REVIEW

Prostate organogenesis

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ABSTRACT

Prostate organogenesis begins during embryonic development and continues through puberty when the prostate becomes an important exocrine gland of the male reproductive system. The specification and growth of the prostate is regulated by androgens and is largely a result of cell-cell communication between the epithelium and mesenchyme. The fields of developmental and cancer biology have long been interested in prostate organogenesis because of its relevance for understanding prostate diseases, and research has expanded in recent years with the advent of novel technologies, including genetic-lineage tracing, single-cell RNA sequencing and organoid culture methods, that have provided important insights into androgen regulation, epithelial cell origins and cellular heterogeneity. We discuss these findings, putting them into context with what is currently known about prostate organogenesis.

KEY WORDS: Prostate organogenesis, Prostate development, Androgen signaling, Androgen receptor, Cellular heterogeneity, Prostate culture

Introduction

The prostate is a male sex accessory gland that produces and secretes crucial components of seminal fluid following puberty and is located at the base of the bladder. The prostatic fluid amounts to approximately 30% of the seminal fluid. It contains essential nutrients, including enzymes, zinc and prostate-specific antigen, that aid spermatozoan survival for reproduction. Thus, the prostate has a significant role in male reproductive health and fertility. Although the development of this reproductive organ starts at gestational week (GW) 8 in humans, the prostate becomes a functional exocrine gland after puberty (Buskin et al., 2021).

Prostate organogenesis is composed of many intricate developmental processes that remain an important area of study owing to the prevalence of prostate diseases such as benign prostatic hyperplasia (BPH) and prostate cancer. Analyses of gene expression profiles have identified similarities between prostate organogenesis and disease (Dhanasekaran et al., 2005; Pritchard et al., 2009). Recently, the advent of new technologies, such as single-cell RNA sequencing (scRNA-seq), has added to our understanding of epithelial cell origins and heterogeneity, with implications for understanding prostate cancer initiation, progression and treatment (Crowley et al., 2020; Guo et al., 2020; Henry et al., 2018; Karthaus et al., 2020; Mevel et al., 2020). Additionally, scRNA-seq experiments have also demonstrated the role of androgen receptor (AR) signaling and androgen requirements during prostate

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organogenesis (Lee et al., 2021; Mevel et al., 2020). Other recently developed technologies, including genetic lineage-tracing models and refined organoid culture systems, have provided further insights into prostate progenitor cells during organogenesis (Grossmann et al., 2021; Hepburn et al., 2020; Shibata et al., 2020; Tika et al., 2019). Here, we focus on reviewing these studies and discuss what is currently known about prostate organogenesis. We first introduce the stages of prostate organogenesis and discuss how androgens regulate this process. We then evaluate the cellular heterogeneity within the developing prostate and compare the conservation of prostate organogenesis between humans and mice. Finally, we discuss additional *in vivo*, *ex vivo* and *in vitro* model systems with which to study prostate organogenesis.

Stages of prostate organogenesis

In humans and rodents, prostate organogenesis begins prenatally, following the development and synthesis of testosterone from the testes (Pointis et al., 1979; Staack et al., 2003). The embryonic urogenital sinus (UGS), which consists of the urogenital sinus epithelium (UGE) and the urogenital sinus mesenchyme (UGM), is present in both male and female embryos and emerges as a caudal extension of the hindgut at GW 7 in humans and embryonic day (E) 13.5 in mice. The UGS develops into a division of the vagina in females, and into prostate tissue in males, as a result of the presence of testosterone (Cunha et al., 2018; Robboy et al., 2017).

After prostate specification, epithelial budding into the UGM begins at GW 10 in humans and E16-E18 in mice (Fig. 1A) (Cunha et al., 2018). Although prostate ducts are visible during mouse embryonic development, most growth and branching in the mouse prostate occurs after birth (Sugimura et al., 1986a; Timms et al., 1994).

In the next stage of prostate organogenesis, epithelial buds extend distally, branch and form ducts. Ductal outgrowth begins in humans at GW 11, or after birth in mice, and continues until the prostate reaches its mature size of approximately 20 g in young men (Buskin et al., 2021). The initial prostate buds elongate as solid cords and form distinct ventral, dorsal, lateral and anterior prostate lobes in mice (Fig. 1B). The human prostate is uni-lobular and does not have distinct lobes, but contains peripheral, central and transition zones (Fig. 1C). Epithelial buds elongate through cell replication at the distal tips and branch at bifurcation points (Sugimura et al., 1986b). Recent studies in the mouse prostate have further characterized the properties of epithelial progenitor cells at these sites of prostate growth (Tika et al., 2019). Prostate ducts are formed as lumens through canalization, after which epithelial cells further differentiate and produce prostatic secretions (Cunha et al., 2018).

