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Authors
Wang, ZA
Toivanen, R
Bergren, SK
et al.

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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. Shen1,*

1Departments 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
2Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104, INSERM U964, 67400 Illkirch, France
3Present address: Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA 95064, USA
*Correspondence: mshen@columbia.edu
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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<sup>−/−</sup>; Pten<sup>+/−</sup>, Pten<sup>−/−</sup>, 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-Cre<sup>ERT2</sup> driver (Rock et al., 2009) for lineage tracing of basal cells and the PSA-Cre<sup>ERT2</sup> (Ratnacaram et al., 2008) or CK8-Cre<sup>ERT2</sup> (Van Keymeulen et al., 2011) drivers for tracing of luminal cells, together with the R26R-YFP reporter (Srinivas et al., 2001). Tamoxifen
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-CreERT2 driver to tumors would imply that basal cells were the cell of origin, whereas tumor cells marked by the PSA-CreERT2 or CK8-CreERT2 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-CreERT2; R26R-YFP (which we denote CK5-trace) strictly marks basal cells with 23.6% efficiency, while PSA-CreERT2; R26R-YFP (PSA-trace) marks luminal cells with 11.5% efficiency and CK8-CreERT2; 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 (Figures S3A–S3C; Table S2C). In contrast, 10.8% of the cells in tumor lesions of 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-CreERT2 inducible driver (Figures S4C, S4F, and S4G; Table S1E).

Next, we examined the transgenic ARR2/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 2R; Figures S2A and S2D; Table S1A), while the percentage of YFP+ cells in PIN lesions of CK5-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; Hi-Myc mice (Figures S3H, S3I, and S3J; Tables S1B, S1C, and S1P). Notably, in tumor lesions of CK5-trace; Pten−/− mice, 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-CreERT2 inducible driver (Figures S4C, S4F, and S4G; Table S1E).
Figure 2. Luminal Cells Are Favored Cells of Origin in the NP, Hi-Myc, and TRAMP Models

(A) Experimental time course.

(B) Lineage marking of basal cells (arrowheads) in the AP of CK5-trace; NP mice at 2 months of age.

(C and D) Marking of luminal cells (arrows) in the AP of PSA-trace; NP mice (C) or CK8-trace mice (D) at 2 months.

(E, F, J, and K) H&E staining of NP prostates shows normal histology at 2 months and PIN/carcinoma lesions at 6 months.

(G and L) Clusters of YFP+ cells are rarely detected in CK5-trace; NP tumor lesions at 6 months.

(H, I, M, and N) YFP+ cell clusters in tumor lesions of PSA-trace; NP (H and M) and CK8-trace; NP (I and N) mice at 6 months.

(O and P) Normal AP histology in Hi-Myc mice at 2 months (O) and PIN/carcinoma lesions at 6 months (P).

(Q) Absence of YFP+ cell clusters in CK5-trace; Hi-Myc tumor lesions in the AP at 6 months.

(R and S) YFP+ cell clusters in tumor lesions of PSA-trace; Hi-Myc mice (R) and CK8-trace; Hi-Myc mice (S) at 6 months.

(T and U) Normal histology of the AP in TRAMP mice at 2 months (T) and carcinoma at 5 months (U).

(V) Absence of YFP+ cell clusters in CK5-trace; TRAMP AP tumor lesions at 5 months.

(W and X) YFP+ clusters in AP tumor lesions of PSA-trace; TRAMP (W) and CK8-trace; TRAMP (X) mice at 5 months.

(Y–A’0) Percentage of YFP+ cells in NP (Y), Hi-Myc (Z), and TRAMP (A’) models; Nor = normal, Tum = tumor; **p < 0.001 by Student’s t test; error bars are 1 SD. Arrowheads in (G), (L), (Q), and (V) indicate marked basal cells. Scale bars correspond to 50 μm in (B)–(D), (G)–(I), (L)–(N), (Q)–(S), and (V)–(X) and 100 μm in (E), (F), (J), (K), (O), (P), (T), and (U). See also Figures S1–S4.
CK8-trace; Hi-Myc mice (n = 4) in proportion to the initial luminal marking efficiency (Figures 2S and 2Z; Figure S2I; Tables S1H and S1P). Similar results were found in the DLP and VP of CK5-trace; Hi-Myc and PSA-trace; Hi-Myc mice (Figures S4H–S4M).

