Multipotent and unipotent progenitors contribute to prostate postnatal development

Marielle Ousset¹,⁶, Alexandra Van Keymeulen¹,⁶, Gaëlle Bouvencourt¹, Neha Sharma¹, Younes Achouri², Benjamin D. Simons³,⁴ and Cédric Blanpain¹,⁵,⁷

The prostate is a glandular epithelium composed of basal, luminal and neuroendocrine cells that originate from the urogenital sinus during embryonic development. After birth, the prostate keeps developing until the end of puberty. Here, we used inducible genetic lineage tracing experiments in mice to investigate the cellular hierarchy that governs prostate postnatal development. We found that prostate postnatal development is mediated by basal multipotent stem cells that differentiate into basal, luminal and neuroendocrine cells, as well as by unipotent basal and luminal progenitors. Clonal analysis of basal cells revealed the existence of bipotent and unipotent basal progenitors as well as basal cells already committed to the luminal lineage with intermediate cells co-expressing basal and luminal markers associated with this commitment step. The existence of multipotent basal progenitors during prostate postnatal development contrasts with the distinct pools of unipotent basal and luminal stem cells that mediate adult prostate regeneration. Our results uncover the cellular hierarchy acting during prostate development and will be instrumental in defining the cellular origin and the mechanisms underlying prostate cancer initiation.

The prostate is a secretory gland surrounding the urethra at the base of the bladder that produces approximately 30% of the seminal fluid providing nutrients, ions and enzymes necessary for the survival of the spermatozoids during their journey through the female reproductive tract. Whereas the human prostate is organized into one gland containing a central, transitional and peripheral zone, the mouse prostate consists of four pairs of lobes surrounding the urethra that present distinct patterns of branching and histological appearance¹,². The adult prostate is a pseudo-stratified epithelium composed of three main cell lineages³,⁴: basal cells express basal keratin K5, K14 and the key transcription factor p63 (ref. 5); luminal or secretory cells express K8, K18, androgen receptor and the secretory proteins such as prostate-specific antigen and prostate acid phosphatase⁵; and neuroendocrine cells express synaptophysin and chromogranin A as well as neuropeptides³,⁴. Cells co-expressing markers of both basal and luminal cells, such as the co-expression of K5/K14 and K8/K18/K19, and therefore called intermediate cells, have been reported in mouse and human developing and adult prostate, and are thought to represent either multipotent prostate stem cells or intermediate cells between basal stem cells and luminal progenitors⁷–¹¹.

The prostate arises relatively late during mouse embryonic development from the endoderm anterior urogenital sinus (UGS). It is first visible by the presence of buds in the urogenital sinus epithelium at embryonic day (E)17.5 (refs 12,13). As for many other epithelial appendages, prostate development depends on epithelial to mesenchymal interactions¹⁴,¹⁵. Classical tissue recombination studies, confirmed by genetic experiments, have demonstrated that the first signal necessary for prostate development arises from the underlying mesenchyme in an androgen-dependent manner, and androgens also act later in prostate epithelial cells to sustain their growth and differentiation as well as to promote the survival of most adult luminal cells¹⁶,¹⁷. The initial buds, which correspond to solid epithelial outgrowth, invade the underlying mesenchyme and undergo multiple rounds of branching events and canalization, leading to the pseudostratified appearance of the prostate epithelium. At birth, the main ducts of the ventral prostate have already undergone 2–3 branching events and during the first two weeks of postnatal life, most branching has already occurred and the ductal branching is complete within 60–90 days¹⁸. Morphogenesis of the dorsolateral prostate is slightly delayed in comparison with the growth of the main ducts: branching occurs during the first two weeks, but the terminal differentiation of the secretory epithelium is complete only after the first month¹⁸.

Although the prostate development and structure have already been extensively studied, the lineage hierarchy that governs prostate...
basal, luminal and neuroendocrine cells during early postnatal development. (a) Confocal imaging of immunostaining of K8 (red) and K5 (green) in the prostate gland at birth (P1). (b) Scheme summarizing the genetic strategy used to target YFP expression in K14-expressing cells at birth (P1). (c) Scheme summarizing the protocol used to study the fate of K14 basal cells during early prostate development. (d–h) Immunostaining of K5 (d,g), K8 (e,h) or p63 (f) (red) and YFP (green) 3 days (d–f) or 4 weeks (g,h) after doxycycline injection at birth. (i) Percentage of YFP+ cells in K5+ and in K5−K8+ cells 3 days and 4 weeks after doxycycline injection at birth in K14rtTA/TetOCRE/RosaYFP mice. (j–l), Immunostaining of Nkx3-1 (g), androgen receptor (AR; k) or synaptophysin (Syp; l) (red) and YFP (green) 4 weeks after doxycycline injection at birth. Scale bars, 10 µm. Histograms and error bars represent the mean and s.e.m. (n = 3). lu, lumen. See Supplementary Table S1 for further data.

