

Basal Progenitors Contribute to Repair of the Prostate Epithelium Following Induced Luminal Anoikis

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<http://dx.doi.org/10.1016/j.stemcr.2016.03.007>

SUMMARY

Contact with the extracellular matrix is essential for maintenance of epithelial cells in many tissues, while in its absence epithelial cells can detach and undergo anoikis. Here, we show that anoikis of luminal cells in the prostate epithelium is followed by a program of tissue repair that is mediated in part by differentiation of basal epithelial cells to luminal cells. We describe a mouse model in which inducible deletion of E-cadherin in prostate luminal cells results in their apoptotic cell death by anoikis, in the absence of phenotypic effects in the surrounding stroma. Quantitative assessments of proliferation and cell death in the luminal and basal compartments indicate that basal cells can rapidly generate luminal cells. Thus, our findings identify a role for basal-to-luminal differentiation in prostate epithelial repair, and provide a normal context to analogous processes that may occur during prostate cancer initiation.

INTRODUCTION

Epithelial integrity and tissue homeostasis are severely challenged by wounding or a range of pathological states. Under such conditions, epithelial cells can detach from the underlying basement membrane and undergo apoptotic cell death through a process known as anoikis (Chiarugi and Giannoni, 2008; Frisch and Francis, 1994; Frisch and Screaton, 2001; Taddei et al., 2012). Subsequently, the missing epithelial cells can be replaced through the activity of endogenous stem/progenitor cells. In the prostate, insults such as bacterial or viral infection can result in inflammation and epithelial cell death, but the process of repair has been poorly studied to date.

The identity of epithelial stem/progenitor cells within the prostate remains a subject of intense study (Shibata and Shen, 2015). The prostate epithelium comprises luminal, basal, and neuroendocrine cells, with both luminal and basal compartments containing stem/progenitor activity (Shen and Abate-Shen, 2010). In addition, rare “intermediate” cells that co-express basal and luminal markers have been proposed to correspond to stem/progenitor cells (De Marzo et al., 1998; Verhagen et al., 1988; Wang et al., 2001; Xue et al., 1998), or a transitional state between basal progenitors and luminal descendants (Bonkhoff and Remberger, 1996; Litvinov et al., 2006; Ousset et al., 2012; van Leenders et al., 2000).

Stem/progenitor activity has been observed in distinct contexts during prostate development and homeostasis in vivo. In the hormonally naive adult prostate epithelium,

luminal and basal compartments are maintained by unipotent progenitors (Choi et al., 2012; Lu et al., 2013; Wang et al., 2013), while during prostate organogenesis some basal progenitors are multipotent, giving rise to luminal and neuroendocrine progeny (Ousset et al., 2012; Wang et al., 2014a). In addition, rare bipotential populations exist within both the luminal and basal compartments during androgen-mediated regeneration of the regressed prostate (Lee et al., 2014; Wang et al., 2009, 2013, 2015).

In contrast, both luminal and basal populations display considerable lineage plasticity in specific contexts. Explanted luminal cells can generate basal cells in organoid culture (Chua et al., 2014; Karthaus et al., 2014), whereas basal cells can generate luminal cells in sphere formation assays, and after recombination with embryonic urogenital mesenchyme in renal grafts (Burger et al., 2005; Goldstein et al., 2008, 2010; Hofner et al., 2015; Lawson et al., 2007; Richardson et al., 2004; Wang et al., 2013). Basal-to-luminal differentiation can also occur in pathological contexts, such as during prostate cancer initiation (Choi et al., 2012; Lu et al., 2013; Wang et al., 2013, 2014b), and after acute inflammation in bacterial prostatitis (Kwon et al., 2014b).

Taken together, these findings indicate that basal-to-luminal differentiation can occur in prostate organogenesis, pathogenesis, and ex vivo assays, but rarely during normal tissue homeostasis. Thus, it has been unclear to what extent the plasticity of endogenous adult prostate basal cells in ex vivo models and disease states reflects an in vivo activity. In this study, we introduce a mouse model in which a tamoxifen-inducible Cre driver is used to delete E-cadherin in prostatic luminal cells, which are highly

