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REVIEW

Prostate organogenesis: tissue induction, hormonal regulation and cell type specification

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ABSTRACT

Prostate organogenesis is a complex process that is primarily mediated by the presence of androgens and subsequent mesenchyme-epithelial interactions. The investigation of prostate development is partly driven by its potential relevance to prostate cancer, in particular the apparent re-awakening of key developmental programs that occur during tumorigenesis. However, our current knowledge of the mechanisms that drive prostate organogenesis is far from complete. Here, we provide a comprehensive overview of prostate development, focusing on recent findings regarding sexual dimorphism, bud induction, branching morphogenesis and cellular differentiation.

KEY WORDS: Androgen signaling, Development, Differentiation, Induction, Prostate

Introduction

The human prostate is a small walnut-sized organ that is located just below the bladder and surrounds the urethra (Fig. 1). It contains a system of branching ducts comprising pseudo-stratified epithelium surrounded by a fibromuscular stroma. The prostate is a male sex accessory gland that functions by producing and secreting fluids that contribute to the ejaculate, and thereby significantly enhances male fertility. Intriguingly, the prostate is highly susceptible to oncogenic transformation at a frequency significantly greater than that of other male secondary sexual tissues, such as the seminal vesicles. Indeed, approximately one in seven men will be diagnosed with prostate cancer during their lifetime (Siegel et al., 2016). Consequently, this disease has been the focus of intense investigation in order to understand its pathobiology and to provide improved treatment (Attard et al., 2016; Shen and Abate-Shen, 2010). It has long been hypothesized that malignancy, including that of the prostate, occurs due to a re-awakening of the developmental processes that occur during organogenesis. Although this concept that tumors are caricatures of organogenesis has been proposed for some time (Pierce and Speers, 1988), more recent studies have demonstrated key similarities in gene expression programs between prostate organogenesis and cancer (Pritchard et al., 2009; Schaeffer et al., 2008). Thus, there is considerable rationale to gain a comprehensive understanding of the fundamental mechanisms behind prostate development.

Here, we review what is currently known about the cellular and molecular mechanisms of prostate development, highlighting areas that require further investigation. We describe successive stages of prostate organogenesis, from the specification of prostate identity and epithelial bud induction to cell lineage specification and differentiation. In particular, we focus on the major signaling pathways that mediate androgen function and mesenchymal-epithelial interactions. Although other signaling pathways are also relevant for prostate organogenesis, these will not be described in detail and are instead summarized in Table 1. Finally, it should be noted that a large proportion of our knowledge regarding prostate organogenesis has been elucidated using rat and mouse models, so we also discuss similarities and differences in prostate organogenesis between rodents and humans.

An overview of prostate development and structure

During embryogenesis, the primitive urogenital sinus – the structure from which the prostate arises – forms as a caudal extension of the hindgut. Indeed, lineage tracing has shown that the entire length of the primitive urogenital sinus, including the distal urethra, has an endodermal origin (Seifert et al., 2008). Both the urogenital sinus and hindgut are initially joined as a single excretory tract at the embryonic cloaca. The subsequent division of the cloaca into separate urogenital and anorectal tracts occurs by 8 weeks of gestation in humans and 13.5 days post coitum (dpc) in the mouse (Hynes and Fraher, 2004); interestingly, this process has long been thought to occur by formation of a urorectal septum, but an alternative model has recently been proposed (Huang et al., 2016). The primitive urogenital sinus is then subdivided into the bladder at its rostral end, the urogenital sinus (UGS) in the middle, and the penile urethra caudally. The prostate forms through epithelial budding from the UGS starting at approximately 10 weeks of gestation in humans (Kellokumpu-Lehtinen et al., 1980), and at 17.5 dpc in the mouse (Bhatia-Gaur et al., 1999; Cunha et al., 1987; Lung and Cunha, 1981). Prostate organogenesis then continues under the influence of circulating androgens through birth and pre-pubertal stages, until the prostate reaches its mature size during puberty. Notably, a detailed description of the anatomy of the developing urogenital system in the mouse has been recently published (Georgas et al., 2015).

Conceptually, the process of prostate organogenesis can be divided into four stages (Fig. 2). In the first stage, prior to epithelial budding, developmental cues that are directly or indirectly mediated by androgens result in prostate induction in males. In the second stage, after prostatic fate is determined, the urogenital sinus epithelium (UGE) buds into the surrounding urogenital sinus mesenchyme (UGM), initiating tissue outgrowth and branching morphogenesis to form a system of ducts composed of solid epithelial cords. This process involves paracrine signaling to the UGE from the UGM, in which androgen receptor (AR) function is necessary to mediate epithelial outgrowth. In the third stage, ductal outgrowth is associated with a process of branching morphogenesis, which gives rise to the mature ductal network. In the mouse, this process results in the formation of four sets of prostatic lobes

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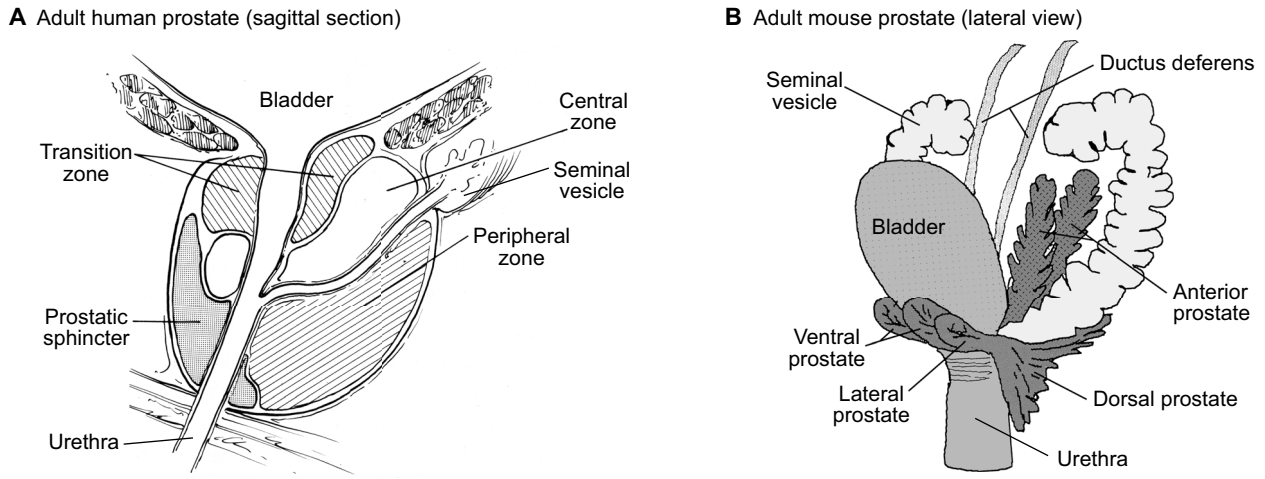

Fig. 1. Overview of prostate anatomy. (A,B) Schematics of the adult human (A) and adult mouse (B) prostate gland. Key structures and regions of the prostate are indicated. Adapted from Cunha et al. (1987) and McNeal (1969) and reproduced from Abate-Shen and Shen (2000).

(Fig. 1B), each with distinct patterns of ductal branching, whereas in humans this process leads to the formation of different prostatic zones (Fig. 1A) within a uni-lobular organ (Cunha et al., 1987; Timms, 2008). In the final stage, the solid epithelial cords undergo canalization to form the ductal lumen and cytodifferentiation to give rise to functional glandular epithelium with fully differentiated cell types.

The mature prostate epithelium contains several distinct cell types that differ in their morphology (Figs 2, 3). The luminal cells are tall columnar epithelial cells that express cytokeratins (CK; also known as KRT) 8 and 18 as well as secretory proteins such as prostate specific antigen (PSA; also known as KLK3) (Liu et al., 1997; Verhagen et al., 1988, 1992; Wang et al., 2001). Below the luminal layer are non-secretory basal cells that line the basement membrane and express CK5, CK14 and p63 (Trp63) (Signoretti et al., 2000; Verhagen et al., 1988, 1992; Wang et al., 2001). Notably, mouse and human prostate basal cells express low or undetectable levels of AR compared with luminal cells, nearly all of which express high levels of AR (El-Alfy et al., 1999; Mirosevich et al., 1999). Within the basal layer are occasional intermediate cells that co-express luminal and basal markers as well as additional markers such as CK19 (De Marzo et al., 1998; Wang et al., 2001; Xue et al., 1998); despite considerable speculation, it remains unclear whether intermediate cells represent a functionally distinct cell type. Finally, rare neuroendocrine cells correspond to basally localized cells that express secreted neuropeptides and other hormones, and often display a dendritic-like process that contacts the glandular lumen (Abrahamsson, 1999).

The mesenchymal compartment of the prostate also contains a number of differentiated cell types (Fig. 3). For example, cells of the embryonic UGM form a layer of smooth muscle, which lines the epithelium and exhibits contractile activity to aid expulsion of prostatic fluid into the ejaculate (Hayward et al., 1996b). The adult prostate stroma also contains a large population of mature fibroblasts that secrete extracellular matrix, consisting of fibrillar proteins, glycoproteins and proteoglycans that form a structural network and mediate growth factor signaling (Tuxhorn et al., 2001). Finally, other components of the stroma include blood vessels, lymphatics, nerves, and immune cells, which have been implicated in stem cell regulation as well as tumorigenesis within the prostate.