postnatal development remains elusive. It is unclear whether the prostate develops from multipotent or distinct classes of unipotent progenitors. p63 is the key transcription factor required for the development of numerous epithelia. p63 is expressed in the basal prostate cells and p63-null mice do not develop a prostate, suggesting that both basal and luminal cells arise from basal p63+ progenitors. However, embryonic UGS mice could differentiate into luminal secretory cells and neuroendocrine cells but not into basal cells, suggesting that basal cells were not required for the generation of a prostate-like tissue in transplantation assays. Chimaeric embryonic studies between wild-type and p63-deficient embryonic stem cells demonstrated that prostate epithelium including basal and luminal cells was derived only from p63+ cells, suggesting that, either during normal embryonic development, all prostate cells arise from p63+ basal progenitors, or that p63+ embryonic prostate progenitors present a strong selective advantage in generating prostate epithelium compared with p63-deficient cells.

Androgen deprivation through castration induced the regression of adult prostate, by the preferential apoptosis of luminal cells, which can regenerate a new fully developed prostate on androgen re-administration. The massive renewing capacities of the prostate, as demonstrated by its ability to undergo multiple cycles of regression/regeneration, demonstrate the presence of castration-resistant stem cells within the adult prostate epithelium. Different models have been proposed to account for the regeneration of the adult prostate after castration. One model suggests that the prostate regenerates from the proliferation and differentiation of multipotent stem cells. Transplantation experiments showed that basal cells are able to regenerate prostate tissue including basal and luminal cells when transplanted together with rat embryonic UGS mesenchyme under the kidney capsule of immunodeficient mice. A single ScAl+ /CD133+/CD44+/CD117+ basal cell can regenerate prostate tissue on transplantation, supporting the existence of adult basal multipotent prostate stem cells. Another model suggests that both basal and luminal stem cell pools are maintained and regenerated independently. Recent studies of lineage tracing indeed showed the presence of castration-resistant basal and luminal unipotent progenitors during prostate regeneration, whereas the presence of a very small proportion (<1%) of bipotent luminal progenitors has been described only in one report.

Although transplantation and castration studies are important to define the resistance to androgen deprivation as well as the differentiation potential of stem cells, these assays mimic a regenerative state that may affect the lineage hierarchy operating under physiological conditions. In addition, the relevance of the regression/regeneration and transplantation experiments to prostate development is unknown at present. Here, we used a lineage-tracing approach in mice to investigate the cellular hierarchy that contributes to prostate postnatal development under physiological conditions.

RESULTS

Basal cells give rise to basal, luminal and neuroendocrine cells during postnatal development

During the first day of postnatal prostate development, the prostate rudiment is composed of epithelial buds that co-express basal keratins (K5 and K14) and luminal keratins (K8/18 and K19). In the main ducts that already presented a lumen, there is a clearer separation between the outer layer of cells expressing basal markers and the inner layers expressing luminal markers, although the luminal keratins were also expressed in the outer layer at a lower level (Fig. 1a).