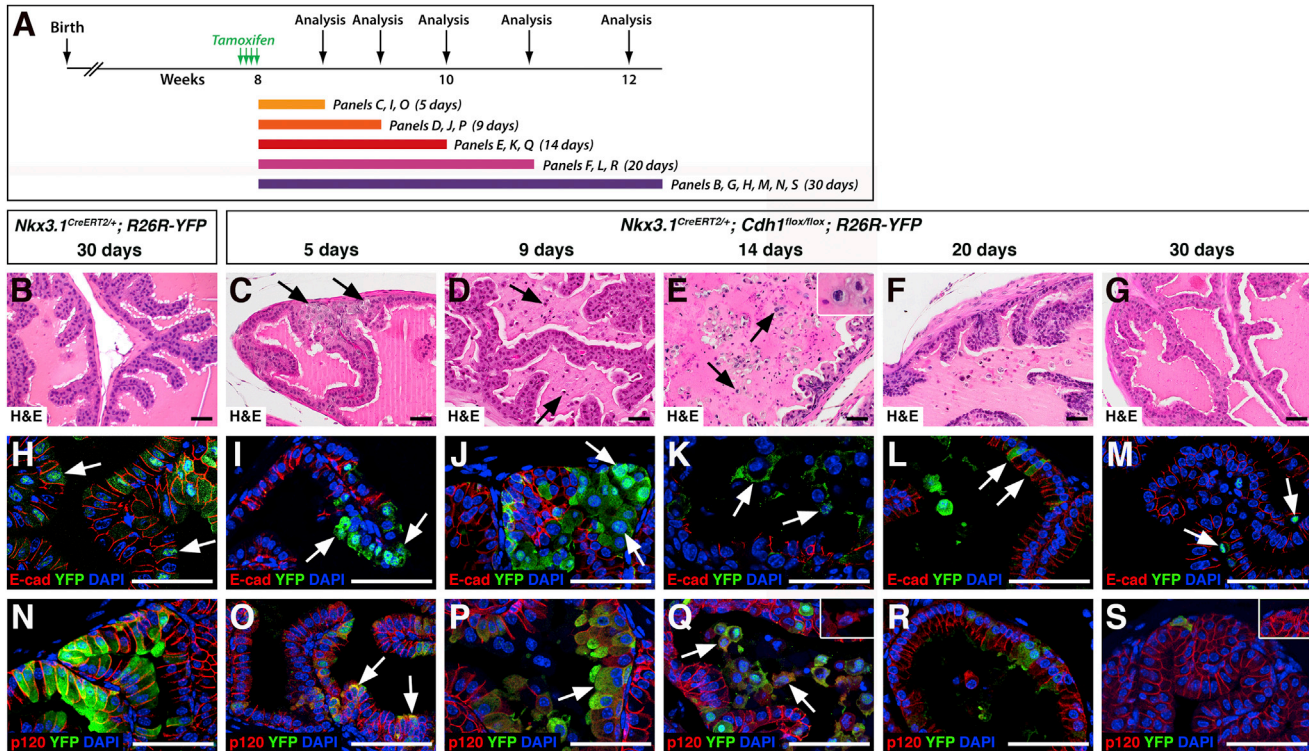


Figure 1. Inducible Deletion of E-Cadherin in the Prostate Epithelium

(A) Schematic timeline of the experiment.

(B–G) H&E staining of histological sections from the anterior prostate. Arrows in (C) show patches of atypical cells, and arrows in (D), (E), and inset in (E) show cells sloughing into the lumen in *Cdh1^{del}* prostates.

(H–M) Immunofluorescence staining for E-cadherin and YFP. Arrows in (H) show intact E-cadherin expression in control mice; arrows in (I), (J), (K), and (M) show E-cadherin loss in YFP⁺ cells of *Cdh1^{del}* prostates; and arrows in (L) indicate rare YFP⁺ cells in which E-cadherin was not deleted.

(N–S) Immunofluorescence staining for p120 catenin and YFP. Arrows in (O), (P), and (Q) show cytoplasmic p120 staining in YFP⁺ cells of *Cdh1^{del}* prostates.

Numbers of mice examined: n = 7 for (B), (E), (H), (K), (N), and (Q); n = 5 for (C), (I), and (O); n = 4 for (D), (F), (G), (J), (L), (M), (P), (R), and (S). Scale bars, 50 μm. See also [Figures S1 and S2](#); [Tables S1 and S2](#).

susceptible to anoikis (Kwon et al., 2014a). This results in rapid sloughing and death of luminal cells, followed by repair of the damaged epithelium. We show that basal-to-luminal differentiation contributes to tissue repair, providing a new approach for studying prostate stem/progenitor activity and epithelial specification.