Androgen regulation of prostate organogenesis
AR signaling from mesenchymal cells during prostate specification and budding

Fetal testicular androgens and AR signaling are essential for prostate induction and bud initiation. Testosterone is produced in Leydig cells of the testis as early as GW 6 in humans and E13 in mice

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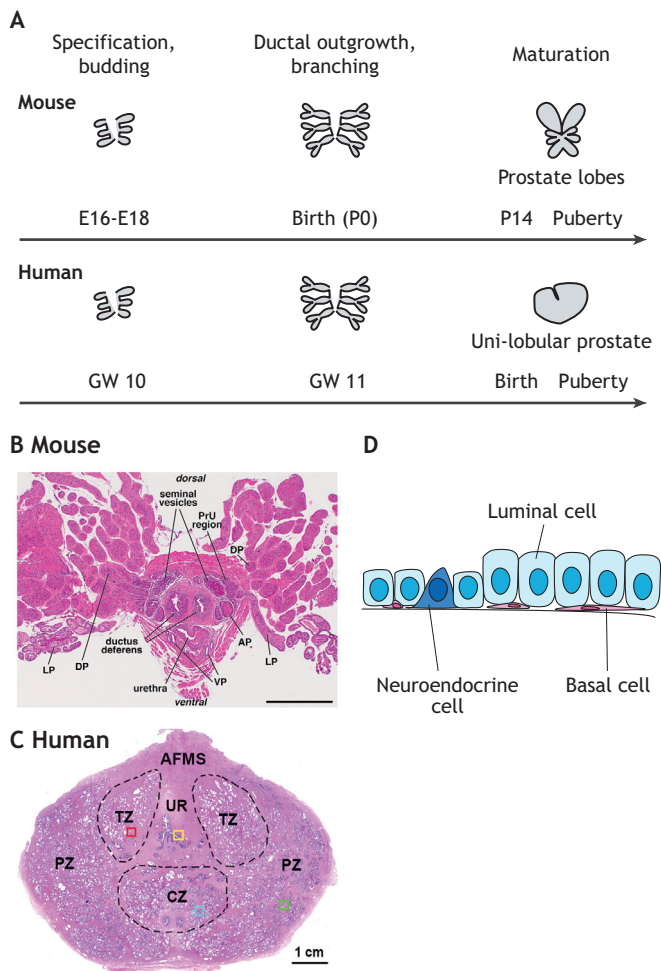
Fig. 1. Prostate growth prior to and after puberty. (A) Illustrations of mouse and human prostate tissue (gray) development. After the prostate is specified from the urogenital sinus, epithelial budding into the urogenital sinus mesenchyme occurs between E16 and E18 in mice and at GW 10 in humans. The epithelial buds then extend and branch to form ducts after birth in mice or from GW 11 in humans. (B) Transverse sections of the adult mouse prostate stained with Hematoxylin and Eosin (reproduced from Crowley et al., 2020). The mouse prostate has ventral (VP), dorsal (DP), lateral (LP) and anterior prostate (AP) lobes. Scale bar: 2 mm. (C) Transverse sections of the adult human prostate stained with Hematoxylin and Eosin (reproduced from Henry et al., 2018). The adult human prostate is uni-lobular with peripheral (PZ), central (CZ) and transition (TZ) zones. Scale bar: 1 cm. (D) Luminal (blue), basal (pink) and neuroendocrine (dark blue) mammalian prostate epithelial cell types. AFMS, anterior fibromuscular stroma; PrU, periurethral; UR, urethra.

(Cunha et al., 2018; Pointis et al., 1979; Tapanainen et al., 1981). In the UGS, testosterone is converted by 5α-reductase to dihydrotestosterone (DHT), which binds to AR with high potency. Upon DHT binding, AR functions as a nuclear transcription factor (Russell and Wilson, 1994).

AR activity is required in the UGM but not the UGE for prostate induction and growth. This was first shown in tissue recombination experiments with AR null testicular feminized (*Tfm*) mice (He et al., 1991), where wild-type and *Tfm* epithelial and stromal cells were separated, recombined and regrafted under the renal capsule of adult male mice (Cunha and Lung, 1978). Consistent with the initial requirement for AR activity in stromal cells, AR expression is low in prostate epithelial cells during the initial stages of prostate organogenesis (He et al., 2018; Shibata et al., 2020; Takeda and Chang, 1991). It has been thought that AR expression in the UGM

precedes expression in the UGE. Intriguingly, *in vivo* genetic lineage-tracing experiments using an *Ar-Cre* recombinase and fluorescent reporters have shown that transient *Ar* expression is detected in the UGE at E12.5, before expression in the UGM (Lee et al., 2021). The functional significance of this early AR expression in the UGE remains unclear.

In vivo lineage-tracing experiments support the requirement for AR in stromal cells, but not in epithelial cells, for mouse prostate bud formation. Prostate buds do not form during embryonic development when AR is deleted in Shh-responsive *Gli1*-expressing fibroblast and smooth muscle cells (Le et al., 2020). In contrast, deletion of AR in epithelial cells expressing keratin 8 (*Krt8*, also known as *Ck8*) or transformation-related protein 63 (*Trp63*, also known as *p63*) has confirmed that AR is not required in prostate epithelial cells for prostate bud formation (Lee et al., 2021). However, functional AR in the UGE is necessary for epithelial and mesenchymal cell differentiation and the production of secretory proteins in the adult prostate (Cunha et al., 1987; Simanainen et al., 2007; Wu et al., 2007).