We also investigated the TRAMP model, which expresses the SV40 large T antigen under the control of the probasin promoter, giving rise to aggressive tumors (Greenberg et al., 1995). We found that the AP in TRAMP mice appeared mostly normal at 2 months but developed invasive, poorly differentiated adenocarcinoma by 5 months (Figures 2T and 2U). In tumor lesions of CK5-trace; TRAMP mice (n = 3), YFP⁺ cell clusters were not observed, whereas the frequency of YFP⁺ cells in nontumor regions was unaffected (Figures 2V and 2A; Figures S2J, S3H, and S3I; Tables S1I and S2F). However, YFP⁺ cell clusters were found in tumor lesions of PSA-trace; TRAMP mice (n = 5) and CK8-trace; TRAMP mice (n = 3) in percentages similar to the initial luminal marking efficiencies (Figures 2W, 2X, and 2A; Figures S2K and S2L; Tables S1J, S1K, and S1P). Similar results were observed in the DLP and VP of TRAMP mice, although these lobes were already hyperplastic at 2 months of age (Figures S4N–S4S). Taken together, these findings show that luminal cells are the favored cell of origin in each of the genetically engineered mouse models examined.

Given the potential caveat that cancer initiation might occur prior to adulthood in these genetically engineered models, we investigated the cell of origin in a hormonal carcinogenesis paradigm (Ricke et al., 2008; Wang et al., 2000), in which lineage marking unequivocally takes place prior to prostate tumor initiation (Bosland et al., 1995; Noble, 1977; Ricke et al., 2008; Wang et al., 2000). After lineage marking of basal cells in CK5-trace mice or luminal cells in PSA-trace and CK8-trace mice (Figures 3A–3D; Tables S1L, S1N, and S1P), we treated the mice with a combination of testosterone (T) and estradiol-17β (E2) for 4 months, resulting in formation of low-grade PIN lesions in all prostate lobes (Figures 3E, 3I, and 3M). Using this protocol, we found that YFP⁺ clusters were rare in PIN lesions of CK5-trace mice (n = 5), while the frequency of YFP⁺ cells was unaffected in untransformed regions (Figures 3F, 3J, 3N, and 3Q; Figures S2M, S2P, S2S, S3J, and S3K; Tables S1M and S2G). In contrast, YFP⁺ clusters were present in PIN lesions of PSA-trace (n = 4) and CK8-trace mice (n = 3) (Figures 3G, 3H, 3K, 3L, 3O, 3P; Figures S2N, S2O, S2Q, S2R, S2T, and S2U), with the percentage of YFP⁺ cells similar to the initial efficiency of luminal cell marking (Figure 3Q; Tables S1O and S1Q). These results indicate that carcinogenesis induced by T+E2 treatment leads to prostate cancer initiation from luminal cells.

Previous studies have concluded that basal cells are cells of origin for human prostate cancer using renal grafting methods (Goldstein et al., 2010; Stoyanova et al., 2013; Taylor et al., 2012). To determine whether the potential discrepancy between these studies and our findings might be due to the different methodologies employed, we tested whether basal cells in our mouse models of prostate cancer could give rise to tumors after renal grafting. We performed tamoxifen induction of CK5-trace; Hi-Myc mice at 7 weeks of age and isolated basal cells by flow sorting for YFP (Figures 4A and 4B). The sorted basal cells were recombined with rat urogenital sinus mesenchyme andrafted under the renal capsule of immunodeficient NOD.Cg-Prkdcscid Il2rgtm1Sug/JicTac (NOG) mice, followed by analysis after 3 months (Figure 4G). We observed extensive regions of YFP⁺ epithelium, which contained PIN lesions that were mostly composed of luminal cells, indicating that basal to luminal differentiation had taken place (Figures 4D–4F). We obtained similar results for basal cells isolated from CK5-trace; TRAMP mice (Figures 4G–4I), as well as from CK5-trace; Pten⁻/⁻ mice, in which the graft PIN lesions were also positive for phospho-Akt (Figures 4J–4L). Finally, we performed renal grafting of YFP⁺ basal cells isolated from tamoxifen-induced CK5-trace mice, followed by treatment of the NOG graft recipients with T+E2 for 3 months (Figure 4M). In the resulting grafts, marked basal cells could give rise to PIN lesions that mostly contained luminal cells (Figures 4N and 4O). Taken together, our results show that prostate basal cells are not favored as the cell of origin in their native microenvironment for any of the mouse models analyzed but nonetheless can give rise to tumors in renal grafts.

DISCUSSION

In principle, the cell of origin for cancer might be context specific, depending upon the oncogenic pathways being activated. In our studies, we have employed a novel lineage-tracing methodology for systematic assessment of the cell of origin for prostate cancer in a diverse range of mouse models. Using this “agnostic” lineage-tracing approach, we have unexpectedly found that luminal epithelial cells are consistently observed as the cell of origin.