RESULTS

E-Cadherin Is Essential for Maintenance of Prostate Luminal Cells

To delete *E-cadherin* (*Cdh1*) in the prostate epithelium, we used mice carrying the tamoxifen-inducible *Nkx3.1^{CreERT2}* driver (Wang et al., 2009), the conditional *Cdh1^{flox}* allele (Boussadia et al., 2002), and an *R26R-YFP* reporter (Srinivas et al., 2001). We administered tamoxifen to

Nkx3.1^{CreERT2/+} or *CreERT2/CreERT2*; *Cdh1^{flox/flox}*; *R26R-YFP* (designated *Cdh1^{del}*) mice at 8 weeks of age, and analyzed prostate tissues at 5, 9, 14, 20, and 30 days thereafter (Figure 1A). In parallel, control *Nkx3.1^{CreERT2/+}*; *R26R-YFP* mice were treated with tamoxifen and analyzed at 5, 14, and 30 days later.

Histological analyses of the anterior prostate lobes showed that controls maintained a normal phenotype after tamoxifen treatment (Figures 1B, S1A, and S1B), whereas *Cdh1^{del}* prostates underwent epithelial damage and repair. At 5 days after tamoxifen administration, we observed foci of atypical cells in *Cdh1^{del}* prostates (Figure 1C), and at 9 and 14 days after tamoxifen treatment, many cells had detached from the epithelium and were present in the lumen (Figures 1D and 1E). However, by 20 days after treatment, fewer detached cells were observed, and at 30 days the normal epithelial phenotype was restored



(Figures 1F and 1G). Similar results were observed in the ventral prostate (VP) and dorsolateral prostate (DLP) of control and *Cdh1^{del}* mice (Figure S2).

To confirm these phenotypes were due to E-cadherin deletion, we analyzed co-expression of E-cadherin and YFP by immunofluorescence staining of control and *Cdh1^{del}* prostates. In control prostates, E-cadherin displayed intact membrane localization in YFP⁺ cells (Figures 1H, S1C, and S1D). However, YFP⁺ cells in *Cdh1^{del}* mice started to lose E-cadherin expression by 5 days after tamoxifen treatment (Figure 1I), and was lost in 93% of YFP⁺ cells at 9 days (Figure 1J and Table S1). Moreover, the detached cells in the prostate lumen of *Cdh1^{del}* mice at 14 and 20 days after treatment expressed YFP, but not E-cadherin (Figures 1K and 1L). In contrast, by 30 days the epithelium displayed a normal distribution of membrane-localized E-cadherin, but YFP⁺ cells were nearly absent (Figure 1M). Notably, the few remaining YFP⁺ cells were localized to basal positions, comprising approximately 2% of the prostate epithelium (Figure 1M and Table S2).

To confirm deletion of E-cadherin we also examined expression of p120 catenin, a binding partner of E-cadherin that becomes localized to the cytoplasm following loss of E-cadherin (Shibamoto et al., 1995). Consistent with E-cadherin expression, control prostates displayed normal membrane localization of p120 catenin (Figures 1N, S1E, and S1F), whereas *Cdh1^{del}* prostates at 5 days after tamoxifen treatment contained sporadic luminal cells with increased cytoplasmic localization of p120 (Figure 1O). At 9 and 14 days, numerous luminal cells showed strong cytoplasmic localization of p120 (Figures 1P and 1Q), which were reduced in frequency at 20 days (Figure 1R). By 30 days, the *Cdh1^{del}* prostates showed essentially normal p120 expression, with rare cells that displayed cytoplasmic p120 localization still detectable (Figure 1S).

E-Cadherin Deletion in Luminal Cells Results in Anoikis Followed by Rapid Epithelial Repair

We anticipated that E-cadherin deletion would be limited to the luminal compartment in *Cdh1^{del}* prostates, since the *Nkx3.1^{CreERT2}* driver is mostly restricted to luminal cells (Wang et al., 2009). To examine the specificity of E-cadherin deletion, we compared expression of YFP with specific luminal and basal markers in control and *Cdh1^{del}* prostates at the most prominent stage of epithelial damage (14 days), and after repair (30 days). As expected, YFP expression was predominantly observed in CK18-positive luminal cells of control mice at 30 days (34.9% of CK18⁺ cells versus 0.7% of CK5⁺ cells) (Figure 2A and Table S3). In *Cdh1^{del}* prostates, the detached YFP⁺E-cadherin⁻ cells in the prostate lumen at 14 days were also CK18⁺ (Figure 2B), confirming that these sloughed cells were luminal. Notably, at 30 days the *Cdh1^{del}* luminal cells displayed

membrane-localized E-cadherin but were YFP⁻ (Figure 2C), indicating replacement of sloughed luminal cells by newly formed luminal cells.