The identity of the paracrine signals from stromal cells that are required for prostate specification remains a topic of investigation (Box 1). Recent scRNA-seq analysis of stromal cells from the UGS of male mice at E17.5 revealed several clusters of fibroblasts; of these clusters, peri-epithelial fibroblasts surrounding epithelial buds have been examined in detail due to their proximity to epithelial bud cells. Peri-epithelial fibroblasts express elevated *Ar,* bone morphogenetic protein 7 (*Bmp7*), *Wif1* and *Wnt5a*, supporting previous reports of the role of AR, Bmp and Wnt/β-catenin pathways in providing epithelial-mesenchymal signals for prostate bud growth (Lee et al., 2021). The enrichment of many developmental signaling pathways in *Gli1*-expressing fibroblasts further suggests that paracrine signals from stromal cells may work in combination to induce prostate budding. Additional information on studies of the signaling pathways known to be important in prostate organogenesis, including Bmp, Wnt/β-catenin, fibroblast growth factor (FGF), Hedgehog and Notch signaling, have been summarized in other reviews (Buskin et al., 2021; Francis and Swain, 2018; Montano and Bushman, 2017; Toivanen and Shen, 2017).

AR signaling from mesenchymal cells during duct formation, branching and puberty

Following a surge of testosterone after birth, circulating androgens remain low throughout prepubertal development in the mouse (Donjacour and Cunha, 1988; Jean-Faucher et al., 1978; Motelica-Heino et al., 1988). However, androgens continue to play a role in the modulation of branching morphogenesis of prostate ducts (Donjacour and Cunha, 1988).

Similar to the role of AR signaling in mesenchymal cells during prostate budding, AR signaling from stromal cells also contributes to prostate growth during puberty in mice (Lee et al., 2021). Deletion of AR in Shh-responsive *Gli1*-expressing cells during puberty leads to a significant reduction in prostate growth and irregular morphogenesis as a result of decreased proliferation of epithelial and stromal populations (Olson et al., 2021). This reduction is more severe compared with previous studies of AR deletion in prostate fibroblasts and smooth muscle cells (Lai et al., 2012; Yu et al., 2012), suggesting a role for mesenchymal AR signaling from Gli1-expressing cells that is necessary for the maintenance of stem cell niches during prostate organogenesis (Olson et al., 2021). Although these recent data suggest a relationship between the AR and Shh signaling pathways, a direct

> **Box 1. The andromedin and smooth muscle hypotheses**
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> The 'andromedin model' proposes that androgens influence the production and release of paracrine factors, or andromedins. These andromedins then regulate prostate epithelial growth and differentiation (Thomson, 2008). Therefore, andromedins should be released by mesenchymal cells, be responsive to androgens, and activate prostate epithelial outgrowth and differentiation (Toivanen and Shen, 2017). Although many prospective andromedins have been investigated, no single molecule has met these criteria. A genomic analysis of AR binding combined with transcriptomic analysis of rodent and human mesenchymal cell populations during prostate organogenesis has revealed differences in AR genomic binding in female and male tissues, with the enrichment of AR in intergenic regions in male tissues (Nash et al., 2019). A strong upregulation of male-enriched genes in mesenchymal cells in response to androgen signaling, as predicted by the andromedin hypothesis, has not been found. Instead, a significant number of genes are repressed by androgen signaling. Combined with information from patients with androgen-insensitivity syndrome, these findings suggest that androgens may regulate multiple signaling pathways as opposed to a single target (Nash et al., 2019).
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> The 'smooth muscle hypothesis' proposes a function for the smooth muscle layer in regulating prostate specification and epithelial budding. Whereas a dense barrier of smooth muscle exists surrounding the epithelium in the adult prostate, the smooth muscle layer is discontinuous during early prostate development, allowing for interactions between the epithelium and mesenchyme (Thomson et al., 2002). The smooth muscle hypothesis suggests that androgens regulate smooth muscle differentiation and allow epithelial growth and budding to occur until the smooth muscle layer develops and obstructs additional epithelial expansion. Such a role of smooth muscle differentiation in regulating branching morphogenesis has been demonstrated in mouse lung development (Goodwin et al., 2019).
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> Although the smooth muscle hypothesis and andromedin model propose distinct pathways for how androgens influence epithelial budding, both models may work in combination (Thomson, 2008; Toivanen and Shen, 2017).

mechanistic link has not been discovered and would be an interesting area of future investigation.

Epithelial cell-type specification and cellular heterogeneity

During mammalian prostate organogenesis, as the developing prostate ducts begin to branch and canalize, prostate epithelial cells differentiate into three major cell types: luminal cells, basal cells and neuroendocrine cells (Fig. 1D). In the mammalian adult prostate, luminal cells express KRT8, KRT18 and AR, and include secretory columnar cells. Basal cells, distinguished by their expression of KRT5, KRT14 and TRP63, occupy an intermittent, supportive layer between the basement membrane and luminal cells. The progenitor relationships of luminal and basal epithelial cell types have been widely studied owing to their importance in understanding prostate tumorigenesis (Lu et al., 2013; Wang et al., 2013). In the adult prostate, genetic lineage-tracing studies have demonstrated that homeostasis is generally maintained by unipotent basal and luminal progenitor cells (Choi et al., 2012; Liu et al., 2011; Wang et al., 2013). Although rare 'intermediate' cells that co-express luminal and basal markers have been described in the basal layer of the adult prostate (Wang et al., 2001, 2013; Xue et al., 1998), distinct clusters of intermediate cells have not been identified in scRNA-seq studies and it is not clear whether they represent a unique cell type. Neuroendocrine cells express synaptophysin and chromogranin A and are enriched in the proximal region of the prostate (Cheng et al., 2013; Kwon et al., 2021). Lineage tracing of basal cells during prostate organogenesis has shown that basal cells can give rise to rare neuroendocrine cells (Ousset et al., 2012; Wang et al., 2020).