Overall, we have analyzed a representative sample of widely used mouse models of human prostate cancer (Irshad and Abate-Shen, 2013; Ittmann et al., 2013; Shappell et al., 2004). However, there may be specific caveats associated with each model; for example, tumor initiation might conceivably occur in basal cells prior to 7 weeks of age in the transgenic models, resulting in early basal-to-luminal differentiation that would escape lineage marking. This possibility seems unlikely, since all tumor initiation would have to occur prior to 7 weeks of age to avoid detection of subsequent tumor formation from basal cells by lineage tracing. Nonetheless, our analysis has yielded the remarkably consistent result that luminal cells are favored as the cell of origin, and consequently we believe that this finding is likely to reflect the biology of prostate cancer, rather than a coincidence of intrinsic biases in each model. However, we note that basal cells could nonetheless act as cells of origin for prostate adenocarcinoma in other experimental contexts. In addition, the ability of inflammation to enhance basal-to-luminal differentiation in vivo (Kwon et al., 2014) suggests that alterations of the tissue microenvironment could influence the cell of origin (Goldstein and Witte, 2013).

To date, the cell of origin has usually been assayed by conditional gene targeting to generate oncogenic insults within a specific cell type. However, if the targeted cell type is a stem/progenitor cell, it can be difficult to discern whether tumor initiation takes place within the stem/progenitor itself or instead within its differentiated progeny. In this situation, it can be useful to distinguish between a “cell of origin” and a “cell of mutation” as distinct entities (Liu et al., 2011; Liu and Zong, 2012). In
particular, a progenitor that initially acquires a mutation may not directly transform and hence be a “cell of mutation,” while its lineage-restricted progeny may inherit the mutation and subsequently undergo oncogenic transformation and thus would represent a “cell of origin.” For example, lineage tracing of gliomas in a \( p53; Nf1 \) mouse model has shown that neural stem cells act as a cell of mutation, whereas their descendant oligodendrocyte progenitors correspond to the cell of origin (Liu et al., 2011).

In this regard, prostate basal cells removed from their normal tissue microenvironment can acquire facultative bipotential progenitor properties after combination with embryonic urogenital mesenchyme, resulting in the differentiation of luminal cells (Goldstein et al., 2008; Lawson et al., 2007, 2010; Wang et al., 2013), while transformed basal cells give rise to luminal tumors in renal grafts (Goldstein et al., 2010; Stoyanova et al., 2013; Taylor et al., 2012). Our findings are consistent, since lineage-marked basal cells in each of our mouse models can give rise to prostate cancer in the context of renal grafts. Consequently, we propose that mutated basal cells do not usually act as a cell of origin in prostate tissue in situ but can function as a cell of mutation in renal grafts by acquiring facultative progenitor

![Figure 3. Luminal Cells Are the Favored Cell of Origin of Tumors Induced by T+E2 Hormonal Treatment](image)
properties and thereby generating luminal progeny that are authentic cells of origin.

Notably, previous studies have shown that targeted deletion of Pten in basal cells results in formation of tumors in situ, albeit with a temporal delay that appears to be associated with basal-to-luminal differentiation, and which are less aggressive than tumors arising from targeting of luminal cells (Choi et al., 2012; Wang et al., 2013). Interestingly, PIN lesions arose from targeted basal cells by 3 months of age, in contrast with the absence of contribution from lineage-marked basal cells in NP

Figure 4. Basal Cells Can Give Rise to Prostate Cancer in Renal Grafts

(A) Experimental design.
(B) Representative flow-sort of YFP+ basal cells (2.8% of total prostate cells) from CK5-trace; Hi-Myc mice.
(C) Kidney from recipient NOG mouse containing graft with YFP fluorescence (arrow).
(D–F) Grafted basal cells from CK5-trace; Hi-Myc (D–F) or CK5-trace; TRAMP (G–I) mice generate PIN lesions (D and G), which contain mostly luminal cells (E and H) and some basal cells (arrowheads, F and I).
(J–L) Basal cells from CK5-trace; Pten+/- mice generate PIN lesions (J) that contain mostly luminal cells (K) and express phospho-Akt (L).
(M) Experimental design for T+E2 treatment of grafts.
(N and O) Grafted basal cells give rise to PIN lesions (N) that contain mostly luminal cells (O) after T+E2 treatment.

Scale bars correspond to 25 μm in (E), (F), (H), (I), and (K), 5 mm in (C), and 50 μm in (D), (G), (J), (N), and (O).
and Pten<sup>-/-</sup> mice. These findings are potentially consistent with a “competition” model, which is not mutually exclusive with the cell of mutation model. Thus, if Pten loss occurs in both luminal and basal cells, transformed luminal cells might emerge before basal cells can be transformed, and might suppress subsequent basal cell transformation in a non-cell-autonomous manner.

