

# The many ways to make a luminal cell and a prostate cancer cell

Douglas W Strand and Andrew S Goldstein<sup>1</sup>

Department of Urology, University of Texas Southwestern, Dallas, Texas, USA

<sup>1</sup>Department of Molecular and Medical Pharmacology, Department of Urology, David Geffen School of Medicine, Broad Stem Cell Research Center, Jonsson Comprehensive Cancer Center, University of California, Los Angeles, California, USA

Correspondence  
should be addressed  
to A S Goldstein

**Email**  
AGoldstein@mednet.ucla.edu

## Abstract

Research in the area of stem/progenitor cells has led to the identification of multiple stem-like cell populations implicated in prostate homeostasis and cancer initiation. Given that there are multiple cells that can regenerate prostatic tissue and give rise to prostate cancer, our focus should shift to defining the signaling mechanisms that drive differentiation and progenitor self-renewal. In this article, we will review the literature, present the evidence and raise important unanswered questions that will help guide the field forward in dissecting critical mechanisms regulating stem-cell differentiation and tumor initiation.

### Key Words

- ▶ prostate
- ▶ carcinoma

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## Introduction

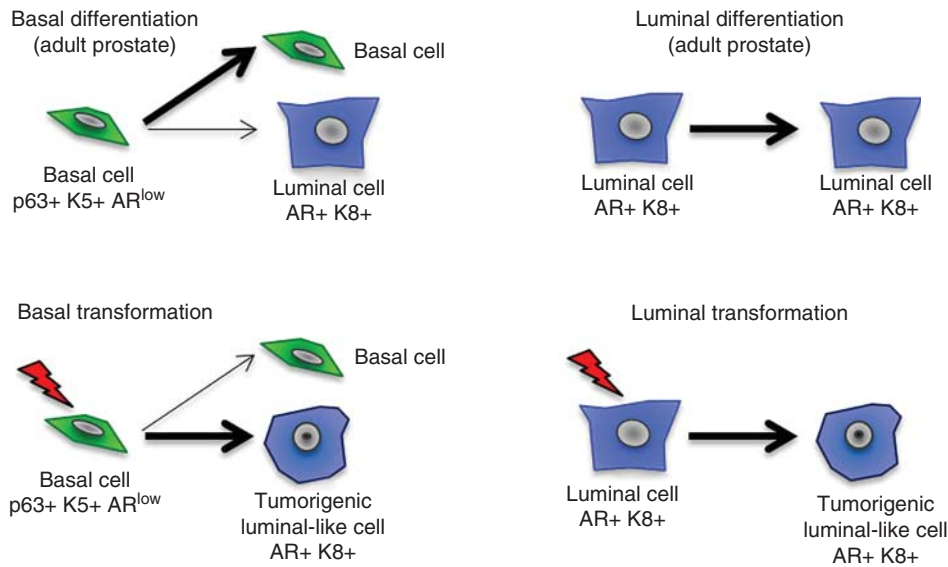
The study of the control of epithelial differentiation began with the so-called ‘Hen’s tooth’ experiment. Primitive epithelium from chicken beak could be instructed to develop into teeth when recombined with mouse embryonic dermal papilla, even though the ancestor of modern birds lost the ability to grow teeth ~100–80 million years ago (Kollar & Fisher 1980). This ingenious experiment demonstrated that epithelial differentiation was not simply a cell-autonomous event, and it was subsequently demonstrated across multiple organs that tissue interactions maintain the stem cell niche and dictate epithelial cell fate. Multiple lines of evidence have now demonstrated that organs harbor tissue-restricted multipotent progenitors into adulthood. The regulation of these progenitors has been the subject of intense research and debate with implications for a wide range of diseases, including those of the prostate.