A comparison of rodent and human prostate development, anatomy and histology

Although analyses of archival human tissue samples have provided descriptive insights into prostate development, functional and mechanistic studies of human prostate organogenesis have been limited, and have depended on the use of animal models, particularly genetically engineered mice. Key features of androgen-mediated prostate induction, epithelial budding, branching morphogenesis and differentiation, as well as the pathways that drive these processes, are similar in rodent and human prostate organogenesis. However, there are significant differences between rodents and humans in terms of the temporal and spatial regulation of these processes. In humans, prostate epithelial budding occurs relatively early during embryogenesis, followed by interrupted phases of organogenesis at postnatal and pubertal stages (Cunha et al., 1987). In contrast, prostate epithelial budding initiates at late fetal stages in the mouse and rat, but the remainder of organogenesis occurs continuously during postnatal stages through puberty.

The gross morphology and histology of the human and rodent prostates (Fig. 1) also display several important differences (Ittmann et al., 2013; Shappell et al., 2004). One of the first studies of human prostate development observed that epithelial buds emerge from the UGE in defined pairs, suggesting that the human gland comprises multiple lobes (Lowsley, 1912). In the adult, however, such lobes are no longer recognizable (Price, 1963). Instead, the human prostate is uni-lobular, containing three zones – the central, transition and peripheral zones – that are believed to originate from five pairs of epithelial buds (McNeal, 1988). The central zone branches anteriorly from the prostatic urethra to surround the ejaculatory duct and constitutes approximately 20% of the prostate. The transition zone encircles the urethra and comprises approximately 10% of the prostate volume; it also represents the site of benign prostatic hyperplasia (BPH), a tissue enlargement that is unrelated to malignancy (McNeal, 1978; Timms and Hofkamp, 2011). Finally, the peripheral zone constitutes approximately 70% of the prostate, and represents the most common site of malignancy (McNeal, 1981). In addition to these epithelial components, the human prostate also has an anterior fibromuscular stroma that covers the glandular tissue, as well as a fibrous capsule that covers the exterior of the organ (McNeal, 1988).

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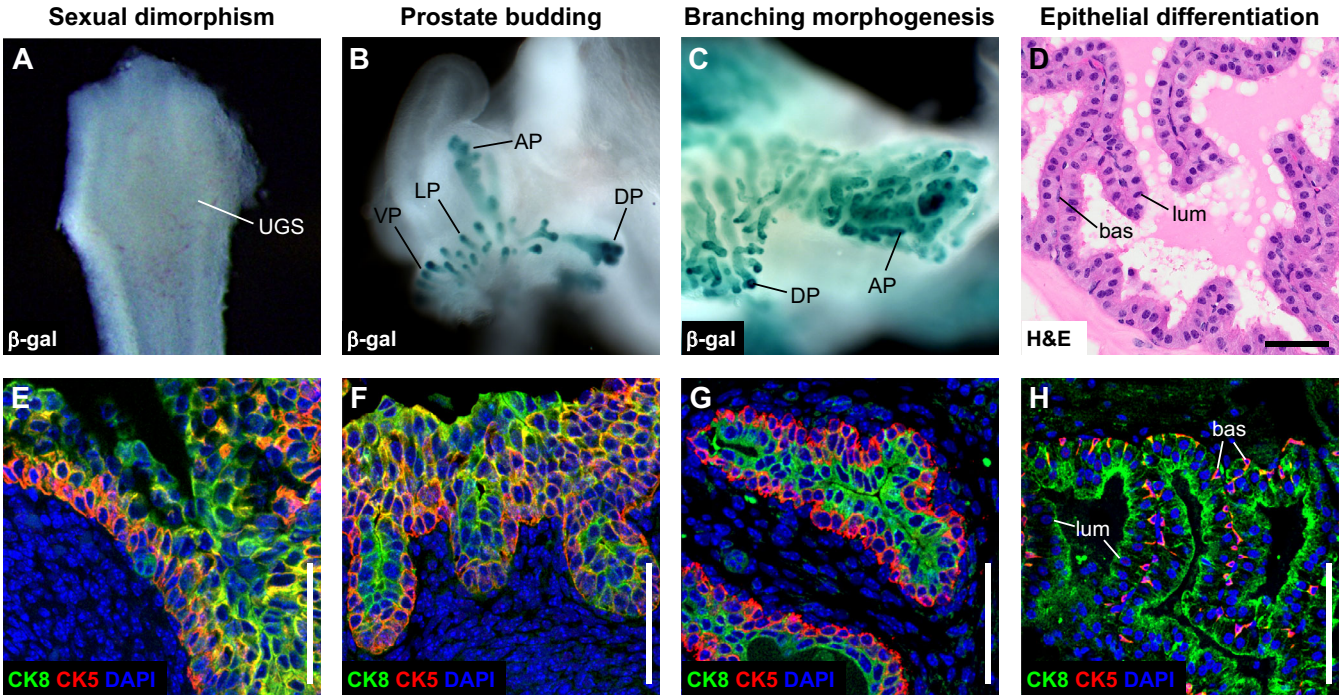

Fig. 2. Stages of prostate organogenesis. Whole-mount images of *Nkx3.1*$^{lacZ/+}$ mouse UGS stained for β-galactosidase (β-gal) activity, at 16.5 dpc (A), P2 (B) and P14 (C). (D) Hematoxylin & Eosin (H&E) staining of the adult mouse prostatic duct. (E,F) Immunofluorescence staining for cytokeratin 5 (CK5) and cytokeratin 8 (CK8) in the mouse prostate epithelium, at 16.5 dpc (E), P2 (F), P14 (G) and 8 weeks old (H). These images illustrate four stages of prostate organogenesis: sexual dimorphism, budding, branching morphogenesis and epithelial differentiation. At 16.5 dpc, the male UGS is devoid of epithelial buds (A) and the majority of urethral epithelial progenitors express CK5 and CK8 (E). Epithelial budding is induced shortly afterwards under the influence of androgens, resulting in the main ductal structures of the four prostatic lobes (B), during which CK5 and CK8 co-expression remains abundant (F). Extensive branching morphogenesis continues during postnatal development (C), when basal and luminal populations become segregated (G), although subsets of intermediate cells are still observed. In the adult prostate, the basal and luminal cells express specific cytokeratin profiles (H), and prostatic excretions can be observed in the ductal lumen (D). AP, anterior prostate; bas, basal; DP, dorsal prostate; LP, lateral prostate; lum, luminal; VP, ventral prostate. Scale bars: 50 µm.

In contrast, the rodent prostate is composed of distinct pairs of lobes (Fig. 1B), termed the anterior prostate (AP), ventral prostate (VP), lateral prostate (LP) and dorsal prostate (DP), which differ significantly in their branching patterns, histology and expression of secretory proteins (McNeal, 1983; Shappell et al., 2004; Thomson and Marker, 2006); the dorsal and lateral lobes are frequently referred to together as the dorsolateral lobes (DLPs). Consistent with the distinct identity of these lobes, several genetically engineered mouse mutants display lobe-specific mutant phenotypes, including null mutants for *Nog* (noggin), *Sox9* and *Wnt5a*, as well as mutants with conditional deletion of *Fgfr2* in the prostate epithelium (Table 1). Interestingly, despite the differences in adult prostate anatomy between mouse and human, their patterns of prostate epithelial budding during early organogenesis have significant similarities (Timms, 2008). However, the relationship of the early budding pattern of the human prostate to the zonal architecture of the adult tissue remains unclear. In this regard, although previous studies have suggested that the mouse DLP most closely resembles the human peripheral zone (Berquin et al., 2005; Price, 1963), the current consensus among veterinary and human pathologists is that there is no clear correspondence between mouse prostate lobes and human prostate zones (Ittmann et al., 2013; Shappell et al., 2004).

There are also differences between the human and rodent prostate in terms of the architecture of their epithelial and stromal compartments. Notably, although luminal, basal, intermediate and neuroendocrine cell populations are present in both the human and rodent prostate epithelium, the basal cell layer appears to be continuous in histological sections of the human prostate, but not the mouse prostate; instead, rodent basal cells may contact each other through long cytoplasmic processes (Hayward et al., 1996c). Thus, there is a 1:1 ratio of basal to luminal cells in the human prostate, whereas the ratio in mouse and rat is approximately 1:7 (El-Alfy et al., 2000). Furthermore, the stromal tissue surrounding the epithelium also differs between the two species. Human prostate ducts are contained within a continuous thick mass of fibromuscular stroma; in contrast, rodent prostate ducts contain epithelium surrounded by a thin smooth muscle layer and are joined by loose connective tissue to neighboring ducts (Ittmann et al., 2013; Shappell et al., 2004), except for the most proximal prostate ducts, which are adjacent to a thicker stromal layer. As a consequence, although the stromal to epithelial ratio varies between the mouse and rat lobes as well as human zones, there is less stromal content in the rodent compared with the human prostate, where stromal and epithelial cells are present in equal numbers (Bartsch and Rohr, 1980).

Prostate induction: sexual dimorphism in the urogenital sinus

Sexual dimorphism of the UGS is reflected in its ability to form the prostate in males versus a portion of the vagina in females. Formation of the prostate is initiated by the circulating androgen testosterone, which is synthesized at significantly higher levels in male embryos than in female embryos (Pointis et al., 1979, 1980).