However, the function of neuroendocrine cells during organogenesis and in the adult prostate remains unclear.

Progenitor cells during prostate organogenesis

Urogenital epithelial progenitor cells give rise to basal progenitors and luminal progenitors (Fig. 2) (Ousset et al., 2012; Shibata et al., 2020; Toivanen and Shen, 2017). In human and mouse prostates, prostate bud epithelial cells in newly formed prostate buds express both basal and luminal markers (Shibata et al., 2020; Wang et al., 2001). 'Inner' bud cells have reduced TRP63 expression and higher KRT8 expression, whereas 'outer' bud cells generally express TRP63 and high KRT5 (CK5). During early neonatal stages of prostate organogenesis, the mouse prostate contains both bipotent basal and bipotent luminal progenitors that can each generate both basal and luminal cells (Ousset et al., 2012; Shibata et al., 2020; Tika et al., 2019; Wang et al., 2020). Recent studies examining the role of AR in epithelial cells during neonatal prostate organogenesis have shown that cell-autonomous AR signaling is not required for the bipotency of prostate progenitor cells (Shibata et al., 2020). However, it remains unknown whether AR requirements differ along the proximal-to-distal axis of the prostate duct during organogenesis.

As prostate organogenesis progresses, bipotent basal stem cells become increasingly limited to the distal region, where most of the growth and branching occurs following puberty (Tika et al., 2019). Whereas bipotent basal cells can generate luminal cells until puberty, lineage restriction of luminal progenitors occurs earlier, prior to puberty (Fig. 2) (Shibata et al., 2020). In summary, these lineage-tracing studies indicate the presence of distinct bipotent and unipotent progenitor populations in the developing and adult prostate.

Androgen regulation during puberty and in the adult prostate

Androgens and AR play key roles during male sexual maturation at puberty. The rate of prostate growth increases significantly during puberty (Tika et al., 2019). During puberty, both luminal and basal cells proliferate in the mouse prostate, but the frequency of proliferation becomes higher in luminal cells. After puberty, epithelial cell proliferation decreases, shifting the ratio of luminal to basal cells from 1:1 at the onset of puberty to 10:1 in the adult prostate (Tika et al., 2019).

Luminal cells in the mammalian adult prostate are considered androgen dependent because they undergo apoptosis in response to castration. For this reason, androgen deprivation therapy (ADT) remains a primary treatment for prostate cancer in humans. By reducing androgen function in the prostate, ADT targets neoplastic prostate cells that are dependent on androgens. Although ADT is successful in reducing prostate tumor growth initially, tumors can develop resistance through mechanisms that restore or bypass AR signaling (Watson et al., 2015). Additionally, genetic lineage-tracing experiments have shown that with inactivation of *Pten*, the gene encoding the tumor suppressor protein phosphatase and tensin homolog, AR is not required for tumor initiation from prostate basal or luminal cells (Xie et al., 2017).

Although AR is not cell-autonomously required in all adult mouse luminal cells during prostate homeostasis, AR deletion in luminal cells promotes prostate inflammation (Zhang et al., 2016). During androgen-mediated regeneration, AR is also not cell-autonomously required in some luminal cells (Xie et al., 2017), whereas in castration-resistant Nkx3-1-expressing cells (CARNs), rare NKX3-1-expressing luminal cells with stem cell properties in the regressed prostate, require AR cell-autonomously (Chua et al.,
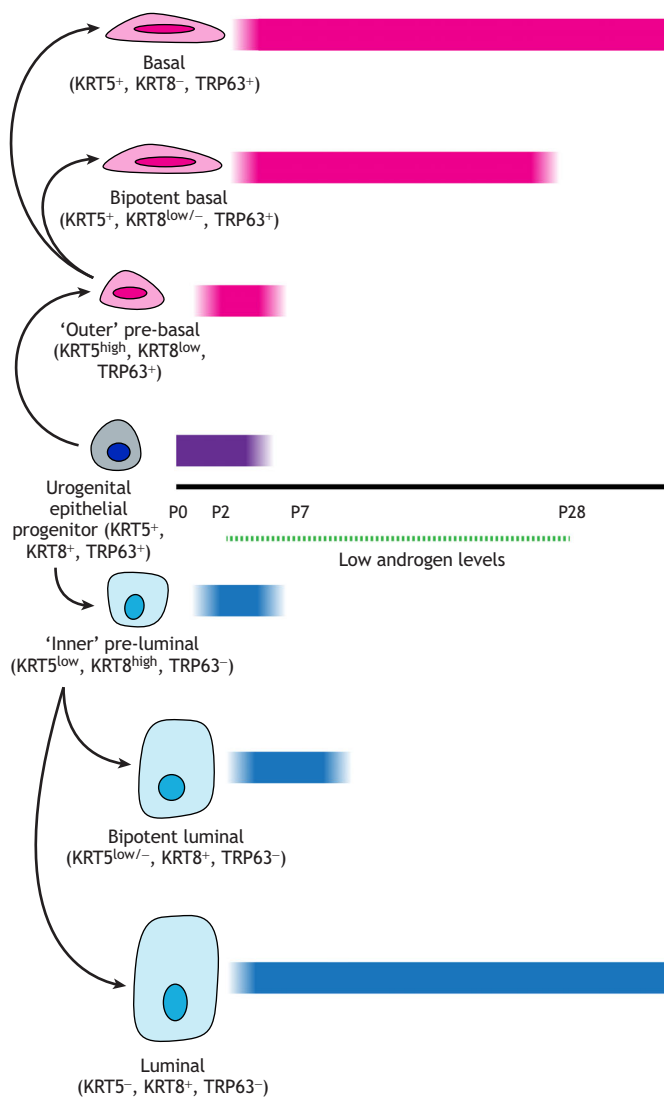
Fig. 2. Model for specification of basal and luminal cell types during prostate organogenesis in the mouse. Urogenital epithelial progenitor cells (purple) express both basal (pink) and luminal (blue) markers. Lineage tracing indicates that bipotent basal and bipotent luminal progenitors are present (shown by colored bars) during neonatal stages of prostate organogenesis (P2-P28), when androgen levels are low (indicated by the dotted green line). Modified from Shibata et al. (2020).