However, at 30 days we observed rare surviving YFP⁺ cells that occupied basal positions, but did not resemble normal basal cells (Figure 2C); these may correspond to *Cdh1* null luminal cells that survived due to their ectopic localization. Consistent with this interpretation, these residual YFP⁺ cells were mostly negative for the basal marker CK5 (Figures 2D–2F and S3A; Table S2). Furthermore, the residual YFP⁺ cells were rarely intermediate (CK5⁺CK8⁺), did not express the neuroendocrine marker synaptophysin, and did not express N-cadherin, suggesting that they were not undergoing an epithelial-mesenchymal transition (Figures S3B–S3D and Table S2).

Since deletion of E-cadherin resulted in sloughing of luminal cells, we examined whether these cells underwent anoikis. We found that the apoptotic marker cleaved caspase-3 (CC3) was rarely detected in control or *Cdh1^{del}* prostates at 30 days, but was readily detectable in the detached luminal cells of *Cdh1^{del}* prostates at 14 days (Figures 2G–2I). Taken together, these data indicate that E-cadherin is essential for survival of luminal epithelial cells, and the *Cdh1^{del}* mice provide a model for studying anoikis and epithelial repair in the prostate.

Loss of E-Cadherin in Prostate Luminal Cells Does Not Affect the Surrounding Stroma

Since another model of prostate epithelial repair has suggested a role for reactive stroma (Kwon et al., 2014b), we examined whether epithelial deletion of E-cadherin might result in stromal defects. Previous studies have characterized reactive stroma as displaying loss of smooth muscle-expressing α -actin and an increase in fibroblasts that express vimentin (Kwon et al., 2014b; Tuxhorn et al., 2001; Tuxhorn et al., 2002). Therefore, we compared the expression of smooth muscle α -actin (SMA) and vimentin in control and *Cdh1^{del}* prostates. Immunostaining of SMA in control and *Cdh1^{del}* prostates showed continuous smooth muscle bands surrounding the epithelial ducts, with no loss of smooth muscle organization (Figures 2J–2L). In addition, we did not observe an increase in vimentin staining in the stroma of *Cdh1^{del}* mice (Figures 2M–2O), indicating that the process of epithelial repair occurs without a phenotypically reactive stroma.

Prostate Basal Cells Contribute to Epithelial Repair after E-Cadherin Deletion in Luminal Cells

Next, we investigated the cell type(s) that were responsible for epithelial repair in *Cdh1^{del}* prostates. First, we quantitated the proportion of basal (CK5⁺), luminal (CK8⁺), and intermediate (CK5⁺CK8⁺) cells during tissue repair (Table S4). We found that the proportions of basal and luminal