To determine the role of basal cells during postnatal prostate development, we first assessed the contribution of K14-derived cells during the first stage of prostate development (from birth to the beginning of puberty), which is marked by a major increase in the number of prostate epithelial cells. To this end, we performed inducible genetic lineage tracing of K14-expressing cells during the earliest step of postnatal development (Fig. 1b,c). Administration of a low dose of doxycycline at postnatal day (P)1 to K14rtTA/TetOCRE/RosaYFP mice induced YFP expression in about 30% of the outer basal layer expressing high levels of K5 and low level of K8 (Fig. 1d,i) and not in the inner luminal cells expressing high levels.
of K8 and negative for K5/K14 (Fig. 1e). We assumed that the 30% of labelled basal cells with this dose are representative of the whole population of basal cells. This dose allows us to study whether these labelled cells expand, self-maintain or decrease over time. YFP-targeted cells expressed the previously described basal prostate stem cell markers, p63 and Sca1, in the proximal region of the prostate (Fig. 1f and data not shown). Interestingly, four weeks after the initial labelling of K14+ basal cells, YFP-expressing cells expanded considerably and comprised around 60% of both K5+ and K8+ luminal cells, including cells expressing Nkx3-1, a previously described luminal stem cell marker, and differentiated luminal cells expressing androgen receptor (Fig. 1g–k). To determine the contribution of K14+ basal cells to the neuroendocrine lineage, we assessed the frequency of YFP expression in neuroendocrine cells. The overall frequency of neuroendocrine cells within the prostate epithelial cells is very low (around 0.4%). We found that 25% neuroendocrine cells (19/76) were YFP+, in good accordance with the proportion of basal cells initially labelled, demonstrating that during physiological postnatal development, neuroendocrine cells arise from basal progenitors (Fig. 1i,l), consistent with the targeting of multipotent basal progenitors expressing K14.

At this stage of prostate development, most basal prostate cells co-expressed K5 and K14, although a small fraction of the basal cells expressed K5 but were negative for K14 (Supplementary Fig. S1a), suggesting that basal cells are a heterogeneous population. To investigate whether there is functional heterogeneity within the basal cells during early postnatal prostate development, we performed inducible lineage tracing using K5CREER/RosaYFP mice and analysed the fate of K5-targeted basal cells during postnatal prostate development (Supplementary Fig. S1b,c). Tamoxifen administration to newborn K5CREER/RosaYFP mice induced YFP expression in 10% of basal K5+ prostate epithelial cells and not in K8+ luminal cells (Supplementary Fig. S1d,e,i). Four weeks after tamoxifen administration, YFP expression was observed in basal and luminal cells (Supplementary Fig. S1f,g,i), confirming the presence of multipotent basal progenitors that contribute to the expansion of the basal as well as luminal cell lineages. K5 lineage tracing also led to the labelling of 18% of neuroendocrine cells (10/54) (Supplementary Fig. S1h). However, in contrast to K14 lineage tracing, the expansion of basal marked cells was much greater than the expansion of luminal cells (Supplementary Fig. S1l), which is seen by the numerous isolated basal marked cells (Supplementary Fig. S1l), and the relative paucity of luminal clones, suggesting that, at this stage of prostate development, K5+ basal cells contained both multipotent and unipotent basal progenitors. As tamoxifen administration slightly and transiently delayed the postnatal development, it is possible that the difference between the K14 and K5 lineage tracing results from a slight delay in the prostate luminal development induced by tamoxifen administration.

As the different prostate lobes do not develop synchronously, we investigated the fate of K14rtTA/TetOCRE/RosaYFP labelled cells at two weeks of age separately in the different prostate lobes. Doxycycline administration to K14rtTA/TetOCRE/RosaYFP mice resulted in the labelling of K5+ cells in the anterior (A; i,j), the dorsolateral (DL; k,l) or the ventral lobes (V; m,n) 4 weeks after doxycycline injection in 2-week-old mice. (o) Percentage of YFP+ cells in K5+ and in K8+ cells in the anterior, dorsolateral and ventral lobes 4 weeks after doxycycline injection in 2-week-old K14rtTA/TetOCRE/RosaYFP mice. (p) Percentage of K8+ cells in K14+ and in K8+ cells in the anterior, dorsolateral and ventral lobes in 5-week-old mice. Histograms represent the mean (n = 2). Scale bars, 10 μm. A, lumen. See Supplementary Table S2 for further data.
Figure 3  Multipotent and unipotent progenitors revealed by clonal analysis. (a) Scheme summarizing the protocol used to perform clonal analysis of basal prostate cells during postnatal development. (b–f) Confocal analysis of immunostaining of K5 and YFP three days (b) and three weeks (c–f) after doxycycline injection in K14rtTA/TetOCRE/RosaYFP mice at birth. These data show the presence of bipotent clones (arrowheads in c and d), unipotent basal clones (dashed arrow in c and arrowhead in e) and unipotent luminal clones (arrows in c and arrowhead in f). Scale bars, 10 μm. (g) Percentage of basal cells with different fates (clones containing one or more basal or luminal cells, and bipotent clones) at 10 days and three weeks after doxycycline administration. The number of clones counted is 126 and 204 respectively for the analyses at 10 days and three weeks. (h) Distribution of the clone size at 10 days and three weeks after doxycycline administration. For both time points, the first column relates to the basal content of both unipotent and mixed basal/luminal cell clones; the second column relates to the size of clones that contain only luminal cells; and the third column relates to the total size of clones including both basal and luminal cells. The number of clones counted is the same as in g. See Supplementary Table S3 for further data.