Because of the resistance of prostate progenitor cells to current anti-androgen therapies, research has focused on the identification of cells of origin for benign and malignant prostatic growth. Striking similarities in the

regulation of epithelial stem cell niches have been recognized across various tissues (Blanpain *et al.* 2007), which may provide clues to the regulation of epithelial differentiation and androgen-independent growth of initiated stem cells in the prostate. There is ample evidence that multiple cell types can act as progenitors for fully differentiated secretory luminal cells but also as cells of origin for prostate tumors (Fig. 1), which may relate to the genotypic heterogeneity in prostate cancer. We suggest that a deeper understanding of the mechanisms that govern cell fate decisions in prostate development, homeostasis and disease may provide new avenues for patient-specific treatments.

### Prostate diseases

The normal prostate is around 20 g in men between 21 and 30 years old. Benign prostate hyperplasia (BPH) of the transition zone is a common age-related disorder, observed histologically in 50% of men over 50 with doubling time of 4.5 years in men between 51 and 70

**Figure 1**

Model of prostate epithelial homeostasis and cancer initiation. In the benign prostate, basal cells and luminal cells predominantly self-renew to make more of themselves, while rare basal cells differentiate into the luminal lineage. Upon basal cell transformation, basal to luminal

differentiation is enhanced and cancers become driven by tumorigenic luminal cells in the absence of the initiating basal cells. Luminal transformation rapidly gives rise to tumorigenic luminal cells.

years old (Berry *et al.* 1984). Lower urinary tract symptoms (LUTS) due to an enlarged prostate are predominantly treated with 5 alpha reductase inhibitors (5ARI), which reduce prostate volume ~25%, predominantly through apoptosis of androgen-dependent epithelia (The Finasteride Study Group 1993). Baseline prostate volume is the most reliable indicator of future resistance to 5ARI therapy (Roehrborn 2006), making the understanding of androgen-independent prostate growth crucial to slowing symptomatic progression.

Prostate cancer is the most common non-cutaneous malignancy and second leading cause of cancer mortality in Western men, and has been treated with surgical and chemical androgen deprivation therapy for 60 years (Huggins & Hodges 2002). Because of the inability to predict which tumors will progress to cause lethality, biopsy and surgical intervention are necessarily overused (Vickers *et al.* 2011, Schroder *et al.* 2012). Although most men initially respond to androgen deprivation, castration-resistant cancer almost universally recurs (The Leuprolide Study Group 1984). Furthermore, our continued effort at better targeting androgen signaling has not drastically improved survival, due to tumor cell compensatory mechanisms (Antonarakis *et al.* 2014).

The design of new treatments for androgen-independent progression of BPH and prostate cancer relies on a deeper understanding of the extrinsic and intrinsic

regulators of prostate epithelial differentiation. Persistent growth in both benign and malignant disease has led many to suggest that progression is driven either by clonal evolution (Gundem *et al.* 2015, Hong *et al.* 2015) or by androgen-independent progenitors giving rise to androgen-dependent progeny. The search for these progenitors has revealed a deeper understanding of the lineage hierarchy of prostate epithelium, but many questions remain as to the triggers that regulate self-renewal and differentiation. The answers to these questions may provide a way to therapeutically target the tumor-propagating cell types.

### Prostate glandular composition and the origins of disease

Prostate glands are composed of a pseudostratified bilayer of basal and luminal epithelium, which are positional terms that do not fully reflect the cellular subtypes within each layer (Abate-Shen & Shen 2000). The basal epithelial layer is believed to contain a small (<5%) population of multipotent stem cells, which are thought to give rise to committed basal, transit amplifying, intermediate cell phenotypes and the luminal/secretory layer, which also contains a small population (<1%) of progenitors (Uzgare *et al.* 2004, Xin *et al.* 2007, Wang *et al.* 2009, Rane *et al.* 2014). The basal and luminal epithelial layers are thought to be important in the pathogenesis of both benign and