2018). These studies highlight the heterogeneity of prostate luminal cells and their requirements for AR.

Cellular heterogeneity of luminal cells
Several unbiased investigations of prostate tissues using scRNA-seq have provided insights into the cellular heterogeneity of epithelial cells in the prostate, particularly for luminal cells. The heterogeneity of luminal cells and the identification of luminal cell types enriched in specific lobes of the adult mouse prostate have been summarized (Crowley and Shen, 2022). Additionally, several studies identified a distinct population of luminal cells characterized by the expression of *Ppp1r1b*, *Ly6a* (*Sca-1*), *Tacstd2* (*Trop2*) and *Runx1* (Crowley et al., 2020; Guo et al., 2020; Joseph et al., 2020; Karthaus et al., 2020; Mevel et al., 2020). These luminal cells are enriched in the proximal region of the prostate epithelium adjacent to the urethra but are also found as rare clusters in the distal regions of the anterior,

dorsal and lateral prostate lobes, and as more frequent clusters in the distal ventral prostate lobe (Crowley et al., 2020; Guo et al., 2020; Karthaus et al., 2020; Mevel et al., 2020). This proximally enriched population of luminal cells is distinct from distal luminal cell populations as this population does not express *Nkx3-1* in the adult prostate. Supporting previous findings on progenitor properties of proximal luminal cells (Tsujimura et al., 2002; Zhang et al., 2018), proximal luminal progenitors are unipotent and can survive without androgens (Guo et al., 2020; Karthaus et al., 2020). Interestingly, gene expression signatures of *Nkx3-1*-expressing cells in the distal regions become more similar to proximal luminal cells after castration owing to loss of expression of AR-regulated genes (Karthaus et al., 2020). However, the cell types remain distinct, and proximal luminal cells generally do not generate *Nkx3-1*-expressing cells during androgen-mediated regeneration following castration (Guo et al., 2020; Karthaus et al., 2020; Mevel et al., 2020).

scRNA-seq during prostate organogenesis
Findings from scRNA-seq of mouse E15.5 UGS and UGS explant cultures have suggested that the specification of prostate proximal luminal cells, marked by expression of RUNX1, occurs during embryonic stages of prostate organogenesis (Fig. 3) (Mevel et al., 2020).

Although the proximal luminal cell population in the adult prostate does not express NKX3-1, epithelial cells in the proximal prostate have been lineage traced using an *Nkx3-1-Cre* driver, revealing that this population is derived from early prostate bud cells and is prostatic in origin, rather than urothelial (Crowley and Shen, 2022; Thomsen et al., 2008; Zhang et al., 2008). This is further supported by studies reporting *Nkx3-1* expression by *in situ* hybridization starting at E15.5 and by *Nkx3-1-lacZ* reporter expression (Bhatia-Gaur et al., 1999; Kruithof-de Julio et al., 2013). Consistent with these findings, explant cultures initiated at E15.5, before prostate bud formation, contain cells that transiently co-express NKX3-1 and RUNX1 (Mevel et al., 2020). The specification of proximal and distal luminal cell types likely occurs early during prostate organogenesis, because *in vivo* lineage tracing of *Runx1*-expressing cells at 2 weeks of age and lineage tracing in explant culture suggest that *Runx1*-expressing proximal luminal cells do not contribute significantly to the expansion of NKX3-1-expressing cells in the distal prostate (Mevel et al., 2020).

Further analyses are necessary to fully understand the functional significance of luminal cell epithelial heterogeneity in the developing and adult prostate. As discussed previously, the proximal luminal cell population is derived from prostate bud cells, as for its distal luminal counterpart. However, the mechanism of the specification of proximal luminal cells is not known. As this luminal cell population resembles human prostate ductal cells and can be a cellular origin for prostate cancer, future studies should focus on how proximal luminal cell-derived tumors respond to androgen-deprivation therapy, especially given the maintenance of these cells following castration (Crowley et al., 2020; Guo et al., 2020; Karthaus et al., 2020; Mevel et al., 2020).