Basal cells contain multipotent and unipotent progenitors

The ability of basal progenitor cells to give rise to cells of basal, luminal and neuroendocrine fate suggests that either basal cells are truly multipotent and able to give rise at the clonal level to all of the different prostate cell lineages or that basal cells contain multiple types of unipotent progenitor cell already committed to a specific lineage. To discriminate between these two possibilities, we performed clonal analysis by decreasing the dose of doxycycline, so as to label only isolated basal cells and follow the fate of individual marked cells over time by confocal analysis of serial thick sections (Fig. 3a).

Administration of a single dose of doxycycline in 1-day-old K14rtTA/TetOCRE/RosaYFP mice resulted in the labelling of 4% of basal cells, which are separated from each other by around 10 unlabelled cells (Fig. 3b). The analysis of YFP-expressing cells 3 weeks after doxycycline administration demonstrated that the existence of several types of clone consisting of single basal cells, multiple basal cells alone, single luminal cells, multiple luminal cells alone and bipotent clones (Fig. 3c–h). Owing to their relative scarcity, it was not possible to reliably assess the neuroendocrine composition of clones. Surprisingly, at 3 weeks post-induction, most (67%) clones consisted of purely luminal cells without any basal cells (Fig. 3g), suggesting that, at P1, the basal cell compartment contains cells that are already pre-committed to luminal fate. Furthermore, at this time point, around 10% of the clones were truly bipotent, composed of basal and luminal YFP-expressing cells, whereas the remaining 23% were purely basal (Fig. 3g).

To assess the potential heterogeneity of the basal cell compartment at P1, we developed a more quantitative analysis of the clonal fate data to look for signatures of proliferative potential and cell fate choice. Quantification of the total number of cells within each clone revealed a broad spectrum of sizes (Fig. 3c,h). In particular, focusing on clones containing only luminal cells and therefore derived from cells committed to the luminal lineage, the broad distribution of clone sizes is well approximated by a simple exponential with an average clone size of around 6.9 ± 0.6 cells (Supplementary Fig. S3b). Such behaviour is consistent with a single progenitor cell population following a simple pattern of symmetrical cell duplication, with a broad (Poisson) distribution of cell cycle times with an average of around 1.7 weeks (Supplementary Note and Figs S3 and S4).

Although the clonal fate data are consistent with the existence of a heterogeneous population of basal cells containing pre-committed
unipotent luminal progenitors, unipotent basal progenitors and bipotent progenitors (Fig. 3c,g), the presence of bipotent clones alongside purely basal clones may equally result from an equipotent basal progenitor that chooses stochastically between symmetric cell duplication (giving rise to two basal progenitors) and asymmetric cell division (giving rise to one basal and one luminal progenitor). Indeed, if we assume that basal progenitors have equal probability of choosing between symmetric and asymmetric cell fate, we obtain an excellent fit to both the basal-alone and total clone size distributions at 3 weeks post-induction if we again take the distribution of cell cycle times of the basal progenitors to be broad (Poisson distributed) with an average of 3.1 weeks, almost twice that of luminal progenitors (Supplementary Note and Fig. S3b). Significantly, with the same (three) fitting parameters (the two division rates and the balance between symmetric and asymmetric fate), the agreement between measurements of clone size and composition at 10 days post-induction and the model dynamics is equally good (Supplementary Theory and Fig. S3b). Moreover, the results of this analysis are further supported by measurements of continuous 5-bromodeoxyuridine (BrdU) incorporation, which suggest a luminal progenitor proliferation rate a factor of 1.5 higher than basal cells in week-old mice (Supplementary Note and Fig. S4).

However, although the lineage tracing measurements provide evidence for just two progenitor cell populations in the developing prostate, organized in a hierarchy, our clonal analysis cannot unequivocally rule out further proliferative heterogeneity in the basal cell compartment. Indeed, our model does not take into account apoptosis that may be important for the canalization of the ducts and does not address the likely influence of spatial heterogeneity in the cell dynamics, resulting from the branching and the formation of new ductal structures at this early stage of development.