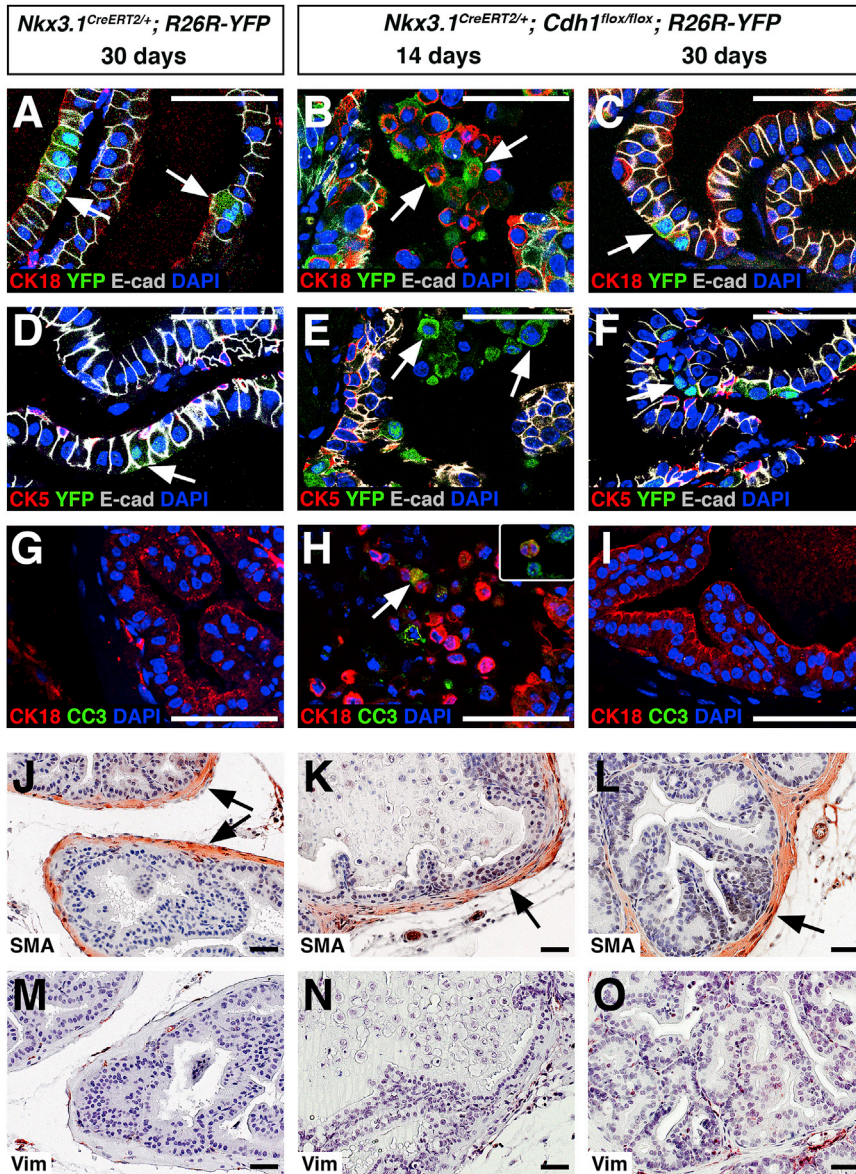


Figure 2. Deletion of E-Cadherin in Prostate Luminal Cells Results in Anoikis without Reactive Stroma

(A–C) Immunofluorescence staining of anterior prostate for CK18, E-cadherin, and YFP; arrows show CK18 expression in YFP⁺ cells.

(D–F) Immunofluorescence staining for CK5, E-cadherin, and YFP; arrows show that YFP⁺ cells do not express CK5.

(G–I) Immunofluorescence staining for CK18 and cleaved caspase-3 (CC3). The arrow in (H) shows co-localization of CC3 and CK18 in a sloughed cell; inset in (H) shows co-localization of CC3 (red) and YFP (green).

(J–L) Immunohistochemical staining for smooth muscle α -actin shows normal appearance of smooth muscle in control and *Cdh1^{del}* prostates (arrows).

(M–O) Immunohistochemical staining for vimentin shows nearly undetectable expression in control and *Cdh1^{del}* prostates. Numbers of mice examined: $n = 3$ for (A), (C), (D), (F), (G), (I), (J), (L), (M), and (O); $n = 4$ for (B), (E), (H), (K), and (N). Scale bars, 50 μ m. See also Figure S3 and Table S3.

cells showed a significant increase and decrease respectively, by 14 days, which then revert to normal proportions by 30 days (Figures 3A–3F, 3M, and 3N). Notably, while control prostates contained only rare intermediate cells, *Cdh1^{del}* prostates showed increasing proportions of intermediate cells by 14 days, which subsequently decreased to normal levels by 30 days (Figures 3A–3F and 3O). Interestingly, we observed intermediate cells appearing to emerge from the basal layer at 9 days (Figure 3C).

Second, we determined the percentages of proliferating and detaching (dying) cells in the basal and luminal compartments (Table S5). In control prostates, there was limited epithelial proliferation that primarily occurred in the luminal compartment (Figures 3G and 3P). However,

in *Cdh1^{del}* prostates, CK5⁺ basal cells showed significantly increased proliferation at 9 and 14 days, before decreasing to control levels (Figures 3H–3L and 3P), while little cell death in basal cells was detected at any time point (Figure 3Q). We also found increased proliferation of luminal cells at later time points (14 and 20 days), before decreasing to control levels (Figures 3H–3L and 3P). Notably, cell death as indicated by detachment into the lumen was only detectable for CK8⁺ luminal cells, and was most significant at 14 days (Figure 3Q).

