Cell Reports
Report

Luminal Cells Are Favored as the Cell of Origin for Prostate Cancer

Zhu A. Wang,[1,3] Roxanne Toivanen,[1] Sarah K. Bergren,[1] Pierre Chambon,[2] and Michael M. Shen[1,*]
[1]Departments of Medicine, Genetics and Development, Urology, and Systems Biology, Columbia Stem Cell Initiative, Herbert Irving Comprehensive Cancer Center, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
[2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104, INSERM U964, 67400 Illkirch, France
[3]Present address: Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA 95064, USA
*Correspondence: mshen@columbia.edu
http://dx.doi.org/10.1016/j.celrep.2014.08.002

SUMMARY

The identification of cell types of origin for cancer has important implications for tumor stratification and personalized treatment. For prostate cancer, the cell of origin has been intensively studied, but it has remained unclear whether basal or luminal epithelial cells, or both, represent cells of origin under physiological conditions in vivo. Here, we use a novel lineage-tracing strategy to assess the cell of origin in a diverse range of mouse models, including *Nkx3.1*$^{+/-}$; *Pten*$^{+/-}$, *Pten*$^{+/-}$, *Hi-Myc*, and *TRAMP* mice, as well as a hormonal carcinogenesis model. Our results show that luminal cells are consistently the observed cell of origin for each model in situ; however, explanted basal cells from these mice can generate tumors in grafts. Consequently, we propose that luminal cells are favored as cells of origin in many contexts, whereas basal cells only give rise to tumors after differentiation into luminal cells.

INTRODUCTION

The identification of cell types of origin for cancer is significant, since distinct cell populations within a tissue may give rise to different cancer subtypes distinguished by their histopathological phenotypes and patient outcomes (Blanpain, 2013; Visvader, 2009, 2011; Wang et al., 2013). Numerous studies have investigated the cell of origin by introducing an oncogenic insult within a defined cell type to determine whether these cells can give rise to cancer. However, such approaches are potentially limited, as the cell type of origin may be dependent on the specific oncogenic insult and/or the model system. To date, no studies have systematically addressed which cell types can serve as cells of origin in multiple contexts of tumor initiation.

In human and mouse prostate epithelium, luminal and basal cells are the two major cell types, together with rare neuroendocrine cells (Shen and Abate-Shen, 2010). Lineage tracing has shown that luminal and basal cells in the adult mouse prostate represent distinct populations that are mostly self-sustaining (Choi et al., 2012; Lu et al., 2013; Wang et al., 2013). Notably, lineage-marked basal cells rarely generate luminal cells during adult tissue homeostasis but display plasticity under the influence of inductive embryonic urogenital mesenchyme in grafting assays, acquiring facultative progenitor properties and generating luminal cells (Choi et al., 2012; Lu et al., 2013; Wang et al., 2013).

For prostate cancer, previous studies have reached differing conclusions regarding the cell type(s) of origin (Goldstein and Witte, 2013; Wang and Shen, 2011; Xin, 2013). Although prostate adenocarcinoma has a luminal phenotype, both basal and luminal cells have been proposed to represent cells of origin. In particular, transformed human basal cells can give rise to prostate cancer in renal grafting models (Goldstein et al., 2010; Stoyanova et al., 2013; Taylor et al., 2012), whereas a luminal stem cell population identified in the regressed mouse prostate can act as a cell of origin in vivo (Wang et al., 2009). More recently, lineage tracing in mice in which the *Pten* tumor suppressor was specifically deleted in either basal or luminal cells has shown that both cell types can act as cells of origin (Choi et al., 2012; Lu et al., 2013; Wang et al., 2013).

However, it remains unclear whether basal or luminal cells, or both, represent cell types of origin in the context of *Pten* deletion occurring throughout the prostate epithelium or whether the cell of origin might vary depending upon specific oncogenic events. We have investigated this issue using a novel lineage-tracing strategy in a diverse range of mouse models that recapitulate important features of human prostate tumorigenesis. Our results indicate that luminal cells are consistently favored as cells of origin for prostate cancer.