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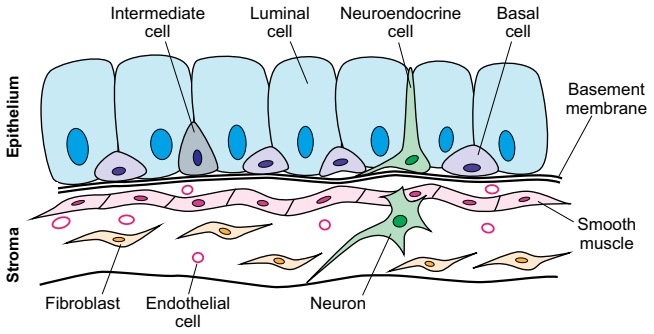

Fig. 3. Differentiated cell types in the prostate. Schematic of cell types in the adult prostate. The epithelial compartment is composed of basal cells that line the basement membrane, secretory luminal cells, and rare intermediate and neuroendocrine cell populations. These epithelial ducts are adjacent to a stromal compartment that includes smooth muscle cells, fibroblasts, and vascular and neural components.

Testosterone is produced by Leydig cells of the testis, starting at approximately 9 weeks of gestation in humans (Siiteri and Wilson, 1974), and at 13-14 dpc in mice (Pointis et al., 1979, 1980). Once testosterone reaches the UGS, it is converted into its more potent metabolite dihydrotestosterone (DHT) by 5α-reductase (Berman et al., 1995; Ekman, 2000; Ferraldeschi et al., 2013). DHT then binds to AR, a steroid hormone receptor that localizes to the nucleus upon ligand binding and acts as a transcription factor.

Owing to the essential role of androgens, sexual dimorphism of the UGS can be generated independently of genetic sex. For example, XY embryos that lack functional AR develop female external genitalia and fail to form a prostate gland (Brown et al., 1988; Lubahn et al., 1989; Lyon and Hawkes, 1970). Conversely, the UGS of XX mouse embryos or XY embryos that are defective in testis development can develop prostatic structures if administered sufficient levels of androgens *in utero* or in organ culture *ex vivo*, or if grafted into male hosts (Berman et al., 2004; Cunha, 1975; Lasnitzki and Mizuno, 1977; Takeda et al., 1986). Importantly, the ability of androgens to induce prostate budding is temporally restricted, as the UGS of XX mice rapidly loses androgen responsiveness during postnatal day (P) 0 to P5, both in grafts and in organ culture (Cunha, 1975; Thomson et al., 2002).

Classical tissue recombination assays have demonstrated that prostate formation requires paracrine interactions between the UGM and UGE (Cunha et al., 1987). In such tissue recombination assays, isolated epithelial and mesenchymal components from the same or different tissues and/or donors are combined and implanted under the renal capsule of immunodeficient hosts (Hayward, 2002). By themselves, UGM or UGE are unable to grow or differentiate in renal grafts, but recombination of UGE with heterologous UGM results in prostate tissue. Importantly, it was shown that prostate specification is largely conferred by the UGM, as recombination of bladder epithelium with UGM also generates prostate tissue (Donjacour and Cunha, 1993). Indeed, epithelial cells of several other endodermal and ectodermal tissue types, including vaginal, mammary gland and skin epithelial tissues, can also be induced by the UGM to form prostate tissue in tissue recombinants (Cunha, 1975; Taylor et al., 2009). These findings suggest that prostate formation occurs as a result of an instructive induction, in which the UGM specifies prostate identity in the adjoining UGE. However, contrary to this, it has been shown that seminal vesicle mesenchyme can induce bladder epithelium to form prostate in tissue recombination assays (Donjacour and Cunha, 1995). This finding that two heterologous tissues can be combined to form prostate suggests that the mesenchymal signal(s) from the urogenital and seminal vesicle mesenchyme is similar, whereas the specificity of the response is at least partially conferred by the epithelium. Additional complexity in mesenchymal signaling has been suggested by a recent study indicating a potential role for the caudal Müllerian duct mesenchyme (CMDM) acting together with the UGM to induce prostate formation and, in particular, to specify prostate versus urethral glands (Brechka et al., 2016).

The specific roles of AR in the UGM and UGE have also been investigated. Tissue recombination studies using *Tfm* (*Testicular-feminization*) mice, which are AR null mutants, have shown that AR is necessary in the UGM for prostate induction; wild-type UGE does not form prostate buds if combined with *Tfm* UGM (Cunha and Lung, 1978; Donjacour and Cunha, 1993). Subsequently, AR is required in the epithelium for the expression of prostatic secretory proteins by the epithelium, and for promoting differentiation of the surrounding mesenchyme into smooth muscle (Cunha et al., 1992; Cunha and Young, 1991; Donjacour and Cunha, 1993; Hayward et al., 1998). Thus, prostate induction requires functional AR expressed by the mesenchyme, but differentiation of both epithelial and mesenchymal compartments requires functional AR in the epithelium. This model is further supported by more recent studies using conditional gene targeting of AR *in vivo*. In particular, deletion of AR in the majority of stromal fibroblasts and smooth muscle cells leads to the formation of prostate ducts with reduced size and defective branching morphogenesis (Lai et al., 2012), consistent with partial loss of stromal AR function.

Interestingly, small paraurethral glands (sometimes termed Skene's glands) that resemble a rudimentary prostate are present in female rats and humans, but not mice (Mahoney, 1940; Zaviacic and Ablin, 1998). Furthermore, these glands express PSA and prostatic acid phosphatase (PAP; also known as ACPP) (Dietrich et al., 2011; Zaviacic and Ablin, 2000), suggesting that at least some aspects of prostate development also occur in females. Although some reports suggest that Skene's glands might not be paralogous to the male prostate, as they are located caudally along the UGS, other studies have identified prostate-like epithelial buds in females that do emerge in regions analogous to prostatic buds in males (Huffman, 1948; Thomson et al., 2002; Timms et al., 1999). It is possible that low levels of androgens in conjunction with AR expression in the XX mesenchyme could be sufficient for induction of prostate-like buds in females (Thomson, 2008). However, studies in rats have suggested that the presence of prostate-like buds in female embryos is more common when embryos are in proximity to other female embryos within the maternal uterus, raising the possibility that residual estrogens play a role in their induction (Timms et al., 1999).

Epithelial budding and specification

Under the influence of androgens, the UGE buds into the surrounding UGM and initiates prostate tissue outgrowth. However, although the activation of AR expressed in the UGM is believed to drive UGE specification and differentiation, the molecular mechanisms mediating this inductive process are poorly defined. In particular, the identity of the intermediary signal(s) from the UGM to the UGE is unclear, and the mechanism of its regulation by AR signaling is unknown. At present, there are two major hypotheses for how androgens mediate prostate epithelial induction and budding: the andromedin model and the smooth muscle model (Fig. 4).

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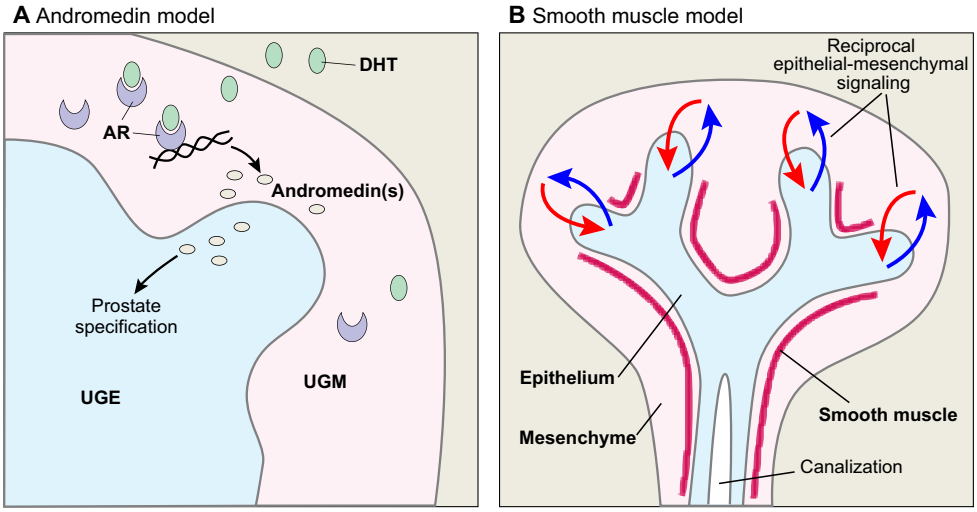

Fig. 4. Models of prostate bud induction. (A) In the andromedin model, androgen signaling through androgen receptor (AR) expressed in the urogenital mesenchyme (UGM) results in the production of one or more signaling factors (andromedins) that directly induce prostate budding in the adjacent urogenital epithelium (UGE). (B) In the smooth muscle model, signals that promote epithelial budding occur constitutively, and androgen signaling within the mesenchyme indirectly controls this process. In this model, androgen signaling is proposed to regulate the differentiation of smooth muscle around the emerging prostate ducts, thereby inhibiting further epithelial budding in regions where smooth muscle has already formed. DHT, dihydrotestosterone.

The andromedin hypothesis

In the andromedin model (Fig. 4A), ligand binding to AR in the UGM results in upregulation of the activity of a signaling factor(s) – an andromedin – that in turn acts directly upon the UGE to mediate prostate formation (Tenniswood, 1986; Thomson, 2008). Thus, in the simplest scenario, candidate andromedins should: (1) be expressed by the mesenchyme, (2) be upregulated by androgen signaling and (3) induce growth and prostate differentiation of the epithelium. Several candidate andromedins have been proposed over the years but, to date, no single candidate fully satisfies all of these criteria. However, it is conceivable that distinct signaling factors might provide andromedin function acting in combination, but this possibility has not yet been tested.