Since the proportion of basal cells returned to normal levels by 30 days, and few if any basal cells underwent anoikis (Figures 3M and 3Q), the increased proliferation of basal cells observed by 9 days (Figure 3P) indicates that basal cells

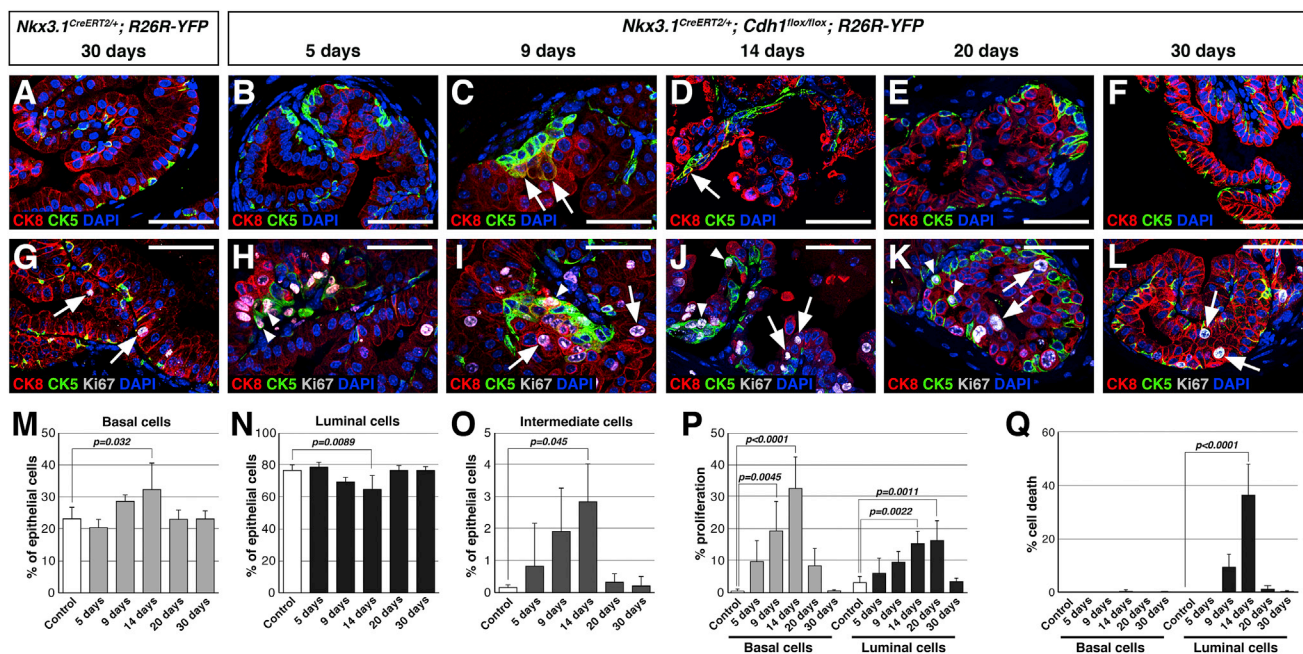


Figure 3. Basal Cells Contribute to Epithelial Repair following E-Cadherin Deletion

(A–F) Immunofluorescence staining for CK5 and CK8 identifies double-positive intermediate cells (arrows). (G–L) Immunofluorescence staining for CK8, CK5, and Ki67 shows low levels of proliferation in luminal (arrows) and basal (arrowheads) cells of control and *Cdh1^{del}* prostates. (M–O) Quantitation of the proportions of basal (M), luminal (N), and intermediate (O) cells in control and *Cdh1^{del}* prostates. Values represent the mean and SD of at least three animals per group. (P and Q) Quantitation of cell proliferation (P) and cell death (Q) in the basal and luminal compartments. Values represent the mean and SD (error bars) of at least three animals per group. Numbers of mice examined: $n = 4$ for (A), (B), (C), (E), (G), (H), (I), and (K); $n = 5$ for (D) and (J); $n = 3$ for (F) and (L). Scale bars, 50 μm . See also Tables S4 and S5.

contribute to repair of the luminal compartment. This interpretation is also consistent with the transient increase in the percentage of intermediate cells at 14 days (Figure 3O). Thus, our findings suggest that prostate basal cells repair the luminal compartment by undergoing differentiation into intermediate cells and then luminal cells, as suggested by the increase in luminal proliferation after basal proliferation.