malignant prostate disease, albeit for different reasons (De Marzo *et al.* 1998). Proliferation of basal/stem cells is thought to contribute to benign prostatic enlargement, although direct evidence for this is still lacking (Dermer 1978, Isaacs & Coffey 1989). Alternatively, primary prostate cancers are characterized by the loss of basal epithelium (Brawer *et al.* 1985) and the multiclonal expansion of luminal epithelial foci (Grisanzio & Signoretti 2008, Lindberg *et al.* 2013); however, after androgen deprivation therapy, residual tumor-propagating cells repopulate the de-bulked tumor (Germann *et al.* 2012) and lead to lethal monoclonal metastases (Liu *et al.* 2009). BPH nodules also appear to be clonal (Blackwood *et al.* 2011, Gaisa *et al.* 2011) and many men are, or become resistant to SARI therapy (McConnell *et al.* 2003), which may be due at least in part to androgen-independent growth mechanisms in basal cells (Isaacs 2008, Bauman *et al.* 2014, Lin-Tsai *et al.* 2014).

Early evidence that basal cells could give rise to luminal cells in the adult was shown by [<sup>3</sup>H]-thymidine uptake and Ki67 immunoreactivity in BPH tissue, which showed that actively dividing cells were predominantly localized to the basal compartment (Dermer 1978, Bonkhoff *et al.* 1994). This observation led to the hypothesis that a resident stem cell within the basal compartment could give rise to a luminal cell since the ratio of basal to luminal cells was unchanged (Isaacs & Coffey 1989). Many have since demonstrated that an indigenous, androgen-independent prostate progenitor cell survives castration and can repopulate the luminal layer upon re-administration of androgen (English *et al.* 1987, Verhagen *et al.* 1988, Wang *et al.* 2009, Germann *et al.* 2012, Shi *et al.* 2014). The development of cell lineage tracing in mouse models coupled with antibody-based cell sorting and *ex vivo* culturing have more specifically identified both basal and luminal progenitors that contribute to glandular development, adult homeostasis and post-castration regeneration (Collins *et al.* 2001, Wang *et al.* 2009, Ousset *et al.* 2012, Shi *et al.* 2014), and these same markers can be used to identify and study progenitors in human prostate (Goldstein *et al.* 2008, Karthaus *et al.* 2014).

Developmental signaling in the urogenital mesenchyme directs solid cords of p63-positive epithelial progenitors to bud from the urogenital sinus epithelium and give rise to basal, luminal and neuroendocrine cell types (Signoretti *et al.* 2005). Cell lineage tracing has confirmed that these p63<sup>+</sup>, ck14<sup>+</sup> progenitors give rise to all epithelial lineages through asymmetrical divisions during development, but that fully differentiated luminal cells are derived mainly from symmetrical division of luminal

progenitors in the homeostatic adult (Choi *et al.* 2012, Ousset *et al.* 2012, Wang *et al.* 2014a). However, stress conditions during adulthood such as androgen deprivation and inflammation drive luminal cell differentiation from both basal and luminal progenitors (Wang *et al.* 2013, Kwon *et al.* 2014).

In adulthood, a subset of basal epithelial cells are multipotent and have been reported to be enriched or isolated based on either expression of various cell surface markers, including Sca-1, CD44, CD49f,  $\alpha 2\beta 1$  integrin, Trop2, CD117 and CD133, and the capacity for self-renewal in serially passaged 3D cultures (Collins *et al.* 2001, Bhatt *et al.* 2003, Hudson 2004, Richardson *et al.* 2004, Xin *et al.* 2005, Goldstein *et al.* 2008, Leong *et al.* 2008, Garraway *et al.* 2010, Ousset *et al.* 2012). The luminal layer also contains a small population of CK18<sup>+</sup>, Nkx3.1<sup>+</sup> progenitors that are resistant to castration and can contribute to the repopulation of luminal secretory cells after re-administration of androgen (English *et al.* 1987, Leong *et al.* 2008, Wang *et al.* 2009, Chua *et al.* 2014, Shi *et al.* 2014). Finally, recent evidence in mice suggests that a label-retaining progenitor population that expands following castration and regeneration is CD133<sup>+</sup>, Sca-1<sup>+</sup>, CD44<sup>+</sup>, CD49f<sup>+</sup> and CD117<sup>+</sup>, but also AR<sup>+</sup> (Shi *et al.* 2014). A similar  $\alpha 2\beta 1^{\text{Hi}}$ , CD133<sup>+</sup> progenitor enriched by FACS from human prostate was also shown to express functional androgen receptor (AR) (Williamson *et al.* 2012), but it's still unclear whether androgen deprivation therapy may inadvertently expand a progenitor population capable of tumor propagation or SARI-resistant BPH.