RESULTS

To determine the cell of origin for a mouse model of prostate cancer, we performed lineage marking of either basal or luminal cells in apparently normal tissue to determine whether their progeny contribute to the tumors that subsequently arise (Figure 1). Since the lineage-tracing methodology uses inducible Cre recombinase, we analyzed mouse models in which the tumor phenotype is not driven by Cre. We used the *CK5-CreER*^{T2} driver (Rock et al., 2009) for lineage tracing of basal cells and the *PSA-CreER*^{T2} (Ratnacaram et al., 2008) or *CK8-CreER*^{T2} (Van Keymeulen et al., 2011) drivers for tracing of luminal cells, together with the *R26R-YFP* reporter (Srinivas et al., 2001). Tamoxifen

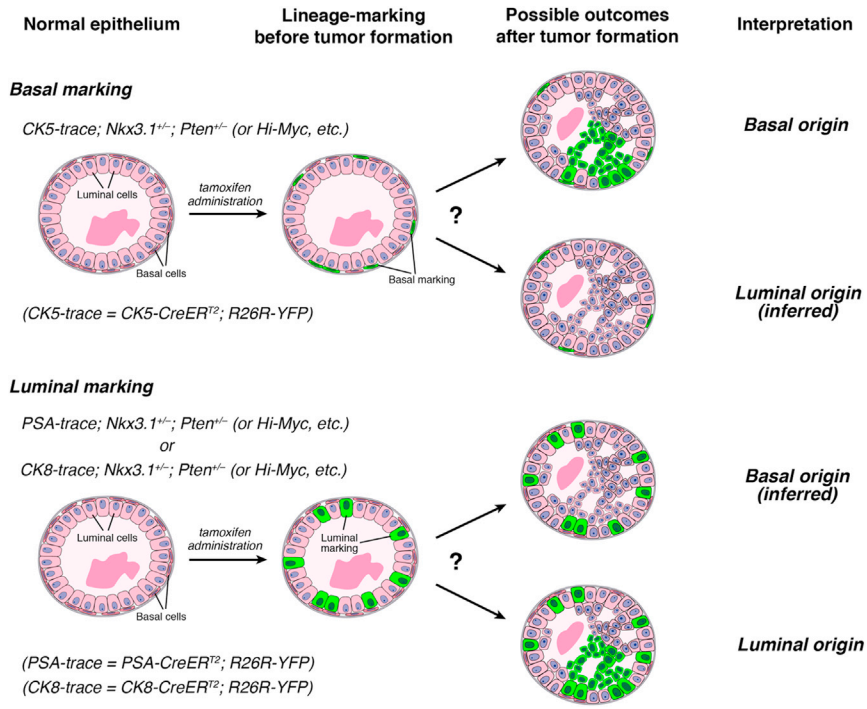

Figure 1. Experimental Design for Analysis of Cell of Origin

The inducible *CK5-CreER*^{T2} driver can lineage mark basal cells by YFP expression in different prostate cancer models prior to overt cancer formation. Similarly, the inducible *PSA-CreER*^{T2} and *CK8-CreER*^{T2} drivers can mark luminal cells in phenotypically normal epithelium. The presence of YFP$^+$ cell clusters in subsequent PIN/cancer lesions indicates that the marked cell type acts as the cell of origin in the mouse model analyzed.

induction for lineage marking was performed in young adult male mice at 7 weeks of age, when the basal and luminal lineages have been established as largely self-sustaining compartments (Choi et al., 2012; Ousset et al., 2012; Wang et al., 2013). Contribution of cells marked by the *CK5-CreER*^{T2} driver to tumors would imply that basal cells were the cell of origin, whereas tumor cells marked by the *PSA-CreER*^{T2} or *CK8-CreER*^{T2} drivers would indicate a luminal origin (Figure 1). Notably, our approach dissociates the time of lineage marking from the onset of tumorigenesis and allows multiple models to be analyzed using the same overall strategy.