Intermediate cells are associated with luminal commitment
Intermediate cells are basal cells that co-express K5 and K8, which represent about 15% of basal cells (108/695; Fig. 4a,b). To determine the contribution of intermediate cells to luminal differentiation of basal bipotent cells, we assessed the frequency of intermediate cells in K14 clonal analysis experiments. Whereas intermediate cells were not found in unipotent basal clones, about 25% of bipotent clones contained intermediate cells 4 weeks after doxycycline administration in 2-week-old mice (Fig. 4c–f), suggesting that intermediate cells are strongly associated with luminal differentiation of basal cells. The absence of intermediate cells in some bipotent clones suggests...
that either some basal cells give rise to luminal cells without passing through an intermediate cell stably co-expressing K5 and K8, or that K5 and K8 expression is dynamically regulated and the co-expression of K5 and K8 occurred only transiently during luminal differentiation of basal cells.

Unipotent luminal progenitors contribute to luminal lineage expansion during postnatal development

To determine whether the luminal compartment also contains progenitors that contribute to prostate postnatal epithelial expansion, we performed inducible lineage tracing of luminal cells using the K8CREER/RosaYFP protocol used to study the fate of K8 luminal prostate cells during late postnatal development. (c,d) Immunostaining of K5 (c) or K8 (d) (red) and YFP (green) 1 week after tamoxifen injection in 2-week-old mice. (e–j) Immunostaining of K5 (e,g,i) or K8 (f,h,j) (red) and YFP (green) in the anterior (A; e,f), the dorsolateral (DL; g,h) or the ventral (V; i,j) lobes 4 weeks after tamoxifen injection in 2-week-old mice. (k) Percentage of YFP+ cells in K5+ and in K8+ cells 4 weeks after tamoxifen injection in 2-week-old K8CREER/RosaYFP mice in the anterior, the dorsolateral lobes and the ventral lobes. (l) Immunostaining of K5 (red) and YFP (green) 4 weeks after tamoxifen injection in 2-week-old K8CREER/RosaYFP mice in the anterior lobes of the prostate. Most YFP cells are luminal cells but K8CREER can also label the intermediate K5+K8+basal cells (arrowhead). Scale bars, 10 μm. Histograms represent the mean (n=2). See Supplementary Table S6 for further data.

DISCUSSION

The various lineage tracing experiments of basal and luminal cells performed at different stages of postnatal prostate development demonstrate the existence of multipotent basal progenitors able to differentiate into basal, luminal and neuroendocrine cells, and unipotent luminal progenitors, which together contribute to the massive epithelial expansion that arises during prostate postnatal development. Whether the basal population supports unipotent cells, or whether unipotent clones are the outcome of the stochastic fate of bipotent basal cells, as suggested by the clonal analysis, remains an open question (Fig. 8). Our lineage tracing experiments in mice are consistent with the results of clonal analysis of human prostate that showed the presence of bipotent clones containing basal and luminal Cox2-deficient cells, as well as unipotent basal or luminal clones of Cox2-deficient cells.

The existence of multipotent basal stem cells that contribute to both basal and luminal expansion during postnatal development contrasts with the presence of basal and luminal unipotent stem cells that...
Prostate cancer is the second leading cause of cancer in men. The most frequent prostate cancer is acinar adenocarcinoma, but different specific genetic abnormalities have been found in human prostate cancers including PTEN and/or p53 deletion, loss of Nkx3-1 and genetic translocations such as TMPRSS2-ERG. Different pieces of evidence suggest that both basal and luminal lineages may be involved in prostate tumour initiation. Transplantation of genetically modified basal cells can initiate prostate cancer\(^6\). On the other hand, deletion of PTEN and/or p53 in luminal cells using probasinCRE (refs 36,37), PSACREER (refs 38), Nkx3-1CREER (ref. 29) and K8CREER (ref. 31) induced prostate cancer, demonstrating the ability of luminal cells to initiate prostate cancer on PTEN deletion. A recent study showed that deletion of PTEN in basal cells using K5CREER does not result in advanced prostate cancer as found on PTEN deletion in luminal cells, but can induce prostate intraepithelial neoplasia possibly through the differentiation into luminal intermediates\(^31\). Clearly, more studies are needed to determine whether the different types of prostate cancer arise from the same tumour-initiating cells, whether basal and luminal cells respond differently to different oncogenes and tumour suppressor genes, and whether tumour suppressor gene deletion in multipotent and unipotent progenitors during prostate postnatal development influences prostate cancer initiation and progression.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**ACKNOWLEDGEMENTS**