## Models for the characterization of progenitors

There is still considerable controversy over the identity of the tumor-propagating cell in prostate cancer, predominantly because of the various techniques used to define progenitor cell properties. Flow cytometry, serial passaging of *ex vivo* 3D spheres, tissue regeneration with inductive mesenchyme and genetic lineage tracing are each used to characterize whether a cell displays the ability to self-renew or differentiate. Prostate stem cells were originally isolated by flow cytometry using cell surface markers that were enriched either in functionally analogous epidermal stem cells (Collins *et al.* 2001) or after castration (Lawson *et al.* 2007, Goldstein *et al.* 2008).

Functional characterization of the self-renewal and differentiation capacity of these putative prostate stem cell populations is accomplished using tissue regeneration with inductive mesenchyme followed by kidney capsule xenografting (Xin *et al.* 2003) as well as serial passaging

as 3D spheres (Collins *et al.* 2001, Xin *et al.* 2007). These studies clarify that only a subpopulation of basal cells has the capacity to self-renew or give rise to differentiated progeny *in vivo*. However, lineage tracing studies of basal cell self-renewal and differentiation in the homeostatic adult indicate that, when removed from their endogenous tissue microenvironment, the plasticity of basal cells in 3D cultures or tissue regeneration, xenografting causes an overestimation of the physiological relevance of their contribution as progenitors of luminal epithelium, mimicking non-homeostatic conditions such as castration, inflammation or initiation (Wang *et al.* 2013, 2014b). We will look at the meaningful differences and overlapping contributions of each model system.

### Mouse models

Due to the emergence of castration-resistant lethal cell types after hormonal therapy, there has been an intense search for the tumor cell of origin in order to therapeutically target the tumor-propagating cell type. In contrast to reductionist cell culture models, mouse models provide an experimental system to test the effects of genetic manipulation in a cell's native environment. For many years, oncogenes and tumor suppressor genes were driven or knocked out in mice using the probasin promoter, which is predominantly expressed in luminal epithelium (Grabowska *et al.* 2014). The realization that the basal epithelium contains a stem cell population and many of the tumor suppressors (e.g., PTEN, Notch, p53, p63, Bcl-2) and oncogenes (e.g., c-MYC,  $\beta$ -catenin) altered in human prostate cancer progression prompted the generation of mouse models using promoters from genes expressed in basal cells (CK5, CK14) in order to determine whether a basal cell could be a tumor cell of origin (Abate-Shen & Shen 2000). Direct comparisons of genes using basal and luminal promoters suggests that basal and luminal cells can each serve as targets of prostate cancer initiation (Wang *et al.* 2006, Korsten *et al.* 2009, Choi *et al.* 2012), but that tumors propagated by transformed luminal cells more closely resemble human prostate carcinomas (Wang *et al.* 2014b).

### Tissue regeneration

Early work by Cunha & Lung (1978) identified presumptive stroma, or mesenchyme, as essential for prostate-specific epithelial differentiation. Cunha *et al.* (1983a,b) went on to show, using heterotypic tissue recombination, that mesenchyme from any species could instruct

epithelium from other endodermal lineages to differentiate into a new cell type. Various androgen-regulated stromal factors were shown as paracrine factors (insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), Wnt) that could regulate epithelial differentiation, though these 'andromedins' are still poorly characterized (Thomson 2008). These studies demonstrated the powerful extrinsic control of tissue interactions on epithelial differentiation during development and paved the way for the study of stromal alterations that contribute to prostate cancer progression (Strand *et al.* 2010).