In control experiments to examine the specificity of the inducible Cre drivers in a wild-type background, we found that *CK5-CreER*^{T2}*; R26R-YFP* (which we denote *CK5-trace*) strictly marks basal cells with 23.6% efficiency, while *PSA-CreER*^{T2}*; R26R-YFP* (*PSA-trace*) marks luminal cells with 11.5% efficiency and *CK8-CreER*^{T2}*; R26R-YFP* (*CK8-trace*) marks 4.1% of luminal cells (Tables S1L, S1N, and S1P), consistent with previous studies (Ousset et al., 2012; Ratnacaram et al., 2008; Wang et al., 2013). Importantly, the percentage of lineage-marked cells in the *CK5-trace* and *PSA-trace* mice does not change between 2 months of age, shortly after labeling, and 6 months of age, when most of our tumor analyses are mostly performed (Figure S1; Tables S2A and S2B), indicating that the lineage-marked cell populations are stable in a nontumorigenic background.

We first investigated the cell of origin for the high-grade prostatic intraepithelial neoplasia (PIN) lesions in the *Nkx3.1*$^{+/-}$*; Pten*$^{+/-}$ (which we denote *NP*) model that is heterozygous for null alleles of the *Nkx3.1* homeobox gene and of *Pten* (Kim et al., 2002). As reported previously, the anterior prostate (AP) and dorsolateral prostate (DLP) of *NP* mice appear normal at 2 months of age (Figures 2E and 2J) but frequently display high-grade PIN/carcinoma lesions at 6 months (Figures 2F and 2K). Quantitation of initial lineage marking in *CK5-trace; NP* mice and *PSA-trace; NP* mice revealed similar efficiencies as mice with a wild-type background (Figures 2B, 2C, and 2Y; Tables S1A and S1B). Notably, in tumor lesions of *CK5-trace; NP* mice at 6 months of age, we found that yellow fluorescent protein (YFP)$^+$ cells in clusters (defined as containing at least three YFP$^+$ cells) were rarely observed (0.5%, n = 6 mice) (Figures 2G, 2L, and 2Y; Figures S2A and S2D; Table S1A), while the percentage of YFP$^+$ cells in untransformed regions was unaffected (Figures S3A–S3C; Table S2C). In contrast, 10.8% of the cells in the tumor lesions of *PSA-trace; NP* mice (n = 4) and 4.5% of the cells in tumor lesions of *CK8-trace; NP* mice (n = 3) were YFP$^+$ (Figures 2H, 2I, 2M, 2N, and 2Y; Figures S2B, S2C, S2E, and S2F; Tables S1B, S1C, and S1P). Furthermore, we found that YFP$^+$ clusters were also rare in PIN lesions of 6-month-old *CK5-trace; Pten*$^{+/-}$ mice, whereas the frequency of YFP$^+$ cells was unchanged in nontumor regions (n = 3) (Figures S3D, S3E, S4A, S4B, S4D, S4E, and S4G; Tables S1D and S2D). However, the percentage of YFP$^+$ cells in PIN lesions of *PSA-trace; Pten*$^{+/-}$ mice (n = 3) was similar to the percentage initially marked by the *PSA-CreER*^{T2} inducible driver (Figures S4C, S4F, and S4G; Table S1E).

Next, we examined the transgenic *ARR*$_2$*/probasin-Myc* (*Hi-Myc*) model, in which expression of c-Myc is driven in both luminal and basal compartments, leading to invasive adenocarcinoma (Ellwood-Yen et al., 2003). Consistent with previous studies (Ellwood-Yen et al., 2003), the histology of the AP in *Hi-Myc* mice was mostly normal at 2 months of age (Figure 2O), although the DLP and ventral prostate (VP) were hyperplastic (Figures S4H and S4K). In the PIN/carcinoma lesions in the AP of *CK5-trace; Hi-Myc* mice at 6 months, YFP$^+$ cell clusters were rare, whereas the percentage of YFP$^+$ basal cells in untransformed regions was unaffected (n = 5 mice) (Figures 2P, 2Q, and 2Z; Figures S2G, S3F, and S3G; Tables S1F and S2E). In contrast, 13.1% of the cells within the PIN/carcinoma lesions of 6-month-old *PSA-trace; Hi-Myc* mice (n = 6) were YFP$^+$, similar to the initial percentage (12.6%) of luminal cells marked at 2 months (Figures 2R and 2Z; Figure S2H; Table S1G). Similarly, YFP$^+$ cells were present in PIN/carcinoma lesions of