Fibroblast growth factor (FGF) 7 and FGF10 were among the first molecules to be suggested as candidate andromedins (Lu et al., 1999; Yan et al., 1992). Both *Fgf7* and *Fgf10* are expressed in the mouse UGM *in vivo*, and addition of exogenous FGF7 or FGF10 can promote epithelial growth and branching in UGS explants in culture (Finch et al., 1995; Sugimura et al., 1996; Thomson and Cunha, 1999). Despite these similarities, FGF7 and FGF10 also display notable differences, as FGF7 can stimulate epithelial budding and ductal branching in the absence of DHT in neonatal prostate organ culture (Sugimura et al., 1996), whereas FGF10 is unable to induce epithelial budding in the absence of DHT (Donjacour et al., 2003). Furthermore, although *Fgf7* null mutants lack a prostate phenotype (Guo et al., 1996), *Fgf10* null mutants either lack budding completely or occasionally form rudimentary prostate buds (Donjacour et al., 2003); interestingly, the epithelial-specific deletion of their cognate receptor *Fgfr2* results in a small prostate that lacks anterior and ventral lobes (Lin et al., 2007). However, *Fgf7* and *Fgf10* do not appear to display sexually dimorphic expression and are not regulated by androgens (Thomson and Cunha, 1999; Thomson et al., 1997), suggesting that they are not andromedins. Instead, FGF10 might act to stabilize and promote the growth of nascent prostate buds, rather than be required for their initial formation (Donjacour et al., 2003).

There is evidence that at least some potential andromedins show sexually dimorphic expression. In particular, several Wnt ligands are upregulated during the period of prostatic bud formation (Mehta et al., 2011; Pritchard et al., 2009; Zhang et al., 2006). Furthermore, analyses using sensitive transgene reporters have shown that Wnt signaling activity is present in the mouse UGM prior to prostate budding and subsequently in both the mesenchyme and ductal tips during outgrowth (Kruithof-de Julio et al., 2013; Mehta et al., 2011). Consistent with these findings, downstream targets of canonical Wnt signaling such as *Lef1* are expressed in epithelial cells of prostate ductal tips (Francis et al., 2013; Mehta et al., 2013; Wu et al., 2011). Notably, conditional targeting of β-catenin, the central mediator of canonical Wnt signaling, results in formation of prostate rudiments that lack expression of the early prostate-specific epithelial marker *Nkx3.1* (*Nkx3-1*) (Francis et al., 2013; Mehta et al., 2013; Simons et al., 2012). However, there is currently no direct evidence that Wnt ligands are androgen regulated, and thus they do not currently fulfill all of the criteria for being considered andromedins. Interestingly, the Wnt inhibitor Wif1 is expressed at higher levels in the UGM of males and is androgen regulated, but *Wif1* null mutants lack a prostate phenotype (Keil et al., 2012b), and consequently the role of Wif1 in prostate formation remains unclear.

It is possible that andromedins, although not showing sexually dimorphic expression themselves, might elicit sexually dimorphic responses through downstream components of their signaling pathways. For example, retinoic acid activity is high in the mouse UGM and can promote epithelial budding in culture (Vezina et al., 2008). Formation of retinoic acid is catalyzed by aldehyde dehydrogenases (ALDH), and ALDH inhibitors block prostate bud formation by the UGS in explant culture in the presence of DHT (Bryant et al., 2014). Interestingly, *Aldh1a1* is only expressed in the male but not female UGS prior to prostate budding *in vivo*, whereas treatment of the female UGS with DHT in culture will induce *Aldh1a1* expression (Bryant et al., 2014). Similarly, other candidate andromedins such as FGFs might also exert their effects through sex-specific expression of downstream pathway components (Schaeffer et al., 2008; Thomson and Cunha, 1999; Thomson et al., 1997).

The smooth muscle hypothesis

The smooth muscle hypothesis (Fig. 4B) proposes that androgen signaling has indirect effects on epithelial growth by regulating the differentiation of smooth muscle, which forms a barrier between the inductive mesenchyme and the UGE to block further epithelial budding and outgrowth (Hayward et al., 1996b; Thomson and Marker, 2006). This model could be particularly applicable for the formation of the ventral prostate lobe, in which the inductive capabilities of the UGM appear to be concentrated in a region of dense peripheral mesenchyme cells termed the ventral mesenchymal pad (VMP) (Timms, 2008; Timms et al., 1995).

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Androgen signaling reduces the extent of smooth muscle formation, which might allow signaling factors that are constitutively active in the VMP to reach the epithelium (Chrisman and Thomson, 2006; Thomson et al., 2002).

However, androgens also appear to be essential for the differentiation of smooth muscle during prostate development, and castration of adult hosts results in a loss of smooth muscle cells (Hayward et al., 1996b). Furthermore, at least some of the signals that control smooth muscle formation might be epithelial, as the UGE is essential for smooth muscle differentiation by the UGM in tissue recombinants (Cunha et al., 1983, 1992). Moreover, the species-specific properties of the smooth muscle in tissue recombinants are conferred by the species origin of the epithelium, rather than the mesenchyme (Hayward et al., 1998).

In summary, these two models for how androgens induce prostate epithelial budding are not mutually exclusive, and might even synergize. It is likely that the mesenchyme produces secreted factors that induce epithelial growth, and that androgen signaling regulates some of these factors or downstream pathway components. Moreover, the diffusion and/or activity of these secreted signaling factors could be blocked by the formation of smooth muscle. Notably, smooth muscle differentiation might reflect an inhibitory feedback loop mediated by androgen signaling within the stroma, or might instead correspond to a reciprocal paracrine feedback loop involving signaling from prostate epithelium that has responded to mesenchymal andromedins (Fig. 4B).

Mechanisms of epithelial specification

Although the studies described above have identified factors that might signal between the mesenchyme and epithelium during initial prostate budding, relatively little is known about how these paracrine signals regulate the activity of transcriptional regulators to mediate epithelial specification. However, studies in genetically engineered mice have implicated several transcriptional regulators as key players in the specification and differentiation of the prostate epithelium (Table 1).

Of particular interest is the winged-helix transcription factor Foxa1, which is broadly expressed in endodermal derivatives, including in the UGE prior to prostate induction and subsequently in the prostate luminal epithelium (Mirosevich et al., 2005). Although *Foxa1* null mouse mutants have a neonatal lethal phenotype, prostate rudiments can be rescued by renal grafting, thereby allowing the analysis of prostate tissue phenotypes (Gao et al., 2005). Such rescued prostate tissue displays basal cell hyperplasia, loss of luminal secretory cells, absence of ductal canalization, and an increased number of intermediate cells co-expressing basal and luminal markers, suggesting a defect in epithelial differentiation (Gao et al., 2005). Furthermore, prostate-specific deletion of *Foxa1* results in prostate epithelial hyperplasia, as well as expression of seminal vesicle marker genes, consistent with loss of terminal differentiation (DeGraff et al., 2014). In the prostate, Foxa1 functions at least in part as a 'pioneer' transcription factor that opens chromatin to recruit AR to target promoters (Cirillo et al., 2002; Gao et al., 2003). Although Foxa1 is broadly expressed in endodermal tissues, its activity in the prostate epithelium may be mediated by interactions with tissue-specific regulators, including members of the NFI family of transcription factors, which co-occupy many AR/Foxa1 binding sites on target promoters (Grabowska et al., 2014).

The homeodomain transcription factor Nkx3.1 is also important for prostate epithelial specification. In mice, *Nkx3.1* null mutants display epithelial hyperplasia, altered prostate secretory protein expression, and expression of seminal vesicle markers, indicating a defect in epithelial differentiation (Bhatia-Gaur et al., 1999; Dutta et al., 2016). *Nkx3.1* is the earliest known specific marker of the prostate epithelium (Bhatia-Gaur et al., 1999; Keil et al., 2012a). In the neonatal prostate epithelium, *Nkx3.1* is expressed by all epithelial cells, whereas its expression in the adult prostate is found in all luminal cells as well as a subpopulation of basal cells (Bhatia-Gaur et al., 1999; Chen et al., 2005; Kruithof-de Julio et al., 2013; Wang et al., 2009). Interestingly, studies of a *lacZ* knock-in allele have shown that Nkx3.1 expression is strongest in the ductal tips and mediates a positive-feedback loop with canonical Wnt signaling during organogenesis, consistent with a central role for Nkx3.1 in prostate outgrowth and morphogenesis (Kruithof-de Julio et al., 2013). The similarity between *Foxa1* and *Nkx3.1* mutant phenotypes is consistent with biochemical studies that have shown that Foxa1 and Nkx3.1 can interact with AR to form components of an 'enhanceosome' that regulates expression of prostate epithelial target genes (He et al., 2010; Tan et al., 2012), although AR-independent roles of Nkx3.1 are also likely to be important. In particular, *Nkx3.1* expression can re-specify seminal vesicle epithelium to form prostate in tissue recombinants; this activity is mediated by interaction of Nkx3.1 with the G9a histone methyltransferase (Ehmt2) to activate expression of the target gene *Uty*, which encodes a histone demethylase (Dutta et al., 2016).