Lineage tracing in mouse models demonstrates that initiation of basal cells causes both progenitor cell enrichment (Mulholland *et al.* 2009) and basal-to-luminal differentiation (Lu *et al.* 2013, Wang *et al.* 2013). Concordant results are seen when murine or human basal epithelium isolated by FACS and initiated with oncogenes *ex vivo* recapitulate the histological and molecular features of human prostate cancer upon tissue regeneration with inductive fetal mesenchyme (Goldstein *et al.* 2010, Lawson *et al.* 2010). Furthermore, luminal tumors can be serially propagated in the absence of basal cells (Stoyanova *et al.* 2013). What is still unclear is whether the serially passaged tumor is propagated by an emergent intermediate or luminal progenitor derived from the transformed basal cell.

While the majority of differentiation events occur from basal to luminal cells, luminal to basal cell differentiation has also been observed in both lineage-traced mice and in *ex vivo* organoid cultures (Karthaus *et al.* 2014). Regardless of the representative numbers of specific progenitor populations *in vivo*, the functional capacity of distinct basal and luminal progenitors to give rise to fully differentiated luminal progeny, especially under inflamed or castrate conditions, is of utmost importance for developing therapeutic strategies to target androgen-independent progenitors.

### Cell culture

Because of the limited access to patient samples and the limited amount of starting material to work with, one of the most promising advances in prostate stem cell research is the optimization of culture conditions for expanding and differentiating stem cells in culture (Sato *et al.* 2011, Clevers *et al.* 2014). The inductive power of fetal mesenchyme in driving epithelial differentiation is still poorly understood (Cunha & Lung 1978), so optimizing the media conditions necessary for feeder-free cultures will

provide the reductionist systems necessary for dissecting the mechanisms responsible for cell fate decisions.

The cancer stem cell hypothesis states that a genetically unstable progenitor retains unlimited self-renewal while a subset of its progeny still matures to a luminal-like secretory cell phenotype (Visvader & Lindeman 2012). Prostate carcinomas display luminal exocrine, neuroendocrine and intermediate cell phenotypes. The transit amplifying or intermediate cells are proposed to be the progenitors within the tumor and consequently targets for androgen-independent progression (van Leenders & Schalken 2003).

In order to understand the underlying mechanisms of self-renewal and differentiation, a variety of different culture models have been attempted for the propagation and differentiation of prostate epithelial progenitors. Litvinov *et al.* show that low-calcium, serum-free media can select for CD133<sup>+</sup>/ABCG2<sup>+</sup>/α2β1<sup>Hi</sup>/p63<sup>+</sup>/PSCA<sup>-</sup>/AR<sup>-</sup>/PSA<sup>-</sup> stem cells from primary human prostate as well as immortalized human prostate epithelial cultures, and that the selected stem cells could give rise to both neuroendocrine and CD133<sup>-</sup>/p63<sup>+</sup>/PSCA<sup>+</sup> intermediate cell lineages. However, the intermediate cell lineage could not be fully differentiated into CD133<sup>-</sup>/p63<sup>-</sup>/PSCA<sup>-</sup>/AR<sup>+</sup>/PSA<sup>+</sup> secretory luminal epithelium even after the addition of dihydrotestosterone (DHT) (Litvinov *et al.* 2006).

Taking this work one step forward, Heer *et al.* (2006) used flow cytometry to sort human prostate CD133<sup>-</sup>/α2β1<sup>Hi</sup> transit amplifying cells and CD133<sup>+</sup>/α2β1<sup>Hi</sup> stem cells to determine the mechanisms that cause terminal differentiation into luminal secretory cells. They demonstrate that keratinocyte growth factor (KGF) can drive transit amplifying progenitor (TAP) differentiation by downregulating β1 integrin through p38 activation. In addition, they demonstrate that CD133<sup>-</sup>/α2β1<sup>Hi</sup> transit amplifying cells express AR mRNA, but AR protein is under constant proteasomal degradation. The CD133<sup>+</sup>/α2β1<sup>Hi</sup> stem cell population did not express AR mRNA or protein (Heer *et al.* 2007).