scRNA-seq and genetic lineage-tracing studies have also revealed information on basal progenitor cell identity and function during prostate organogenesis (Lee et al., 2021; Wang et al., 2020). During postnatal prostate organogenesis, *Zeb1* expression marks a multipotent basal stem cell population (Wang et al., 2020). *Zeb1*-expressing epithelial cells have also been detected in E17.5 UGS tissue, with trajectory analysis suggesting a distinct differentiation branch for these cells (Lee et al., 2021). Although *Zeb1*-expressing cells have been shown to be enriched in human BPH and prostate
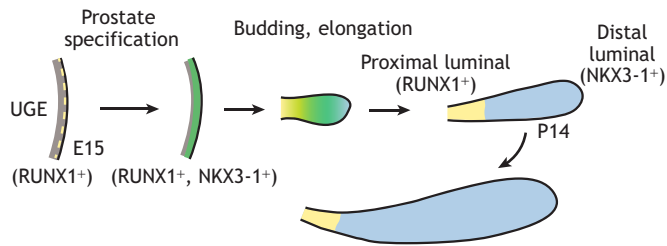
Fig. 3. Model for specification of luminal cell types in the mouse prostate. RUNX1 (yellow) is expressed in the pre-bud urogenital sinus epithelium (UGE). In urogenital sinus (UGS) explant culture, early prostate bud cells transiently co-express RUNX1 and NKX3-1 (green). *Nkx3-1* (blue) is one of the earliest markers of prostate specification. After transient co-expression of markers of proximal (RUNX1) and distal (NKX3-1) luminal cell types, proximal and distal luminal cell types remain distinct (yellow and blue domains, respectively).

cancer (Wang et al., 2020), the role of these cells in prostatic disease remains unclear.

Comparison of human and mouse prostate organogenesis

Although most of our understanding of prostate organogenesis derives from rodent studies, analysis of human prostate development remains important for clinical applications. Many early developmental processes are conserved in mice and humans, despite stark contrasts during the later stages of prostate organogenesis. In both humans and mice, androgens induce prostate formation and are an essential regulator of prostate development (Kellokumpu-Lehtinen et al., 1981). Early stages of organogenesis, including prostate epithelial budding from the UGE, ductal elongation and marker expression, are similar in human and mouse (Cunha et al., 2018; Timms, 2008; Wang et al., 2001). However, the morphological organization of the mouse and human prostates differ significantly during the latter stages of organogenesis and in adult prostates (Cunha et al., 1987; Toivanen and Shen, 2017).

Morphology and histology of the human prostate

Despite similarities in the initial emergence of epithelial buds early in development, the adult human prostate is uni-lobular, whereas the mouse prostate has several distinct lobes (Fig. 1) (Buskin et al., 2021; Cunha et al., 2018; McNeal, 1988). Although the human prostate is composed of separate zones, these zones do not correspond to specific lobes of the mouse prostate (Fig. 1) (Ittmann, 2018). However, analysis of gene expression profiles of luminal cells from scRNA-seq suggests that mouse lateral prostate cells most resemble human peripheral zone cells (Crowley et al., 2020).

In addition to gross morphological differences, the adult human and mouse prostate differ histologically. Like the mouse prostate, human prostatic ducts are composed of luminal, basal and rare neuroendocrine cells; however, whereas basal cells form a continuous layer in the human prostate with the ratio of basal to luminal cells being 1:1, in the adult mouse prostate this ratio is 1:10 (El-Alfy et al., 2000; Tika et al., 2019). The increased presence of basal cells in the human prostate may reflect a more influential role in maintaining the luminal compartment and ductal structure.

Ductal morphogenesis in the human prostate

Recent analyses of human prostate organogenesis using mitochondrial DNA mutations and cytochrome c oxidase (CCO) deficiency as a reporter, or whole-genome sequencing and

mathematical modeling, have revealed epithelial cell lineage relationships during human prostate development (Grossmann et al., 2021; Moad et al., 2017). Analysis of CCO-deficient clones in serial sections of whole human prostates has revealed long clonal patches, some of which can be traced from the proximal region of the prostate adjacent to the urethra to the distal acini (Moad et al., 2017). Most clones contain a mixture of basal and luminal cells, suggesting a contribution of bipotent progenitors to human prostate organogenesis, as observed in the mouse prostate (Moad et al., 2017; Ousset et al., 2012; Shibata et al., 2020). Basal-only (7%) and luminal-only (5%) clones have also been identified but are rare and restricted to the proximal region of the prostate (Moad et al., 2017).

Using laser-capture microdissections of epithelial cells from ducts and acini, somatic mutations in progenitor cells generated over a human lifespan have been identified and traced (Grossmann et al., 2021). More mutations have been identified in distal regions than in proximal, suggesting that distal prostate cells have undergone more cell divisions (Grossmann et al., 2021). It is estimated that 5-10 progenitor cells generate each duct of the human prostate during embryogenesis; there are approximately 30 individual ducts, with local proliferation of side and terminal branches occurring during puberty (Grossmann et al., 2021). The distribution of mutations suggests that embryogenesis and puberty are key time points for ductal morphogenesis and growth of the human prostate (Grossmann et al., 2021).

Methods for modeling prostate organogenesis

In vivo grafting and *ex vivo* culture of prostate cells remain important for expanding knowledge on prostate organogenesis and for studying prostate disease. Explant culture, tissue recombination and organoid culture of rodent and human prostate tissues allow for the analysis of cell growth and function in varying conditions or following drug treatment (Table 1). Models of prostate organogenesis are particularly important for studies of human prostate organogenesis, for which access to human tissues is limited (Buskin et al., 2021). In addition, *in vitro* models allow for the manipulation of culture conditions, including androgen levels. The development and use of these technologies have enhanced our understanding of prostate organogenesis.