Finally, our finding that luminal cells are the favored cell of origin in multiple mouse models raises the possibility that most human prostate adenocarcinomas arise from luminal cells. In particular, cytological examination of human PIN lesions suggests that early initiating events occur in luminal cells, including c-Myc upregulation and telomere elongation (Gurel et al., 2008; Meeker et al., 2002). Moreover, human prostate luminal cells may be prone to cancer initiation due to a decreased DNA damage response (Jäämaa et al., 2010). Our results also imply that cell of origin analyses for human cancer may be inherently difficult using grafting assays, due to the plasticity of basal cells. Instead, approaches such as retrospective lineage tracing using mitochondrial mutations may provide insight into human prostate cancer origins (Blackwood et al., 2011; Gaïsa et al., 2011). Since the cell of origin may be a critical factor in conferring aggressiveness in prostate cancer (Wang et al., 2013), these and other approaches to identify cell types of origin are likely to be important for biomarker identification and disease prognosis.

**EXPERIMENTAL PROCEDURES**

**Mouse Procedures**

Mouse lines were maintained on an inbred C57BL/6N or mixed C57BL/6N-129S6/SvEvTac background. Primer sequences for genotyping are listed in Table S2. For tamoxifen induction, mice were administered 9 mg/40 g tamoxifen (Sigma) suspended in corn oil by oral gavage once daily for 4 consecutive days.

For T/E2 treatment, a 1.0 cm Silastic capsule (No. 602–305 Silastic tubing; 1.54 mm inside diameter, 3.18 mm outside diameter; Dow-Corning #2415669) filled with testosterone (Sigma) and a 0.4 cm Silastic capsule filled with estradiol-17β (Sigma) were implanted subcutaneously. Mice were treated with hormones for 4 months.

All animal studies were performed using protocols approved by the Institutional Animal Care and Use Committee of Columbia University Medical Center.

**Tissue Collection and Flow Cytometry**

Prostate tissue dissection, fixation, and dissociation were performed as described previously (Wang et al., 2013). Cell sorting was performed using a two-sample t test. At least three animals for each experiment or genotype were analyzed.

**Renal Grafting Assay**

For tissue recombinants, 1.0 x 10<sup>7</sup> dissociated YFP<sup>+</sup> cells were mixed with 2.5 x 10<sup>5</sup> dissociated urogenital sinus mesenchyme cells from embryonic day 18.0 rat embryos. Tissue recombinants were cultured in Dulbecco’s modified Eagle’s medium/10% fetal bovine serum/10<sup>-7</sup> M dihydrotestosterone overnight, followed by transplantation under the kidney capsules of immunodeficient NOD.Cg-Pkd1<sup>+/+</sup> Pdx1<sup>L204F</sup>/JicTac (NOG) mice (Taconic) and grown for 12 weeks.

**Histology and Immunostaining**

Hematoxylin and eosin (H&E) staining and immunofluorescence staining were performed (Wang et al., 2013) using the following primary antibodies: rabbit CK5 (Covance #PRB-160P, 1:1,000), rabbit CK8 (Abcam #ab53280, 1:250), mouse CK18 (Abcam #ab668, 1:100), chick GFP (Abcam #ab13970, 1:2,000), and rabbit phospho-Akt (Cell Signaling #3787, 1:50). Samples were incubated with secondary antibodies (diluted 1:500 in PBST) labeled with Alexa 488, Alexa Fluor 555, or Alexa Fluor 647 (Invitrogen/Molecular Probes) and mounted with VECTASHIELD medium with DAPI (Vector Labs). Immunofluorescence was imaged using a Leica TCS SP5 spectral confocal microscope.

**Data Quantitation**

Cell numbers were counted using confocal x40 and x63 photomicrographs. For histologically normal tissues at 2 months, the percentage of YFP<sup>+</sup> cells (labeled “Nor” in Figures 2Y–2A and S1G and “Cont” in Figure 3Q) represents the ratio of YFP<sup>+</sup> cells to total luminal or basal cells. For tumor tissues at later ages, the percentage of YFP<sup>+</sup> cells (labeled “Tum” in Figures 2Y–2A and S1G and “T+E2” in Figure 3Q) represents the ratio of clustered YFP<sup>+</sup> cells in tumor lesions to total epithelial cells within these lesions. Statistical analyses were performed using a two-sample t test. At least three animals for each experiment or genotype were analyzed.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes four figures and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.08.002.

**AUTHOR CONTRIBUTIONS**

Z.A.W. and M.M.S. designed the study. Z.A.W. performed the experiments, with contributions from R.T. for H&E and immunostaining, and S.K.B. for renal grafting. P.C. provided PSA-CreER<sup>+</sup> mice. Z.A.W., R.T., and M.M.S. analyzed data and prepared the manuscript.

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