Lamb *et al.* (2010) developed a primary human prostate epithelial cell stratification model where a confluent population of K5<sup>+</sup>/K14<sup>+</sup>/Bcl<sup>-</sup>2<sup>+</sup>/EGFR<sup>+</sup>/AR<sup>-</sup> basal cells would give rise to overlaying patches of fully differentiated K18<sup>+</sup>/K19<sup>+</sup>/AR<sup>+</sup>/Nkx3.1<sup>+</sup>/TMPRSS2<sup>+</sup> secretory luminal cells after 14 days in culture with DHT and the stromal derived factors FGF7 or FGF10. Although the nuclear localization of AR was limited in this model, it still represented an advance over previous attempts to culture fully differentiated luminal epithelium using retinoic acid, insulin or FGFs (Pehl *et al.* 1996, Gustafson

*et al.* 2006). Another group using primary cultured basal (K14<sup>+</sup>/p63<sup>+</sup>) and transit amplifying (K18<sup>+</sup>/AR<sup>-</sup>) epithelial cells in culture showed that the addition of 1,25-dihydroxy-vitamin D<sub>3</sub>, all-trans retinoic acid, and TGF-β1 could induce low levels of AR transcription while the added inhibition of the mitochondrial protein MAO-A with clorgyline increased AR protein levels (Zhao *et al.* 2008). This is consistent with the observation that basal cells are more densely populated with mitochondria than luminal cells (El-Alfy *et al.* 2000) and may suggest that a metabolic rewiring is partially necessary for cellular differentiation.

One of the key difficulties with these culture models is that primary cells eventually undergo senescence after a few passages (Litvinov *et al.* 2006), requiring a constant supply of fresh tissue from patients with inherently variable genetic and clinical backgrounds. A further complication is the limited number of stem cells within normal tissue (1–5% of total cells) and the limited amount of starting tissue. Most immortalized cell lines do not fully recapitulate normal glandular architecture, and while spontaneously immortalized human prostate epithelial cell lines have been developed for the serial study of normal differentiation, their self-renewal properties are difficult to assess given the random duplications and deletions acquired during the immortalization process (Jiang *et al.* 2010). However, it has recently been demonstrated that both basal (Xin *et al.* 2007, Lamb *et al.* 2010, Lukacs *et al.* 2010, Goldstein *et al.* 2011, Hofner *et al.* 2015) and luminal (Wang *et al.* 2009, Karthaus *et al.* 2014) progenitors can be propagated and differentiated *ex vivo* under optimized culture conditions, enabling their molecular, cellular and pharmacological assessment. Using an R-spondin-based organoid technology developed for the culture of a variety of epithelial tissues including the intestine (Sato *et al.* 2009), several groups have identified factors that promote both multi-lineage differentiation and long-term expansion of prostate tissue (Chua *et al.* 2014, Gao *et al.* 2014, Karthaus *et al.* 2014). In this assay, both basal and luminal cells appear multipotent, capable of generating organoids containing markers of both the basal and luminal lineages (Karthaus *et al.* 2014). The organoid system may enable a new approach to investigate lineage hierarchy, transformation and mechanisms of self-renewal and differentiation without the use of animal models.

### Molecular regulation of prostate stem cell self-renewal and differentiation

The optimization of culture conditions for the propagation of stem cells was based on the discovery of common

mechanisms of stem cell self-renewal and differentiation (Blanpain *et al.* 2007, Karthaus *et al.* 2014). In particular, the addition of the Wnt pathway agonist R-spondin1 is necessary to maintain human and mouse organoids (Karthaus *et al.* 2014). The R-spondin receptor Lgr4 is strongly expressed in Sca-1<sup>+</sup>/CD49f<sup>+</sup> prostate progenitor cells and is required for proper luminal differentiation as shown in Lgr4 knockout mice (Luo *et al.* 2013). The authors went on to show that Wnt3a plus R-spondin3 co-treatment promotes  $\beta$ -catenin-mediated p63<sup>high</sup> cell proliferation and differentiation. It may seem counter-intuitive to characterize basal cells as 'differentiated,' but it has been recognized by some groups that the 95–99% of basal cells that do not display self-renewing progenitor characteristics are an independent lineage termed 'committed' basal cells (Maitland *et al.* 2011, Rane *et al.* 2014).