Prostate explant culture

Prostate explant culture describes the *ex vivo* culture of prostate tissue on a membrane or support, whereby tissues are in constant contact with culture media. Although initially used in the 20th century to study the effect of carcinogens on mouse prostates (Lasnitzki, 1951), explant culture has been widely utilized for studies of prostate organogenesis. Culture of human fetal prostate tissue with and without androgens has demonstrated that androgens promote the differentiation and maintenance of the UGM and the ultrastructural arrangement of epithelial cells (Kellokumpu-Lehtinen et al., 1981). Standardized serum-free explant culture conditions have been established for studies of prostate organogenesis (Lopes et al., 1996). With the addition of androgens, prostate buds develop from both male and female UGS tissue explants, although there are some differences in bud formation (Keil et al., 2012).

Culture conditions are easily manipulated to examine the role of cell signaling pathways in regulating prostate organogenesis and explanted tissues can be analyzed using *in situ* hybridization and immunostaining (Berman et al., 2004; Keil et al., 2012; Kruithof-de Julio et al., 2013; Mevel et al., 2020). Explant culture has also been useful for studying the prostate-specific function of genes such as

sonic hedgehog (*Shh*); Shh mutants lack prostates owing to insufficient androgen levels (Berman et al., 2004). Furthermore, the *ex vivo* nature of prostate explant culture allows for the close examination of cellular identity and dynamics throughout prostate development, while also preserving tissue structure. More recently, cultured explants from fetal mouse UGS have been used for scRNA-seq studies, revealing the specification of proximal luminal epithelial cells during early stages of prostate organogenesis (Mevel et al., 2020).

Tissue recombination

The inductive effects of the UGM on the UGE required for prostate specification and epithelial budding were originally demonstrated through tissue-recombination experiments involving the co-culture of prostate epithelial and mesenchymal cells followed by grafting under the renal capsule (Cunha and Lung, 1978). When separated, neither UGE nor UGM alone can form prostate tissue in renal grafts. However, the recombination of UGM cells with UGE, or even UGM cells with bladder epithelium results in prostate tissue, indicating the crucial role of the UGM in regulating prostate specification (Donjacour and Cunha, 1993). Tissue-recombination studies have also demonstrated the conservation of mechanisms of prostate organogenesis between human and rodent prostates, and the role of stromal AR signaling in prostate induction (Cunha et al., 1987).

Similar to prostate explant culture, tissue-recombination assays have been used to investigate the function of embryonic lethal genes, such as *Trp63*, which cannot easily be studied *in vivo* using genetically engineered mouse models in postnatal prostate development (Kurita et al., 2004; Signoretti et al., 2005).

Tissue-recombination assays have been used to generate human prostate tissue from human embryonic stem cells recombined with mouse UGM or rat seminal vesicle mesenchyme grafted under the renal capsule (Taylor et al., 2006). Such tissue recombinants form immature glands at 2-4 weeks, which then develop to mature glands at 8-12 weeks (Taylor et al., 2006). Prostate tissue has been

generated from mouse fibroblasts reprogrammed by transient expression of the pluripotency factors OCT4 (POU5F1), SOX2, KLF4 and MYC followed by differentiation to epithelial cells, infection with lentivirus to express FOXA1, NKX3-1 and AR, and tissue recombination with rat UGM (Talos et al., 2017). Human prostate-derived induced pluripotent stem cells (iPSCs) have also been used in tissue-recombination assays with rat UGM to generate human prostate tissue (Hepburn et al., 2020).

Prostate organoid culture

Challenges in developing cell lines derived from patient specimens have led to the adoption of three-dimensional (3D) culture methods to study prostate epithelial cells. 3D organoids can be formed from embryonic and adult stem cells that organize into structured, multicellular complexes that exhibit functional and organizational similarities to organs, allowing for their use in modeling organogenesis (Clevers, 2016). The methodology for prostate 3D organoid culture continues to develop and improve.

Conditions for 3D culture of prostate cells as prostate spheroids (prostaspheres) were developed before the establishment of prostate organoid culture. Prostasphere culture has been used to measure the self-renewal properties of both human and mouse adult prostate stem cells (Goldstein et al., 2008; Lawson et al., 2007), but favors the growth of basal cells (Huang et al., 2015; Wang et al., 2013).

Initial studies of prostate organoids focused on the generation of organoids from adult prostate cells. Prostate organoids can be generated from normal human and mouse prostate cells as well as prostate tumor tissue (Chua et al., 2014; Gao et al., 2014; Karthaus et al., 2014), and can originate from either luminal or basal cells (Chua et al., 2014; Karthaus et al., 2014). In organoid culture, basal and luminal cells can also serve as bipotent progenitor cells, forming organoids that closely resemble prostate glands (Chua et al., 2014; Karthaus et al., 2014). Among luminal cells, isolated proximal luminal cells have been shown to form organoids more efficiently than other luminal cell populations, leading to the conclusion that

