Single luminal epithelial progenitors can generate prostate organoids in culture

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The intrinsic ability to exhibit self-organizing morphogenetic properties in *ex vivo* culture may represent a general property of tissue stem cells. Here we show that single luminal stem/progenitor cells can generate prostate organoids in a three-dimensional culture system in the absence of stroma. Organoids generated from CARNs (castration-resistant Nkx3.1-expressing cells) or normal prostate epithelia exhibit tissue architecture containing luminal and basal cells, undergo long-term expansion in culture and exhibit functional androgen receptor signalling. Lineage-tracing demonstrates that luminal cells are favoured for organoid formation and generate basal cells in culture. Furthermore, tumour organoids can initiate from CARNs after oncogenic transformation and from mouse models of prostate cancer, and can facilitate analyses of drug response. Finally, we provide evidence supporting the feasibility of organoid studies of human prostate tissue. Our studies underscore the progenitor properties of luminal cells, and identify *in vitro* approaches for studying prostate biology.

Despite the apparent simplicity of cell types in the prostate epithelium, there has long been a dearth of suitable cell culture-based systems for investigating prostate biology1. In the normal prostate, there are three epithelial cell types, corresponding to: luminal cells, which are columnar cells expressing cytokeratin (CK) 8, CK18 and high levels of androgen receptor (AR); basal cells, which express CK5 and p63; and rare neuroendocrine cells2. During prostate tumorigenesis, basal cells undergo progressive loss in pre-neoplastic lesions known as prostatic intraepithelial neoplasia (PIN), and are essentially absent in prostate adenocarcinoma, which typically has a luminal phenotype3,4.

Historically, prostate luminal cells have been difficult to grow in culture, which has hindered the establishment of cell lines from normal or transformed prostate epithelium. One approach to circumvent this limitation has been culture of three-dimensional 'prostaspheres' containing epithelial cells explanted from primary mouse or human prostate tissue5-8. Such prostaspheres can be serially passaged and used in assays for prostate epithelial stem cells and tumour-initiating cells9,10. However, prostaspheres typically originate from basal epithelial cells and fail to exhibit complete luminal differentiation in the presence of androgens9,11-13. Notably, prostaspheres fail to demonstrate strong nuclear AR expression in the presence of androgens or a functional response to androgen deprivation6,9.

Recent work has described alternative explant approaches for three-dimensional culture of epithelial cells in the absence of stroma. Such 'organoid' culture systems contain similar extracellular matrix components to those often used in sphere assays, but also use conditions that enhance the survival, proliferation and/or differentiation of stem/progenitor populations14. In particular, cultured stem cells of the mouse small intestine and colon15,16 can form organoids that exhibit normal epithelial architecture and serve as the basis for tissue repair17, and tumour organoids can be established from transformed colon as a model of colon adenocarcinoma18,19. Additional studies of organoids from intestine20, stomach22, liver22 and pancreas23,24 have demonstrated the general feasibility of this approach.

In previous studies, we identified a luminal epithelial stem/progenitor population known as CARNs (castration-resistant Nkx3.1-expressing cells), which are also cells of origin for prostate cancer25. We also showed that single CARNs can reconstitute prostate ducts in a renal grafting assay25. Below, we introduce an *ex vivo* culture system in the presence of androgens or a functional response to androgen deprivation.
system that can support the growth and serial passaging of epithelial organoids derived from CARNs or more generally from normal prostate epithelium. We show that these prostate organoids are primarily derived from luminal epithelial cells, and exhibit functional AR activity in culture. We demonstrate that mouse tumour organoids can model tumour phenotypes and drug response, and show that organoids can be established from benign human prostate tissue and a luminal prostate cancer cell line. Consequently, we propose that organoid culture represents an excellent system for investigating prostate biology and cancer.

RESULTS

Establishment of prostate epithelial organoids from CARNs

Previously, we identified a rare luminal epithelial population in the regressed prostate epithelium that has stem cell properties in vivo and in tissue reconstitution assays. To pursue further analyses of these CARNs, we sought to establish conditions for their isolation and successful propagation in culture. For this purpose, we surgically castrated adult male Nkx3.11CreERT2+/−; R26R-YFP/+ mice to induce androgen deprivation, followed by tamoxifen induction to lineage-mark CARNs (Fig. 1a). Following dissociation of prostate tissue into a single-cell suspension, we used flow-sorting to isolate CARNs based on their yellow fluorescent protein (YFP) expression (Fig. 1b).