### Paracrine regulation of epithelial differentiation and tumorigenesis

The Wnt/ $\beta$ -catenin pathway is a prime example of the control of stem cell self-renewal and differentiation control by paracrine interactions with stroma. The ability of stroma to drive epithelial transformation in the adult has been demonstrated by tissue recombination using both human and mouse tissues. Hayward *et al.* (2001) showed that freshly isolated human carcinoma associated fibroblasts could drive transformation of a non-tumorigenic human prostate epithelial cell line. At around the same time, the stromal reaction adjacent to sites of prostatic intraepithelial neoplasia, a precursor to prostate cancer, was characterized (Tuxhorn *et al.* 2002), and this stromal reaction could be used to independently predict prostate cancer recurrence (Ayala *et al.* 2003).

Among others, alterations to the Wnt/TGF- $\beta$  pathway have been shown to be critical to the protumorigenic activity of the stroma (Li *et al.* 2008, Placencio *et al.* 2008, Franco *et al.* 2011, Carstens *et al.* 2014). The abrogation of TGF- $\beta$  signaling in a subpopulation of stromal cells alone is sufficient to drive epithelial carcinogenesis in either mouse or human experimental systems (Bhowmick *et al.* 2004, Franco *et al.* 2011), and this is at least partially due to increased stromal Wnt production (Li *et al.* 2008, Placencio *et al.* 2008).

### Regional differences in the human prostate stem cell niche

The development of benign and malignant prostate disease in humans is largely restricted to anatomical zones (transition and peripheral respectively), suggesting

intrinsic differences in control of cytodifferentiation (McNeal *et al.* 1988). Given the concentration of progenitor cells in the proximal prostate in mouse (Tsujiura *et al.* 2002, Goldstein *et al.* 2008), it would be intriguing to determine whether the same anatomical concentration of progenitors in the basal layer could be detected in human prostate transition vs peripheral zones. Since basal cells are more resistant to transformation (Choi *et al.* 2012) and are likely cytoprotective due to their loss in cancer, it would also be informative to determine whether there are molecular differences between the stem cell niches in the transition vs peripheral zones.

### Role of progenitor cells in BPH

It has been postulated that stem cell expansion is responsible for the nodular growth of the transition zone in BPH, but direct evidence for this is still lacking. Although there are a variety of histological phenotypes associated with lower urinary tract symptoms, the treatment of an enlarged prostate is still the most difficult, with only a third of patients responding to 5ARIs (McConnell *et al.* 2003, Roehrborn 2006). Molecular signatures of men who undergo surgery for lower urinary tract symptoms related to benign prostatic hyperplasia/lower urinary tract symptoms (BPH/LUTS) have been generated and are correlated with AP-1 transcription factor expression (Descazeaud *et al.* 2008, Lin-Tsai *et al.* 2014), but analyses of progenitor populations have not been performed. However, recent histological evidence does suggest that activation of the Wnt/ $\beta$ -catenin pathway in hyperplastic basal cells is associated with surgical intervention for BPH/LUTS (Bauman *et al.* 2014), suggesting potential activation of a progenitor pathway.