Table 1. Models of prostate organogenesis

Method	Description	Strengths	Limitations	References
Explant culture	*Ex vivo* culture of prostate tissue on a membrane or support	Preserves tissue structure; offers the ability to manipulate culture conditions; genetic mouse models can be used to investigate effects of gene deletion, conduct lineage tracing, etc.	Prostate tissue can only survive 5-7 days in culture; human tissues for explant culture are difficult to obtain	Berman et al., 2004; Keil et al., 2012; Kruithof-de Julio et al., 2013; Mevel et al., 2020
Tissue recombination	Co-culture of prostate epithelial and mesenchymal cells followed by *in vivo* grafting under the renal capsule	Able to test cell-to-cell interactions by recombining separated cell types; can be used to study the conservation of mechanisms across species by recombining cells from differing species	Relies on the use of rodent UGM; xenografts require use of immunocompromised mouse models; unclear contribution of host cells following renal engraftment	Cunha and Lung, 1978; Donjacour and Cunha, 1993; Hepburn et al., 2020; Talos et al., 2017; Taylor et al., 2006
Organoid culture	Structured, multi-cellular complexes formed from stem/progenitor cells or iPSCs that exhibit functional and organizational similarities to organs	Provides a good representation of *in vivo* prostate epithelial architecture; can be used to study cell autonomous effects; can be used for *in vitro* drug testing and with CRISPR-Cas9 gene editing to test effects of gene mutations; reduces animal usage as organoids can be expanded; prostate organoids can be generated from human iPSCs	Favors growth of certain cell types; the lack of vasculature and immune cells fails to recapitulate the tissue microenvironment	Calderon-Gierszal and Prins, 2015; Chua et al., 2014; Feng et al., 2021; Hepburn et al., 2020; Karthaus et al., 2014

proximal luminal cells exhibit greater multipotent progenitor activity (Crowley et al., 2020; Guo et al., 2020; Karthaus et al., 2020). The regeneration potential of the proximal luminal cell population has also been examined through the removal, and subsequent re-addition, of DHT from organoid cultures (Karthaus et al., 2020).

The co-culture of human organoids with benign prostate stroma can promote the viability and branching of human prostate organoids (Richards et al., 2019). In addition to epithelial growth factor (EGF), other growth factors, such as neuregulin expressed by mesenchymal cells, can also promote the growth of mouse and human luminal cell-derived organoids (Karthaus et al., 2020).

Organoids can also serve as an important model for human prostate organogenesis (Hepburn et al., 2020). When human iPSCs are differentiated to definitive endoderm cells, then co-cultured with rat UGM in 3D culture with Matrigel, solid spherical prostate organoids resembling early stages of prostate organogenesis form after 5 weeks of culture, developing into mature prostate organoids with large lumens by 12 weeks. Interestingly, rare neuroendocrine cells, which have previously not been observed in organoids generated from normal adult prostate epithelial cells (Chua et al., 2014; Karthaus et al., 2014), are present in these organoids. These recent advances in human organoid culture methods highlight the importance of paracrine signaling between the epithelial and mesenchymal cells for the generation and maintenance of prostate tissue.

Conclusion

Processes that occur during normal prostate organogenesis, including signaling pathways, are recapitulated in prostate cancer and BPH (McNeal, 1978; Shen and Abate-Shen, 2010; Vickman et al., 2020). The idea that knowledge from understanding the regulation of cellular growth and differentiation in normal cells should be extrapolated to develop effective disease treatments remains (Pierce and Speers, 1988).

In recent years, novel technologies, most notably the use of scRNA-seq, have provided a wealth of information for the investigation of prostate organogenesis, revealing the heterogeneity in luminal cell populations (Crowley et al., 2020; Guo et al., 2020; Karthaus et al., 2020; Mevel et al., 2020). Although the specification and separation of a distinct unipotent luminal progenitor cell population enriched in the proximal prostate appears to occur early during prostate organogenesis (Mevel et al., 2020), the extent to which this separation of lineages is preserved during aging and in prostate disease remains unclear. In old mice, the percentage of Trop2 (Tacstd2)$^+$ cells increases; whether this is solely due to increased proliferation of Trop2$^+$ cells, or if other luminal cell types acquire the ability to generate Trop2$^+$ cells with age is not known (Crowell et al., 2019). Given that proximal luminal cells become enriched after androgen deprivation and can serve as a cellular origin of prostate cancer, the functional significance of these distinct luminal cell types, and similarities and differences in the regulation of these cell types in prostate organogenesis and disease remain topics of interest and relevant for understanding treatment resistance in castration-resistant prostate cancer.

In addition to cells in the epithelium, knowledge from scRNA-seq, combined with new genetic lineage-tracing models, has advanced the investigation of mesenchymal and stromal cells during prostate organogenesis. BPH initiation has long been proposed to involve a 'reawakening' of the inductive mesenchyme, which stimulates epithelial proliferation and formation of BPH nodules (McNeal, 1978). Whether cells resembling the newly identified population of peri-epithelial fibroblasts that promotes epithelial-mesenchymal signaling and epithelial budding and growth in response to AR signaling during organogenesis (Lee et al., 2021; Olson et al., 2021) contribute to BPH initiation or progression is an interesting question for future investigation.

Although significant progress has been made in the development of new methodologies for modeling human prostate organogenesis, there remains a heavy reliance on the use of rodent UGM cells for tissue recombination and some organoid culture-based studies. A further understanding of how mesenchymal cells contribute to the reprogramming of cells in tissue recombination assays and in organoid culture, and additional development of methods is needed.

Aided by the advent of new technologies, our understanding of prostate organogenesis has improved significantly in recent years. However, many questions remain and further findings will likely continue to provide insights for the treatment of prostatic diseases.

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