To culture CARNs, we developed a protocol based in part on the importance of Matrigel in three-dimensional culture of prostate and mammary epithelium, hepatocyte medium for prostate epithelial cell culture, and ROCK inhibitor to improve the survival of dissociated epithelial cells. The resulting protocol involves low-percentage Matrigel floating culture in the presence of epidermal growth factor, heat-inactivated charcoal-stripped fetal bovine serum, which lacks androgens, and supplementation with dihydrotestosterone (DHT; Methods). Under these conditions, isolated CARNs formed epithelial ‘organoids’ that could grow for at least 3–4 weeks in culture (Fig. 1c), exhibiting a range of morphologies, and varying in size from 15 μm in diameter to greater than 0.5 mm in diameter. Importantly, most organoids were homogeneously composed of YFP-expressing cells, indicating their derivation from lineage-marked CARNs (Fig. 1d), and lacked stroma (Fig. 1e,f). Consistent with their growth in culture, many cells within organoids were positive for Ki67 (Fig. 1g). CARN-derived organoids typically exhibited an outer rim of cells positive for the basal marker CK5, and internal cells positive for the luminal marker CK8 (Fig. 1h); few ‘intermediate’ cells that co-express basal and luminal markers were observed. Notably, the organoids expressed nuclear AR (Fig. 1i), as well as nuclear Foxa1, a transcription factor that is essential for prostate organogenesis (Fig. 1j). Thus, lineage-marked CARNs are able to generate basal cells in organoid culture, similar to their ability in vivo and in tissue reconstitution assays.

To confirm that these organoids retained properties of prostate epithelium, we performed tissue reconstitution assays. CARN-derived organoids were recombined with urogenital mesenchyme from rat embryos, followed by implantation under the kidney capsule of immunodeficient mice. The resulting grafts exhibited prostate ductal structures (Fig. 1k) and expressed both basal (p63) and luminal (CK8) markers (Fig. 1l). Furthermore, the epithelial cells were completely YFP positive and expressed nuclear AR (Fig. 1m), indicating that the CARN-derived organoids could successfully reconstitute prostate tissue.

Establishment of prostate organoids from single CARNs

To determine the efficiency of organoid formation, we assessed the number of organoids formed after 7 days of culture. We found that the average efficiency of organoid formation by lineage-marked CARNs was 1.42% (Fig. 1n and Supplementary Table 1). For comparison, we also assayed non-YFP-expressing epithelial cells from the same mice used to isolate the lineage-marked CARNs. These non-YFP-expressing cells could also form organoids in culture, but at a nearly sixfold lower average frequency of 0.24% (Fig. 1n and Supplementary Table 1; Methods), suggesting that non-CARNs can also form organoids, but at a reduced efficiency.

Given their stem cell properties, we examined whether individual lineage-marked CARNs could form an organoid. To isolate single cells, we used flow cytometry to purify lineage-marked CARNs, and then mouth-pipetted single fluorescent cells into individual wells of a 96-well plate. We imaged each well to confirm plating of single cells, and followed their potential growth every other day (Fig. 1o). We found that organoids formed from 5 out of 300 individual lineage-marked CARNs, with an overall frequency (1.67%) similar to that after plating of CARNs as a population (1.42%).

Organoid formation by prostate epithelial cells from hormonally intact mice

As both CARNs and non-CARNs could form organoids, we investigated whether normal prostate epithelial cells could initiate organoid formation. For this purpose, we performed flow-sorting of dissociated prostate cells to remove non-epithelial EpCAM−E-cadherin− cells (Fig. 2a). The resulting organoids exhibited variable morphology and growth rates, suggesting heterogeneity in the starting population (Fig. 2b,c). Many organoids had a ductal structure resembling that of normal prostate, with a bi-layered epithelium surrounding a lumen (Fig. 2d), whereas other organoids contained multi-layered masses of cells (Fig. 2e). The organoids contained proliferating cells (Fig. 2f), and showed stratification into an outer basal layer and an internal luminal layer (Fig. 2g-i), with intermediate cells co-expressing luminal and basal markers rarely observed; neuroendocrine cells have not been detected so far. Furthermore, the organoids exhibited nuclear expression of AR and Foxa1 (Fig. 2j), and could reconstitute prostate tissue in grafts (Fig. 2k). Importantly, organoids derived from normal prostate epithelium could be grown for at least 13 passages, with no apparent alterations in growth rate or phenotype (Supplementary Fig. 1a–c), and could be frozen and thawed with no loss of viability.