### Transformation of basal cells results in a luminal phenotype

Although prostate tumors share the genetic variability observed in other organs (Watson *et al.* 2013), 95% of human prostate tumors are luminal-like adenocarcinomas (Grisanzio & Signoretti 2008, Wang *et al.* 2014b). Even when basal cells are experimentally transformed for tissue regeneration, luminal adenocarcinomas are mostly propagated rather than basal cell carcinomas, suggesting a differentiation event occurs before full transformation (Goldstein *et al.* 2010, Lawson *et al.* 2010, Stoyanova *et al.* 2013, Wang *et al.* 2013). However, if a basal cell is a tumor cell of origin, as has been shown in experimental animal models, one has to wonder why basal or squamous cell

carcinomas represent such a small percentage of prostate tumor phenotypes (and usually occur in the transition zone) (Ali & Epstein 2007). In both animal models and tissue recombination xenografting experiments, the transformation of the basal epithelium can lead to both basal- and luminal-like tumors, with luminal phenotypes capable of repeated propagation in the absence of the initiating transformed basal cell (Choi *et al.* 2012, Stoyanova *et al.* 2013). This has led many to posit that transformation of basal epithelium simply leads to a differentiation event (Hudson *et al.* 2001, Choi *et al.* 2012, Wang *et al.* 2013). Therefore, the question of whether prostate cancer is a basal or luminal phenotype (Wang *et al.* 2009, Maitland *et al.* 2011, Choi *et al.* 2012) may not be as important as understanding the mechanisms that regulate self-renewal vs differentiation in normal and transformed progenitor cells. In fact, the use of differentiation-promoting histone deacetylase inhibitors (Gottlicher *et al.* 2001) can sensitize stem-like prostate cancer cells to radiation (Frame *et al.* 2013), suggesting that further understanding of the mechanisms promoting differentiation may be useful to enhancing current therapies.

### Neuroendocrine differentiation

Studies in mouse models demonstrate that basal cells and some luminal cells can give rise to neuroendocrine cells in the normal prostate (Goldstein *et al.* 2008, Wang *et al.* 2009). It has yet to be shown whether primary human prostate stem/progenitor cells can generate neuroendocrine cells *in vivo*. Other data indicate that prostate cancer cell lines with a luminal-like adenocarcinoma phenotype can take on neuroendocrine features following androgen deprivation (Burchardt *et al.* 1999). These data suggest that neuroendocrine cells can be derived from neighboring epithelial cells and tumor cells. Given the emergence of castration-resistant tumors with small cell or neuroendocrine features in response to newer therapies capable of suppressing the androgen-signaling axis (Beltran *et al.* 2014), understanding the role of neuroendocrine differentiation from normal and malignant prostate epithelial cells is critical for treating aggressive treatment-resistant disease.

### Difficulty in recapitulating native tissue interactions using human models

*Ex vivo* 3D culturing systems of freshly isolated mouse and human prostate epithelia are being developed to study

differentiation in the absence of interaction with stroma (Karthaus *et al.* 2014). While these reductionist culture conditions will be highly valuable in answering specific questions about the intrinsic control of cellular differentiation, the study of the role of the microenvironment in controlling epithelial differentiation and possibly even tumor genotype still requires an experimental system capable of tissue interactions (Goldstein & Witte 2013). This dichotomy is particularly evident when comparing lineage tracing of progenitors in mouse models to either serial passaging of progenitors in a non-native matrix *ex vivo* or in further tissue regeneration experiments with inductive (reprogramming) mesenchyme. Given that tissue regeneration with human cells requires the use of immunocompromised mice, transgenic mouse models are particularly useful for studying the role of both the stroma and inflammation as paracrine regulators of epithelial differentiation. More work is necessary to improve human models to account for epithelial–epithelial and epithelial–stromal interactions.

### Summary

Using a number of model systems, researchers have demonstrated that a range of cell-types can generate luminal cells *in vitro* or *in vivo*. We hypothesize that any progenitor cell that can give rise to the luminal lineage under experimental conditions can respond to oncogenic transformation by generating malignant luminal progeny. It is now critical to determine whether the cell of origin influences the fate of the tumor (aggressive vs indolent) or whether this is determined by genetic alterations or the tumor microenvironment. This is a difficult question to model in mice given the huge differences between mouse and human prostate epithelial basal-to-luminal ratio and basal cell phenotype. It will also be critical to determine whether there are common mechanisms required to make a luminal cell (or prostate cancer cell) regardless of the starting cell and whether that information can be used to detect, prevent or treat prostate cancer.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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