncovered the role of other transcription factors and signaling pathways that have been implicated in prostate cancer, highlighting the use of organogenesis as a model system. These studies have also begun to dissect the dependencies of normal and tumor prostate cells on the microenvironment, which regulate neoplastic processes such as cellular growth, invasion, and migration. Future developmental studies, including those using new techniques such as single-cell RNA sequencing and high-throughput proteomics, could increase our understanding of organogenesis and help unravel the emerging complex nature of cancer, which is characterized by a high degree of cellular heterogeneity and drug resistance that can arise through multiple different mechanisms.

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Cold Spring Harb Perspect Med 2018; doi: 10.1101/cshperspect.a030353 originally published online December 11, 2017

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