DISCUSSION

Our studies have shown that E-cadherin plays an essential role in the maintenance of prostate epithelial integrity and are consistent with the requirement for E-cadherin in homeostasis of other epithelial tissues. For example, deletion of E-cadherin in the mouse small intestine and uterus in vivo leads to increased apoptosis and abnormal differentiation, underscoring its essential role in maintenance of epithelial architecture and function (Reardon et al., 2012; Schneider et al., 2010). Similarly, we have found that dele-

tion of E-cadherin in prostate luminal cells results in their sloughing into the prostate lumen followed by anoikis. After loss of luminal cells, the prostate epithelium is repaired by the activity of basal progenitors, likely by differentiation through a transitional “intermediate cell” state, together with activated luminal progenitors.

Notably, this process of tissue repair differs from androgen-mediated regeneration of the regressed prostate, which is largely driven by unipotent luminal and basal stem/progenitor cells (Choi et al., 2012; Liu et al., 2011; Lu et al., 2013; Wang et al., 2009, 2013, 2015). Such differences in progenitor behavior may reflect the severity of tissue “damage” (Figure 4). Overall, we propose that the identity of stem/progenitor cells depends upon the tissue context, and that there is no single cell population that can account for all stem cell properties within the prostate epithelium.

Unlike other known contexts in which adult prostate basal cells can generate luminal cells, the surrounding stromal tissue is not profoundly affected in our epithelial

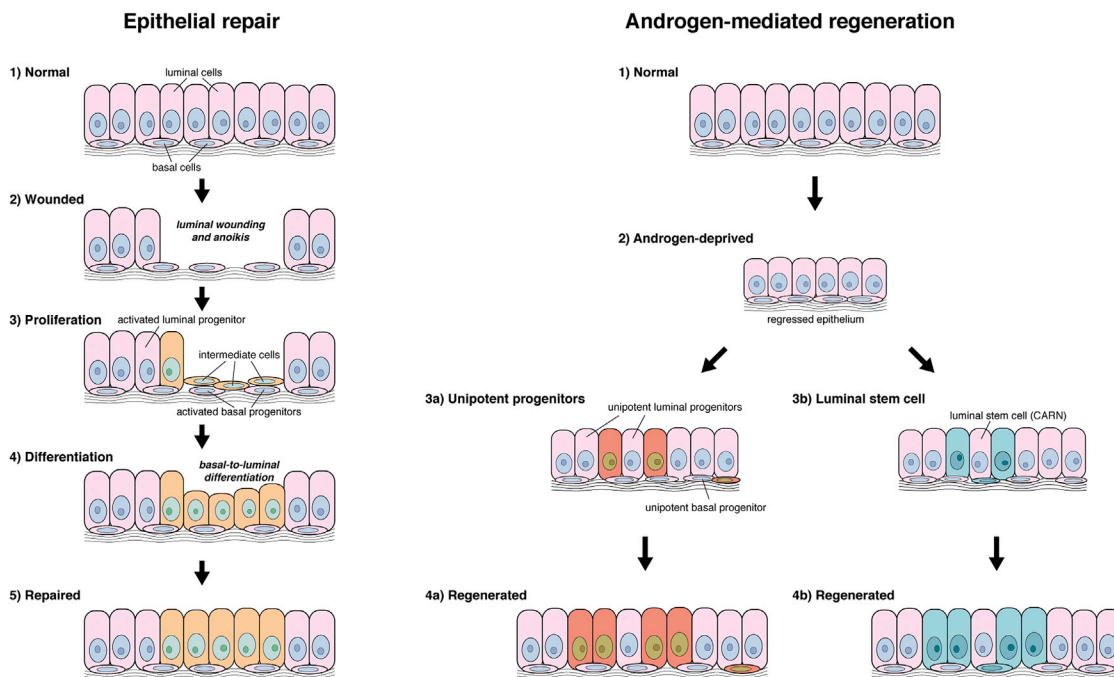


Figure 4. Assay Dependence of Stem/Progenitor Activity in the Prostate Epithelium