The homeobox gene *Hoxb13* encodes a transcriptional regulator that also plays a key role in prostate epithelial specification. Null mouse mutants for *Hoxb13* display normal development of the anterior and dorsolateral lobes, but display defective luminal differentiation and secretory protein production in the ventral lobe, although Nkx3.1 expression is normal (Economides and Capecchi, 2003). Interestingly, *Foxa1* is an essential positive regulator of *Hoxb13* expression in the ventral prostate epithelium of mice (McMullin et al., 2010). It has also been shown that, in human prostate cancer cell lines, HOXB13 protein interacts with AR to either repress or activate downstream targets, depending on the presence of specific HOXB13 response elements, and that HOXB13 is an AR co-regulator that both positively and negatively regulates the recruitment of AR and other AR co-regulators to cognate targets, including the *NKX3.1* enhancer (Norris et al., 2009).

Finally, recent evidence has identified Sox9 as a transcription factor that could play an essential early role in prostate epithelial specification. *Sox9* expression precedes that of *Nkx3.1* in the mouse UGE (Huang et al., 2012; Thomsen et al., 2008), and *SOX9* is also expressed in early prostatic epithelium in human embryos (Wang et al., 2008). Conditional deletion of *Sox9* in mice using a Cre recombinase driven by the *Nkx3.1* promoter leads to severely defective ventral prostate formation and abnormal anterior prostate differentiation (Thomsen et al., 2008). However, deletion of *Sox9* in the UGS prior to prostate budding results in loss of prostate formation in renal grafts, suggesting an essential role for Sox9 at early stages of prostate specification (Huang et al., 2012).

Mechanisms and regulation of ductal outgrowth and branching morphogenesis

Following the formation of prostatic buds, the epithelium undergoes extensive proximal-distal outgrowth and branching morphogenesis, resulting in the morphologically distinct lobes of the rodent prostate or the zones of the human prostate. This phase of organogenesis is believed to be driven by a population of progenitor cells localized at

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the ductal tips, where the majority of proliferative cells are located and bifurcation of the branch points occurs (Sugimura et al., 1986b). In rodents, this stage of organogenesis is initiated during late fetal development, but the most prominent bud outgrowth occurs during the first two weeks postnatally. Analyses of genetically engineered mice, as well as of organ culture assays, have given us insight into the developmental pathways that drive prostate ductal outgrowth and branching morphogenesis. These pathways include the Notch and Activin signaling pathways, and additional studies have suggested roles for glial cell-derived neurotrophic factor (GDNF) and Ephrin signaling as well (Table 1). At present, however, the activities of the Hedgehog (Hh) and bone morphogenetic protein (BMP) signaling pathways in prostate ductal morphogenesis are best understood.

Early studies showed that *Shh* expression in the nascent prostate bud epithelium signals to the surrounding UGM by activating its receptor *Ptc* (*Ptch1*) and downstream Gli transcriptional regulators in the mesenchyme (Doles et al., 2006; Lamm et al., 2002; Wang et al., 2003). Organ culture experiments, together with phenotypic analyses of null mutant and transgenic overexpressing mice, have found that *Shh* is not required for the initial formation or outgrowth of prostate buds, but instead functions to mediate ductal branching and patterning in a stage-specific manner (Berman et al., 2004; Freestone et al., 2003; Yu and Bushman, 2013). Recent studies have also examined Hh signaling during androgen-mediated regeneration of the adult prostate, a process that has similarities with organogenesis, and have found that epithelial Hh activity induces the microRNAs miR26a and miR26b to repress stromal expression of hepatocyte growth factor (HGF), which induces epithelial branching (Lim et al., 2014). Thus, at least in the adult regenerating prostate, regions of low Hh signaling activity might correspond to the location of ductal branch points.

Parallel studies have shown that *Bmp4* is expressed in the UGM and mediates ductal epithelial branching; the addition of BMP4 protein decreases the number of ductal tips in UGS culture, whereas *Bmp4*$^{+/-}$ heterozygotes display increased ductal branching (Lamm et al., 2001). Similarly, BMP7 treatment reduces ductal tip formation in organ culture, and null mutants for *Bmp7* display significantly increased ductal branching (Grishina et al., 2005). Conversely, analyses of the BMP inhibitor noggin have shown that it is expressed in the UGM and that *Nog* mutant mice lack the ventral mesenchymal pad and fail to form the ventral prostate (Cook et al., 2007). Thus, both the Shh and BMP4/7 pathways appear to coordinate epithelial-mesenchymal interactions during prostate branching morphogenesis, but the precise mechanisms involved and their interactions with other relevant signaling pathways are still largely unresolved.

Although the majority of genes known to play a role in prostate branching morphogenesis are regulated by androgens (Table 1), there are likely to be genes and pathways important for this phase of prostate development that are independent of androgen signaling. Indeed, it has been shown that androgen ablation of neonates delays but does not completely abrogate further growth (Donjacour and Cunha, 1988; Lung and Cunha, 1981; Price, 1936). Conversely, administration of exogenous androgens to sexually mature male rodents does not promote further growth and branching of the prostate (Berry and Isaacs, 1984). At present, however, little is known about androgen-independent pathways that promote prostate branching morphogenesis. Estrogen signaling might represent one potential androgen-independent mechanism, as ERα (*Esr1*) null mice display reduced prostate branching morphogenesis (Chen et al., 2009, 2012), but it is not known whether estrogen signaling is truly independent of androgens at these stages of prostate development.

Differentiation of the prostate epithelium

During initial stages of branching morphogenesis, the nascent prostate is composed of solid epithelial cords. However, the epithelial cords subsequently undergo ductal canalization to form glandular structures, and the epithelial cells differentiate into basal, luminal and neuroendocrine lineages.

Mechanisms of epithelial canalization

Unlike the adjacent seminal vesicles that undergo branching morphogenesis as hollow tubes, the developing prostate epithelium undergoes significant branching morphogenesis as solid cords, which then canalize during later stages of development (Hayward et al., 1996a). Although ductal canalization has not been studied in detail in the prostate, it might share molecular similarities with canalization of other tissues such as the mammary and salivary glands (Mailleux et al., 2008). Mammary gland canalization is believed to occur primarily by anoikis, a process in which epithelial cells detach from the extracellular matrix and undergo apoptosis, although non-apoptotic cell death mechanisms might also play a role (Humphreys et al., 1996; Mailleux et al., 2007). Notably, studies of the rat prostate have shown that apoptosis occurs in the center of prostate epithelial cords during early canalization (Bruni-Cardoso and Carvalho, 2007). This process might be regulated by genes such as *Dkk3* and *Mmp2*, which have been previously shown to play a role in normal prostate lumen formation (Table 1) (Bruni-Cardoso et al., 2010b; Kawano et al., 2006; Romero et al., 2013). Further studies are required to determine whether prostate ductal canalization occurs through anoikis and to elucidate the regulatory genes that drive lumen formation.

Lineage specification during organogenesis, homeostasis and regeneration

The transition of undifferentiated epithelial cords of the embryonic prostate into fully differentiated basal and luminal cells in the adult prostate has been an active area of investigation. Below, we describe studies of prostate epithelial progenitors and lineage hierarchy during organogenesis (summarized in Fig. 5). Prostate epithelial progenitors also maintain tissue homeostasis in adulthood, although studies of these progenitors have generally been limited because the adult epithelium is generally quiescent and displays slow turnover. Instead, adult prostate progenitors are more commonly analyzed in experimental contexts in which more rapid epithelial proliferation is induced (see Box 1). Thus, we also mention studies of lineage transitions during tissue homeostasis and androgen-mediated regeneration, but refer the reader to other recent reviews that discuss these studies in more detail (Kwon and Xin, 2014; Shibata and Shen, 2015; Wang and Shen, 2011).

In early studies, the prostate epithelium was reported to display a homogeneous phenotype prior to ductal canalization, displaying co-expression of basal and luminal markers as well as CK19, suggesting that the adult epithelium arises from a population of so-called ‘intermediate’ cells (Wang et al., 2001). By contrast, subsequent analyses of a more comprehensive set of cytokeratins found that the only such marker expressed throughout the developing UGS was the luminal marker CK8, raising the possibility that CK8-expressing cells give rise to the other differentiated cell types (Trompetter et al., 2008). However, these

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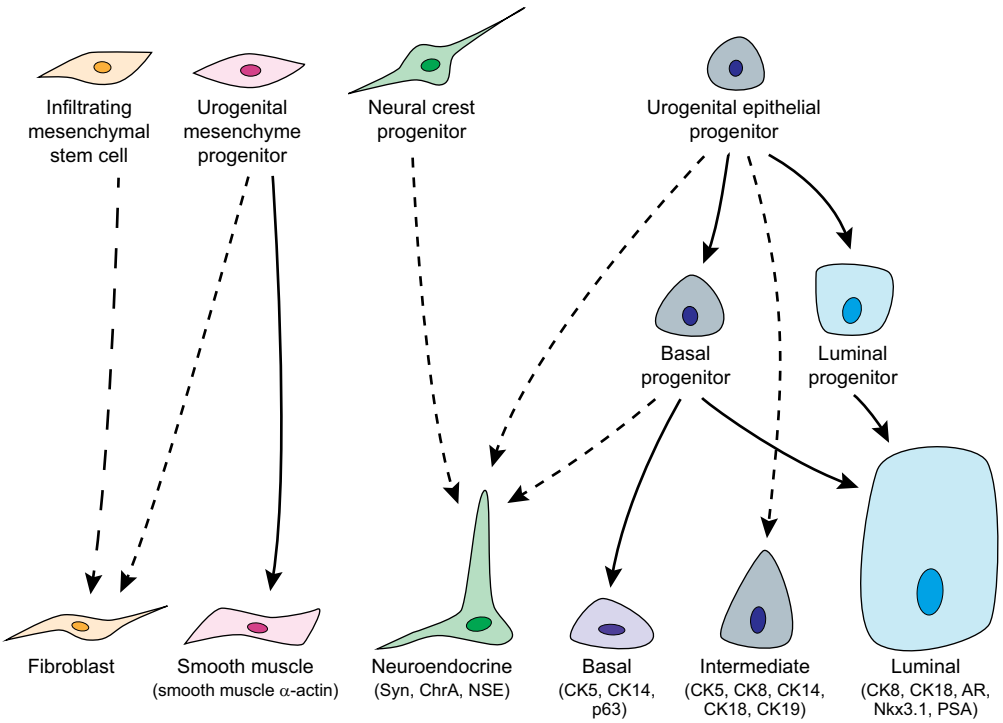