We thank our colleagues who provided us with reagents, which are cited in the text. We thank our colleagues from the Blanpain laboratory and C. Govaerts for their comments on the manuscript and J-M. Vandesande and F. Bollet-Quivogne for their help with confocal imaging. C.B. and A.V.K. are chercheur qualifié, M.O. is collaborateur scientifique of the FRS/FNRS. C.B. is an investigator of WELBIO. This work was supported by the FNRS, TELEVIE, the program d’excellence CIBLES of the Wallonia Region, a research grant from the Fondation Contre le Cancer, the ULB fondation, the fond Gaston Ithier, a starting grant of the European Research Council (ERC) and the EMBO Young Investigator Program.

**AUTHOR CONTRIBUTIONS**

M.O., A.V.K. and C.B. designed the experiments and performed data analysis. M.O. and A.V.K. performed most of the experiments. B.D.S. performed mathematical...
modelling and data analysis. Y.A. generated K8CREER transgenic mice. G.B. and N.S. provided technical support. M.O. and A.V.K. prepared the figures. C.B. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at www.nature.com/doifinder/10.1038/ncb2600
Reprints and permissions information is available online at www.nature.com/reprints

METHODS

Mice. RosaYFP (ref. 39) mice were obtained from The Jackson Laboratory. K14rtTA transgenic mice32 were provided by E. Fuchs, The Rockefeller University, USA. TetOCRE mice41 were provided by A. Nagy, Lunenfeld Research Institute, Canada. The generation of K5CREER, K8CREER and K18CREER mice was described previously32,41. Mice colonies were maintained in a certified animal facility in accordance with European guidelines. The experiments were approved by the local ethical committee (CEBEA).

Targeting YFP expression. For lineage tracing induced at birth, K5CREER/RosaYFP and K8CREER/RosaYFP newborn mice were induced with 1.25 mg tamoxifen (50 μl of 25 mg ml−1 solution; Sigma; diluted in sunflower seed oil, Sigma) and K14rtTA/TetOCRE/RosaYFP were induced with 0.25 mg doxycycline hyclate (Sigma; 50 μl of 5 mg ml−1 solution diluted in sterile PBS) or with 0.005 mg (50 μl of 0.1 mg ml−1 solution diluted in sterile PBS) for clonal analysis, by intraperitoneal injection. For lineage tracing induced at 2 weeks, K5CREER/RosaYFP, K8CREER/RosaYFP and K18CREER/RosaYFP mice were induced with 1 mg tamoxifen and K14rtTA/TetOCRE/RosaYFP with 1 mg doxycycline by intraperitoneal injection.

Histology and immunostaining. Dissected prostates were pre-fixed for 2 h in 4% paraformaldehyde at room temperature. Tissues were washed three times with PBS for 5 min and incubated overnight in PBS/30% sucrose at 4 °C. Tissues where then embedded in OCT and kept at −80 °C. Sections of 5 μm were cut using a HM560 Microm cryostat (Mikron Instruments Inc). Immunostaining was performed as previously described32.

Antibodies. The following primary antibodies were used: anti-GFP (rabbit, 1:1,000, A11122, Molecular Probes), anti-GFP (chicken, 1:4,000, ab13970, Abcam), anti-K8 (rat, 1:500, Tromal, Developmental Studies Hybridoma Bank, University of Iowa), anti-K5 (rabbit, 1:1,000, PRB-160P, Covance), anti-K14 (chicken, 1:1,000, SIG-3476, Covance), anti-synaptophysin (rabbit, 1:500, 18-0130, Invitrogen), anti-androgen receptor (rabbit, 1:500, clone EP670Y, 1852-S, Epitomics), p63 (mouse, 1:100, Clone 4A4, 306-05, Diagnostic BioSystems), anti-Ki67 (rabbit, 1:400, ab15580, Abcam), anti-BrdU (rat, 1:50, clone BU1/75, ab6326, Abcam), Nkx3-1 (provided by M. M. Chen, Columbia University, New York, USA). The following secondary antibodies were used: anti-rabbit, anti-rat and anti-chicken conjugated to AlexaFluor488 (Molecular Probes), to rhodamine Red-X or to Cy5 (JacksonImmunoResearch). Nuclei were stained in Hoechst solution and slides were mounted in DAKO mounting medium supplemented with 2.5% Dabco (Sigma).