Schematic depiction of tissue repair and androgen-mediated regeneration of the prostate epithelium; newly generated cells are indicated by different colors. During tissue repair, luminal epithelial cells are primarily generated by a process of basal proliferation and basal-to-luminal differentiation in which “intermediate” cells represent a transitional state; in addition, there is activity of unipotent luminal progenitors. During androgen-mediated regeneration, tissue growth is largely mediated by unipotent luminal and basal progenitors, with a significant contribution by bipotential luminal stem cells (castration-resistant *Nkx3.1*-expressing cells [CARNs]).

repair model. For example, basal-to-luminal differentiation occurs in sphere formation assays in the absence of stromal tissue, as well as in tissue recombinant grafts with heterologous inductive mesenchyme (Burger et al., 2005; Goldstein et al., 2008, 2010; Hofner et al., 2015; Lawson et al., 2007; Richardson et al., 2004; Wang et al., 2013). Furthermore, a model of bacterial prostatitis also displays acute inflammation and stromal reactivity, which was suggested to be responsible for altered basal cell properties (Kwon et al., 2014b). These previous observations were consistent with the notion that the stroma represents an important niche for basal progenitors. In contrast, basal-to-luminal differentiation occurs in our E-cadherin deletion model when there is no alteration of the surrounding stroma, suggesting that there are signals intrinsic to the prostate epithelium that can promote basal cells to participate in tissue repair. It will be interesting in future studies to determine the identity of such signals that activate basal cell proliferation and differentiation.

Our findings potentially reconcile studies of prostate epithelial stem/progenitor activity with work on injury/repair models in other epithelial tissues (Blanpain and Fuchs, 2014). For example, basal cells can form luminal

secretory and ciliated cell types in the lung airway epithelium after treatment with noxious chemicals (Hogan et al., 2014), and basal cells in the bladder urothelium can generate intermediate and luminal umbrella cells after bacterial or chemical injury (Shin et al., 2011). Thus, our findings contribute to a unifying perspective for a role of basal cells in injury repair of a range of epithelial tissues. Finally, we suggest that the inherent plasticity of prostate basal cells that can be observed in pathological conditions and in cancer reflects latent progenitor activity that normally functions in tissue repair.

EXPERIMENTAL PROCEDURES

Mouse Procedures

Experiments using mice were performed according to protocols approved by the Institutional Animal Care and Use Committee at Columbia University Medical Center. The *Nkx3.1^{CreERT2}* allele was generated previously in our laboratory (Wang et al., 2009), the *Cdh1^{lox}* allele was obtained from the Jackson Laboratory Induced Mutant Resource (B6.129-*Cdh1^{tm2Kcm}/J*) (Boussadia et al., 2002), and the *R26R-YFP* reporter (Srinivas et al., 2001) was kindly provided by Dr. Frank Costantini. For induction of Cre activity, tamoxifen (Sigma catalog #T5648) dissolved in corn oil was



introduced by oral gavage (40 mg/kg) for 4 consecutive days to mice at 2–3 months of age. See also [Supplemental Experimental Procedures](#).

Histology and Immunostaining

Individual prostate lobes were fixed in 10% formalin followed by paraffin embedding, and immunostaining was performed on 5- μ m sections. H&E staining was performed using standard protocols. See also [Supplemental Experimental Procedures](#).

Quantitation and Statistical Analyses

For quantitation of immunofluorescence staining, 63 \times images from a Leica TCS2 AOBS spectral confocal microscope were used for manual counting of the number of cells with the indicated expression patterns. For statistical comparisons in [Figure 3](#), percentages were analyzed by an unpaired t test or one-way ANOVA and Dunnett's multiple comparison test using Prism 6 software (GraphPad).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and five tables and can be found with this article online at <http://dx.doi.org/10.1016/j.stemcr.2016.03.007>.

AUTHOR CONTRIBUTIONS

A.M. initiated mouse and immunostaining experiments, and R.T. performed quantitative analyses and completed experiments for publication. R.T., A.M., and M.M.S. designed experiments, analyzed data, and wrote the manuscript.

ACKNOWLEDGMENTS

We thank Cory Abate-Shen, Maho Shibata, and Flaminia Talos for insightful comments on the manuscript. This work was supported by NIH R01DK076602. A.M. and R.T. were supported by postdoctoral fellowships from the DOD Prostate Cancer Research Program.

Received: May 21, 2015

Revised: March 21, 2016

Accepted: March 22, 2016

Published: April 21, 2016

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