Fig. 5. Lineage relationships between cell types during prostate organogenesis. Lineage relationships between cell types during prostate organogenesis are depicted, showing known (solid arrows) and potential (dashed arrows) relationships. Upon induction of prostate budding, intermediate-like urogenital epithelial progenitors give rise to basal progenitors, which have multipotent and unipotent activity, as well as unipotent luminal progenitors. The origins of intermediate and neuroendocrine cells have not currently been resolved, although several studies suggest that neuroendocrine cells could either have neural crest or epithelial origins. Urogenital mesenchyme progenitors give rise to differentiated smooth muscle cells in the prostatic stroma, but it is not known whether these progenitors also give rise to prostate fibroblasts, or whether the fibroblasts originate from infiltrating mesenchymal stem cells. ChrA, chromogranin A; NSE, enolase 2 (Eno2); Syn, synaptophysin (Syp).

studies of cell type-specific marker expression are inherently limited in their ability to provide definitive information on lineage relationships.

More recently, lineage-tracing studies using specific Cre drivers to mark cell types *in vivo* have suggested that basal progenitors give rise to the mature prostate epithelium during organogenesis. In particular, inducible Cre drivers under the control of CK5, CK14 or CK8 promoters have been used to perform lineage tracing of basal and luminal epithelial cells during postnatal epithelial growth (Ousset et al., 2012). These analyses of early postnatal development showed that both luminal and basal compartments contain unipotent progenitors that only generate progeny of a single cell type, but that there are also multipotent basal progenitors that can give rise to basal, luminal and neuroendocrine cells (Ousset et al., 2012; Wuidart et al., 2016). Consistent with this interpretation, lineage-tracing studies of basal cells using *deltaNp63*^{cre} mice have shown that p63-expressing basal cells in the UGS can give rise to all three prostate epithelial cell types (Pignon et al., 2013). However, there is significant co-expression of basal and luminal markers during early organogenesis, and basally located cells continue to express luminal markers well into postnatal development, which overlaps temporally with the time in which lineage marking occurs in these studies (Fig. 2). Therefore, it is unclear whether the progenitors of luminal and neuroendocrine cells are exclusively basal during early stages of organogenesis. Notably, although *p63* null mutant mice are neonatal lethal, renal grafting of *p63* mutant UGS results in prostate tissue that contains luminal cells but completely lacks basal cells, and can undergo serial androgen-mediated regeneration in the absence of basal cells, indicating tissue maintenance by a luminal progenitor (Kurita et al., 2004). The potential roles of luminal cells as progenitors during prostate organogenesis therefore requires further investigation, particularly given increasing evidence for luminal progenitor activity in adult prostate epithelium (Chua et al., 2014; Karthaus et al., 2014; Kwon et al., 2016; Wang et al., 2009).

Additional studies have focused on the localization of distinct progenitor populations within regions of the prostate lobes. Early studies of ductal outgrowth during organogenesis and androgen-mediated regeneration showed that cellular proliferation is highly enriched at ductal tips, suggesting a distal location for prostate epithelial progenitors (Sugimura et al., 1986a,b). Notably, a distal localization is consistent with the distribution of stem/progenitor cells during ductal outgrowth of tissues such as the lung and mammary gland (Hogan et al., 2014; Sreekumar et al., 2015). Furthermore, expression of Nkx3.1 is elevated in ductal tips (Kruithof-de Julio et al., 2013), and a role for Nkx3.1 in progenitor specification and maintenance has been suggested by the ductal morphogenesis defects in *Nkx3.1* null mutants as well as by the stem cell properties of luminal Nkx3.1-expressing cells (termed CARNs; castration-resistant Nkx3.1-expressing cells) in the regressed adult prostate (Bhatia-Gaur et al., 1999; Wang et al., 2009). In contrast, studies of bromodeoxyuridine (BrdU) label-retaining cells have supported a proximal localization of epithelial progenitors during serial regeneration of the adult prostate (Tsujimura et al., 2002). This finding is also consistent with the recent identification of a luminal progenitor in the proximal region of the adult prostate epithelium (Kwon et al., 2016). Taken together, these findings suggest that the prostate epithelium might contain multiple stem/progenitor populations with distinct spatial distributions and functions during organogenesis and regeneration.

Although studies of prostate epithelial progenitors have focused mostly on basal and luminal cells, they have also given insight into the origin of neuroendocrine cells. Several studies have addressed whether neuroendocrine cells are of epithelial origin, or instead could be derived from the caudal neural crest. In particular, tissue reconstitution experiments have shown that adult epithelial progenitors can generate neuroendocrine cells (Goldstein et al., 2008; Wang et al., 2009, 2013b), and this has been further supported by lineage tracing during prostate organogenesis (Ousset et al., 2012). Consistent with this interpretation, lineage tracing has shown that neuroendocrine cells in the lung and thyroid have an epithelial

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Table 1. Genes and developmental pathways that function during prostate organogenesis

Pathway/gene	Model	Assay	Result	Reference
Androgen				
Ar	*Tfm* mouse	Tissue recombination with *Tfm* or WT UGM or UGE.	WT UGM (with AR) was essential for the formation of prostate.	(Cunha and Lung, 1978)
	Tfm mouse	Tissue recombinants with WT UGM and TFM or WT UGE.	WT epithelium (with AR) was required for production of prostatic secretory proteins.	(Donjacour and Cunha, 1993)
	WT rat	Organ culture of UGS.	Androgen treatment resulted in induced prostate budding from male and female UGS.	(Takeda et al., 1986)
Estrogen				
Esr1 (*ERα*)	*ACTB-Cre; ERα$^{flox/flox}$* mice	Analysis of prostate lobes.	Reduced epithelial branching in DLP and VP. Reduced fibroblast proliferation and differentiation.	(Chen et al., 2009)
	FSP-Cre; ERα$^{flox/flox}$ and *probasin-Cre; ERα$^{flox/flox}$* mice	Analysis of prostate lobes.	Reduced epithelial branching in *FSP-Cre; ERα$^{flox/flox}$* but not *probasin-Cre; ERα$^{flox/flox}$* mice.	(Chen et al., 2012)
FGF				
Fgf7	WT rat	Organ culture of postnatal VP with FGF7, or FGF7 inhibitors.	Culture with FGF7 increased epithelial budding, growth and branching. FGF7 inhibition reduced epithelial growth and branching.	(Sugimura et al., 1996)
Fgf10	WT rat	Organ culture of P0 VP with FGF10.	Increased epithelial growth and branching.	(Thomson and Cunha, 1999)
	Fgf10 null mouse	Analysis, organ culture, and renal transplants of UGS.	UGS analysis and culture displayed reduced epithelial budding. Renal transplant revealed reduced epithelial growth, branching and differentiation.	(Donjacour et al., 2003)
	WT rat	Organ culture of P0 VP and LP with FGF10 protein.	Increased epithelial growth, branching and differentiation.	(Huang et al., 2005)
Fgfr2	*Nkx3.1$^{Cre/+}$; Fgfr2$^{flox/flox}$* mouse	Analysis of prostate lobes.	Reduced DP and LP size, diminished AP and VP. Decreased epithelial branching, proliferation and basal cell abundance.	(Lin et al., 2007)
Frs2	*Nkx3.1$^{Cre/+}$; Frs2α$^{flox/flox}$* mouse	Analysis of prostate lobes.	Reduced prostate growth and epithelial branching.	(Zhang et al., 2008)
Hedgehog				
Gli2	*Gli2* null mouse	Analysis, organ culture, and renal transplants of UGS.	Reduced UGS size, epithelial bud formation and polarization. Epithelial hyperplasia and increased basal cell population in renal grafts.	(Doles et al., 2006)
Shh	*Shh* mutant and *Nkx3.1$^{tm2(lacZ)}$* mice	Analysis, organ culture, and renal transplants of UGS.	Loss of epithelial budding *in vivo*. Morphology was rescued in UGS culture and renal transplants. Culture of *Nkx3.1$^{tm2(lacZ)}$* heterozygote UGS with SHH peptide or cyclopamine resulted in altered ductal morphology.	(Berman et al., 2004)
	WT rat and *Shh* null mice	Organ culture of *Shh* UGS. Organ culture of P0 UGS with SHH or cyclopamine.	*Shh* UGS culture displayed normal prostatic budding. Culture of rat UGS with cyclopamine reduced epithelial growth and increased ductal tip number, whereas culture with SHH reduced ductal tip number and increased the mesenchyme population.	(Freestone et al., 2003)
	WT rat	Organ culture of P2 VPs with SHH or cyclopamine.	Reduced epithelial branching, epithelial and stromal proliferation, and increased luminal populations when cultured with SHH. Increased epithelial branching and proliferation, and decreased luminal population when cultured with cyclopamine.	(Wang et al., 2003)
	WT mice	Renal transplants of UGS with Shh antibody.	Renal grafts with Shh antibody displayed reduced epithelial budding.	(Podlasek et al., 1999a)
Smo	WT rat	Organ culture of UGS with SmoM2-transfected stromal cells.	Reduced epithelial branching.	(Wang et al., 2003)