Microscope image acquisition. Images of immunostaining were acquired using 40× Zeiss EC Plan-NEOFLUAR objectives. Images were acquired on an Axio Observer Z1 microscope, with an AxioCamMR3 camera and using Axiovision software (Carl Zeiss). Confocal images were acquired at room temperature using a Zeiss LSM780 multiphoton confocal microscope fitted on an Axiovirt M200 inverted microscope equipped with C-Apochromat (×40, NA = 1.2) water-immersion objectives (Carl Zeiss). Optical sections 1.024 × 1.024 pixels were collected sequentially for each fluorochrome. The data sets generated were merged and displayed with the ZEN software.

Quantification of YFP+ cells. The percentage of YFP+ cells in K5+ cells and in K8+ cells was counted. The number of mice analysed for each time point was at least three and the number of counted cells is described in Supplementary Tables S1, S2, S4–S8 and S11.

Analysis of cell proliferation. Cell proliferation was analysed by immunofluorescence microscopy after pulsing P1 and 2-week-old mice with BrdU (50 mg kg−1) twice daily for 1, 3 or 7 days. The number of mice analysed for each time point was three and the number of counted cells is described in Supplementary Table S9.

Figure S1. K5CreER/RosaYFP lineage tracing during early prostate postnatal development. a, Confocal analysis of K5 (red) and K14 (green) immunostaining of prostate gland at birth (P1). Most basal cells co-expressed K5 and K14. A small fraction of basal cells expressed K5 but were negative for K14, while no K14 positive K5 negative cells were observed.

b, Scheme summarizing the genetic strategy used to target YFP expression in K5 expressing cells at birth.

c, Scheme summarizing the protocol used to study the fate of K5 basal prostate epithelial cells during early postnatal development. d-h, Immunostaining of K5 (d, f), K8 (e, g) or Syp (h) (red) and YFP (green) 3d (d, e) or 4w (f-h) after TAM injection at birth in K5CreER/RosaYFP mice. i, Percentage of YFP+ cells in K5+ and in K8+ cells 3d and 4w after TAM injection at birth in K5CreER/RosaYFP mice. Scale bars, 10 µm. Histograms and error bars represent the mean and sem (n=3). See supplementary Table 8 for additional data.
Figure S2. K5CREER/RosaYFP lineage tracing during late prostate postnatal development. a, Scheme summarizing the genetic strategy used to target YFP expression in K5 expressing cells in 2 weeks old mice. b, Scheme summarizing the protocol used to study the fate of K5+ basal prostate cells during late postnatal development. c-g, Immunostaining of K5 (c, e), K8 (d, f) or Syp (g) (red) and YFP (green) 1w (c, d) or 4w (e-g) after TAM injection in 2w old mice. h, Percentage of YFP+ cells in K5+ and in K8+ cells 1w and 4w after TAM injection in 2w old K5CREER/RosaYFP mice. Scale bars, 10 μm. Histograms and error bars represent the mean and sem (n=3). See Supplementary Table 9 for additional data.
Figure S3 Clone size distribution.

Clone size distribution following the induction of K14rtTA/TetOCRE/RosaYFP mice at P1 and chased for (a) 10 days and (b) 3 weeks. Following induction, the resulting clones show both unipotency and mixed lineage. To capture this behavior, the data for both time points have been fractionated into three: the top panel of (a) and (b) shows the size distribution focusing on the basal content of all clones involving at least one basal layer cell (points). The line shows the prediction of the model dynamics with division rates and differentiation probabilities defined in Supplementary Note. The middle panel of (a) and (b) shows the clone size distribution of clones comprised entirely of cells in the luminal layer (points). Finally the bottom panel of (a) and (b) show the total clone size distribution, independent of cell type (points). Error bars denote s.e.m. Note that, at 3 weeks post-labelling, both the basal and luminal clones follow an approximately exponential distribution, with averages specified in Supplementary Note.
Figure S4 BrdU incorporation during prostate postnatal development. a, Percentage BrdU+ cells in K5+ and in K5-K8+ cells after continuous BrdU administration twice per day starting at birth until 1, 3 or 7 days later. b, Percentage BrdU+ cells in K5+ and in K5-K8+ cells after continuous BrdU administration twice per day starting in 2 week old mice until 1, 3 or 7 days later. Histograms and error bars represent the mean and sem (n=3). See Supplementary Table 10 for additional data.