Continued

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Table 1. Continued

Pathway/gene	Model	Assay	Result	Reference
Hox				
Hoxa10	*Hoxa10* null mouse	Analysis of prostate lobes.	Reduced AP size and epithelial branching.	(Podlasek et al., 1999c)
Hoxa13	*Hoxa13* null mouse	Analysis of prostate lobes.	Reduced prostate size and epithelial branching in DLP and VP.	(Podlasek et al., 1999b)
Hoxb13	*Hoxb13* null mouse	Analysis of prostate lobes.	Defects in luminal cell morphology and absence of secretory protein in VP.	(Economides and Capecchi, 2003)
Hoxd13	*Hoxd13* null mouse	Analysis of prostate lobes.	Reduced epithelial branching and growth.	(Podlasek et al., 1997)
Hoxa13; Hoxd13	*Hoxa13; Hoxd13* null mice	Analysis of prostate lobes.	Absence of AP and reduced epithelial growth.	(Warot et al., 1997)
TGFβ				
Activin A	WT rats	Organ culture of VP with recombinant activin A.	Reduced epithelial growth and branching, reduced mesenchyme differentiation.	(Cancilla et al., 2001)
Bmp4	WT and $Bmp4^{+/-}$ mice	Organ culture of WT UGS with BMP4. Analysis of $Bmp4^{+/-}$ prostate lobes.	Culture of UGS with BMP4 reduced epithelial budding and proliferation. $Bmp4^{+/-}$ prostate displayed increased epithelial branching.	(Lamm et al., 2001)
Bmp7	WT and $Bmp7^{lacZ/lacZ}$ mice	Analysis of $Bmp7^{lacZ/lacZ}$ prostate lobes. Organ culture of WT UGS with BMP7.	$Bmp7^{lacZ/lacZ}$ prostates displayed increased epithelial branching. Culture of UGS with BMP7 reduced prostate epithelial budding.	(Grishina et al., 2005)
Bmpr1a	$Shh^{CreERT2/+}$; $Bmpr1a^{flox/-}$ mice	Analysis of postnatal and adult prostate.	Increased epithelial proliferation and impaired differentiation.	(Omori et al., 2014)
Fst	Sprague-Dawley rats	Organ culture of VP with follistatin.	Increased epithelial growth and branching, increased stromal differentiation.	(Cancilla et al., 2001)
Nog	*Nog* (noggin) null mice	Renal transplants of UGS.	Lack of VMP and VP; reduced LP and DP ductal tips.	(Cook et al., 2007)
Notch				
Notch1	$Notch1^{NTR\text{-}IRES\text{-}GFP}$ mice	Organ culture of WT and *Notch1-NTR-IRES-GFP* prostates treated with prodrug to delete *Notch1*-expressing cells in transgenic mice.	Inhibited epithelial growth and branching. Decreased epithelial differentiation.	(Wang et al., 2004)
	Mx1-Cre; $Notch^{flox/flox}$ and $Nkx3.1^{Cre/+}$; $Notch^{flox/flox}$ mice; WT rats	Organ culture of P3 WT VP with γ-secretase inhibitors. Analysis of prostate lobes in transgenic mice.	Treatment with γ-secretase inhibitor reduced epithelial branching and differentiation. *Notch1* transgenic mice displayed reduced epithelial differentiation.	(Wang et al., 2006)
Wnt				
Canonical	$Nkx3.1^{lacZ/+}$ *mice*	Organ culture of UGS with Wnt inhibitors.	Reduced epithelial budding, Nkx3.1 expression, proliferation, and differentiation of luminal cells.	(Kruithof-de Julio et al., 2013)
Ctnnb1 (β-catenin)	$Axin2^{CreERT2/+}$; $Ctnnb1^{(ex2\text{-}6)fl/fl}$ (deletion) and $Axin2^{CreERT2/+}$; $Ctnnb1^{(ex3)fl/+}$ (stabilization) mice	Analysis of prostate lobes.	Both loss and gain of function of *Ctnnb1* reduced gland size and lumen secretions, and altered the luminal:basal ratio.	(Lee et al., 2015)
	$Nkx3.1^{Cre/+}$; $Ctnnb1^{(ex2\text{-}6)fl/fl}$ (deletion) and $Nkx3.1^{Cre/+}$; $Ctnnb1^{(ex3)fl/+}$ (stabilization) mice	Analysis and organ culture of UGS.	Deletion resulted in reduced epithelial growth, branching, and number of p63-positive cells. Gain of function resulted in increased epithelial bud size.	(Francis et al., 2013)
	$Shh^{Cre/+}$; $Ctnnb1^{(ex2\text{-}6)fl/fl}$ (deletion) and $Shh^{CreERT2/+}$; $Ctnnb1^{(ex2\text{-}6)fl/fl}$ (deletion) and $Shh^{CreERT2/+}$; $Ctnnb1^{(ex3)fl/+}$ (stabilization) mice	Analysis of UGS.	Loss-of-function UGS displayed loss of prostate specification and epithelial bud induction. Inducible deletion in UGS resulted in reduced epithelial budding and basal cells. Gain of function in UGS resulted in reduced epithelial budding.	(Mehta et al., 2013)
	$Rosa26^{CreER/+}$; $Ctnnb1^{(ex2\text{-}6)fl/fl}$ (deletion) $Nkx3.1^{Cre/+}$; $Ctnnb1^{(ex2\text{-}6)fl/fl}$ (deletion) mice	Organ culture and renal transplant of UGS.	UGS displayed loss of prostate specification and epithelial bud induction. UGS renal grafts failed to develop prostatic structures. PND0 renal grafts displayed reduced prostate epithelial and stromal differentiation.	(Simons et al., 2012)

Continued

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Table 1. Continued

Pathway/gene	Model	Assay	Result	Reference
Lgr4	*Lgr4* null mice	Analysis of prostate lobes.	Reduced prostate size, ductal branching and differentiation.	(Luo et al., 2013)
Rspo2, *Rspo3*	WT mice	Culture of UGS with Wnt inhibitor TCDD and R-spondin-2 or R-spondin-3.	UGS culture with R-spondins increases epithelial budding and protects against growth inhibition by TCDD.	(Branam et al., 2013)
Sfrp1	*Sfrp1*$^{lacZ/lacZ}$ (null) and *PB-SFRP1* (overexpression) mice	Analysis of prostate lobes.	SFRP1^{lacZ} mice displayed reduced branching. PB-SFRP1 mice displayed increased proliferation.	(Joesting et al., 2008)
Wnt5a	*Wnt5a* null and WT mice	Organ culture of *Wnt5a* UGS and WT UGS with WNT5a antibody or rmWNT5a. Renal grafts of WT UGS treated with rmWNT5a.	*Wnt5a*$^{-/-}$ UGS displayed reduced epithelial budding. UGS culture with WNT5a antibody had no effect, whereas treatment with rmWNT5a reduced epithelial budding. UGS renal grafts treated with rmWNT5a displayed loss of ventral prostate ducts.	(Allgeier et al., 2008)
	Sprague-Dawley rats and *Wnt5a* null mice	Organ culture of P0 VP with WNT5a protein. Organ culture and renal transplant of *Wnt5a* UGS.	Culture of UGS with Wnt5a reduced epithelial growth and branching and reduced epithelial and mesenchyme differentiation. Culture of *Wnt5a*$^{-/-}$ UGS in culture displayed disorganized epithelial budding. *Wnt5a*$^{-/-}$ transplants displayed reduced epithelial canalization and cell polarization.	(Huang et al., 2009)
Wif1	*Wif1*$^{lacZ/lacZ}$ (null) and WT mice	Analysis of *Wif1* UGS and WT UGS. Organ culture with recombinant WIF1 protein.	*Wif1* null mutants displayed unaltered epithelial bud formation. Culture with WIF1 protein increased epithelial bud number and growth.	(Keil et al., 2012b)
Miscellaneous				
Cdh1	WT mice	Organ culture of UGS treated with DNA methylation inhibitor.	Treatment with 5-azacytidine reduced epithelial budding, which is restored with addition of CDH1 antibody.	(Keil et al., 2014)
Ephrin B1	Wistar rats	Organ culture of P0 VP with EphrinB1-Fc to stimulate Ephrin forward signaling.	Reduced epithelial growth and branching. Increased ductal tip size.	(Ashley et al., 2010)
Foxa1	*Foxa1* null mice	Renal transplant assay of PND1 prostate.	Reduced graft size, epithelial canalization and differentiation.	(Gao et al., 2005)
Gdnf	WT mice	Organ culture of UGS with GDNF.	Increased UGM and UGE proliferation.	(Park and Bolton, 2015)
Ghr	Growth hormone antagonist mice	Analysis of VP and DP prostate lobes.	Reduced epithelial branching.	(Ruan et al., 1999)
Igf1	*Igf1* null mice	Analysis of VP and DP prostate lobes.	Reduced epithelial growth and branching.	(Ruan et al., 1999)
Il1r1	*Il1r1* null and WT mice	Culture of WT UGS with IL-1. Analyses of prostate lobes in *Il1r1* mice.	Culture of UGS with IL-1 increased epithelial growth. *Il1r1* mice display reduced growth and branching in VP and DLP.	(Jerde and Bushman, 2009)
Mmp2	*Mmp2* null mice	Analysis of VP prostate lobes.	Reduced epithelial growth and branching.	(Bruni-Cardoso et al., 2010a)
	WT rats	Organ culture of UGS with MMP inhibitor GM6001 or *Mmp2* siRNA.	Culture of UGS with inhibitor or siRNA reduced epithelial growth and branching. siRNA also reduced epithelial canalization.	(Bruni-Cardoso et al., 2010b)
Nkx3.1	*Nkx3.1* null mice	Analysis of prostate lobes.	Reduced epithelial branching, secretory production.	(Bhatia-Gaur et al., 1999)
Odc1	WT mice	Organ culture of UGS with ornithine decarboxylase inhibitor DFMO.	Reduced epithelial budding.	(Gamat et al., 2015)
p63	*p63* null mice	UGS analysis and renal transplant assay.	*p63* UGS *in vivo* and in renal grafts had no detectable basal cell population and luminal cells displayed differentiation into mucinous cells.	(Kurita et al., 2004)
	p63 mutant rescued by injection of blastocysts with *p63*$^{+/+}$ embryonic stem cells to generate chimeras	Renal transplant assay of *p63* UGS and analysis of UGS from *p63* chimeras.	Renal transplants of *p63* UGS displayed mucinous luminal epithelium. UGS from *p63* chimeras displayed prostate buds derived from the p63$^{+/+}$ wild-type cell population.	(Signoretti et al., 2005)

Continued

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Table 1. Continued

Pathway/gene	Model	Assay	Result	Reference
Pax2	*Pax2* null mice	UGS culture and renal transplant assay.	Reduced epithelial branching and differentiation.	(Xu et al., 2012)
Pten	*Rosa26*$^{CreER/+}$; *Pten*$^{flox/flox}$ and WT mice	Analysis of prostate lobes and organ culture of UGS with rapamycin.	Treatment with rapamycin increased ductal branching. Inducible deletion of *Pten* resulted in decreased branching.	(Ghosh et al., 2011)
Ptn	WT rats	Organ culture of P0 VP with pleiotrophin protein.	Increased epithelial growth and branching.	(Orr et al., 2011)
Sox9	*CAGG-CreER*^{TM}; *Sox9*$^{flox/flox}$ mice	Organ culture and renal transplant of UGS.	Inhibition of budding in culture and in transplants when induced at 14.5 dpc and reduced budding when induced at 16.5 dpc.	(Huang et al., 2012)
	Nkx3.1$^{Cre/+}$; *Sox9*$^{flox/flox}$ mice	Analysis and renal transplant assay of UGS.	Transplants of *Nkx3.1*$^{Cre/+}$; *Sox9*$^{flox/flox}$ UGS at 15.5 dpc displayed absent VP structures and reduced prostate epithelium when transplants were conducted from 18.5 dpc prostates.	(Thomsen et al., 2008)
Sulf1	WT mice	UGS culture with *Sulf1* overexpression by transfection.	Reduced epithelial budding.	(Buresh et al., 2010)

rm, recombinant; WT, wild type.

origin (Johansson et al., 2015; Song et al., 2012). However, descriptive analyses have revealed that cells positive for chromogranin A, which marks neuroendocrine cells, can migrate into the UGS during development, suggesting that prostate neuroendocrine cells are derived from the neural crest ectoderm (Aumüller et al., 1999, 2001); notably, this conclusion has been supported by a recent lineage-tracing study (Szczyrba et al., 2017). Thus, additional work will be required to clarify these apparent discrepancies concerning the origin of prostate neuroendocrine cells.

Stromal progenitors and differentiation during prostate development

During organogenesis, differentiation of the prostate stroma occurs in parallel with that of the epithelium, and the two processes are coordinated by reciprocal epithelial-stromal signaling. The UGM differentiates from a fibroblastic inductive mesenchyme that promotes epithelial growth into a mature stromal compartment containing multiple specialized cell types. To date, the constituent cell types of the mouse prostate stroma have been characterized to a limited extent during organogenesis (Abler et al., 2011; Georgas et al., 2015), but their precise origin and functions have not been clearly elucidated. In the adult mouse prostate, recent work has defined at least four distinct populations of stromal cells: subepithelial cells, smooth muscle cells, wrapping cells and interstitial fibroblasts (Peng et al., 2013). Subepithelial cells are fibroblast-like cells that lie adjacent to the basement membrane beneath the basal epithelial cells, and are covered by a layer of smooth muscle cells. Wrapping cells are also fibroblast-like, and are tightly associated with the outer part of the smooth muscle layer, whereas interstitial fibroblasts are located between prostate ducts (Peng et al., 2013). In the case of the human prostate, ultrastructural analyses of subepithelial cells have resulted in their subdivision into fibroblasts, interstitial Cajal-like cells and spindle-shaped cells with myoid differentiation (Gevaert et al., 2014).

Tissue recombination studies have suggested that the differentiation of smooth muscle cells from the UGM is regulated by signaling from the adjacent epithelium during organogenesis (Hayward et al., 1997). In particular, the combination of human prostate epithelium with rodent UGM resulted in a smooth muscle phenotype that reflected human and not rodent tissue (Hayward et al., 1998). Interestingly, lineage-tracing studies indicate that these smooth muscle cells are maintained by a unipotent progenitor population during adult tissue homeostasis and regeneration (Peng et al., 2013). However, less is known about the origin of the fibroblast-like populations, which might also differentiate from the UGM or alternatively could be derived from infiltrating mesenchymal stem cells (Shaw et al., 2008).

Concluding remarks

Overall, the study of prostate organogenesis provides a rich and fertile area of investigation, at the intersection of multiple topics of interest to developmental biologists, including: the generation of secondary sexual dimorphism, the relationship of androgen

Box 1. Prostate gland homeostasis and regeneration

The mature prostate is a relatively quiescent organ that exhibits slow epithelial turnover. Lineage-tracing studies in mice have shown that unipotent basal and luminal progenitors are largely responsible for the maintenance of their respective cell compartments during adult tissue homeostasis (Choi et al., 2012; Lu et al., 2013; Wang et al., 2013b). However, basal and luminal cells can display additional properties in other contexts. Notably, androgen deprivation by castration results in significant epithelial cell death in the prostate, whereas subsequent testosterone supplementation results in rapid tissue regeneration. During androgen-mediated regeneration, luminal castration-resistant Nkx3.1-expressing cells (CARNs) as well as basal cell populations can display bipotency and self-renewal indicative of stem cell activity (Lee et al., 2014; Wang et al., 2009, 2013b, 2015). Furthermore, during tissue damage and repair, such as in mouse models of prostatitis or luminal-specific anoikis, basal cells show significant capability to replace lost luminal cells (Kwon et al., 2014; Toivanen et al., 2016). Finally, when either basal and luminal cells are isolated from their native tissue microenvironment and grown as spheroids or organoids in culture, or in tissue reconstitution renal grafting assays *in vivo*, both cell types can show bipotency and reconstitution of epithelial architecture (Burger et al., 2005; Chua et al., 2014; Goldstein et al., 2008, 2010; Hofner et al., 2015; Karthaus et al., 2014; Lawson et al., 2007; Richardson et al., 2004; Wang et al., 2013b). Overall, although prostate epithelial progenitors appear to show limited stem cell properties during homeostasis, considerable plasticity can be activated in both basal and luminal cell compartments under circumstances when rapid epithelial growth is required.

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signaling to tissue morphogenesis, the role of reciprocal epithelial-mesenchymal interactions, and the properties of epithelial progenitors and their role in lineage specification, tissue homeostasis and regeneration. However, the relationship between normal development and prostate tumorigenesis is also of particular interest to cancer biologists, as many of the key transcriptional regulators of normal prostate development have also been implicated in prostate cancer, including Nkx3.1, Foxa1, Hoxb13, Sox9, and of course AR (Baca et al., 2013; Barbieri et al., 2012; Bhatia-Gaur et al., 1999; Ewing et al., 2012; Kim et al., 2002; Network, 2015; Shen and Abate-Shen, 2010; Wang et al., 2008, 2013a; Watson et al., 2015). Moreover, key signaling pathways, such as the canonical Wnt and FGF pathways, that function in the prostate are also known to play crucial roles during tumor progression (Acevedo et al., 2007; Corn et al., 2013; Grasso et al., 2012; Kypta and Waxman, 2012; Memarzadeh et al., 2007; Sun et al., 2012). Elucidating how these signaling pathways and transcriptional regulators are integrated at the molecular and functional levels to mediate prostate specification and differentiation will be of particular relevance for understanding their roles in prostate cancer. For example, mechanisms that drive progenitor cell plasticity in the context of epithelial regeneration and repair could also play a role in prostate tumor plasticity in mediating resistance to cancer therapies. Thus, the field of prostate development is likely to remain of vital interest and importance for continuing investigation.

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Competing interests

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