After passaging, organoids continued to express luminal and basal markers, as well as nuclear AR, and were indistinguishable from low-passage-number organoids (Supplementary Fig. 1d,e).

Interestingly, the efficiency of organoid formation from normal hormonally intact prostate epithelium was 0.30%, significantly lower than from lineage-marked CARNs (Supplementary Table 1), which are isolated from the androgen-deprived regressed prostate. We also found that the efficiency of organoid formation from wild-type regressed prostate epithelium was 0.49%, which is not significantly different from that of hormonally intact epithelium. (Supplementary Table 1). These efficiencies are also similar to that of YFP-negative
Figure 1 Generation of prostate epithelial organoids from lineage-marked CARNs. (a) Time course of lineage-marking of CARNs in Nkx3.1<sup>CreERT<sub>2</sub></sup>:R26R–YFP/+ mice. (b) Isolation of YFP-positive lineage-marked CARNs by flow cytometry. (c,d) Bright-field (c) and epifluorescent (d) views of CARN-derived organoids that are filled or hollow (arrow). (e,f) Haematoxylin–eosin (H&E) staining of CARN organoids at low-power (e) showing a range of phenotypes, and at high-power (f). (g) Uniform YFP expression with Ki67 immunostaining (arrows). (h) The basal marker CK5 is expressed on the exterior (arrowheads), and the luminal marker CK8 is expressed internally. (i,j) Strong nuclear expression of AR (arrows, i) and Foxa1 (j). (k–m) Renal grafts generated by tissue recombination of CARN-derived organoids with rat embryonic urogenital mesenchyme (k) exhibit normal stratification of basal (arrowheads, l) and luminal cells (l), and uniform YFP and nuclear AR immunostaining (m); note that the slightly atypical histology in k probably reflects the heterozygous phenotype of Nkx3.1 mutants<sup>40,41</sup>. (n) Efficiency of organoid formation by lineage-marked CARNs (YFP-positive cells from tamoxifen-induced and castrated Nkx3.1<sup>CreERT<sub>2</sub></sup>:R26R–YFP/+ mice; n = 4 experiments) and non-CARNs (YFP-negative cells from the same mice; n = 3 experiments). Source data are provided in Supplementary Table 1. Error bars represent one standard deviation; the difference between CARNs and non-CARNs is statistically significant (**P < 0.002, two-tailed Student's t-test). (o) Generation of organoids from single CARNs. Time course of paired images shown under bright-field (top) and epifluorescent (bottom) illumination shows organoid growth from an isolated single CARN. Scale bars in o correspond to 25 μm, in f–j,l,m to 50 μm, and in c–e,k to 100 μm.
cells in the CARN lineage-marking experiment (Fig. 1n), suggesting that cells distinct from CARNs can form organoids, but at a lower efficiency.

To examine the effects of androgen deprivation, organoids established from normal prostate epithelium were cultured and passaged in the presence or absence of DHT. We found that organoids could still form in the absence of DHT, but were reduced in size (Fig. 2l,m). Notably, AR immunostaining was nuclear in the presence of DHT, but was weaker and mostly cytoplasmic in the absence of DHT (Fig. 2l,m). Notably, AR immunostaining was nuclear in the presence of DHT (Fig. 2l,m). Notably, AR immunostaining was nuclear in the presence of DHT (Fig. 2l,m). Notably, AR immunostaining was nuclear in the presence of DHT (Fig. 2l,m). Notably, AR immunostaining was nuclear in the presence of DHT (Fig. 2l,m). Notably, AR immunostaining was nuclear in the presence of DHT (Fig. 2l,m).

We next used lineage-tracing to investigate which epithelial cell type(s) can give rise to organoids (Fig. 3a). To mark basal cells, we used the tamoxifen-inducible CK5–CreERT2 transgene in combination with the R26R–YFP reporter allele. For marking of luminal cells, we used the CK8–CreERT2 or CK18–CreERT2 transgenes, either in combination with the R26R–YFP reporter or the R26R–Tomato reporter. Notably, these inducible Cre drivers were highly specific in marking basal or luminal epithelial cells in vivo at efficiencies similar to those previously observed (Supplementary Fig. 2 and Table 2).

Using tamoxifen-induced CK5–CreERT2; R26R–YFP mice (which we term CK5-trace), we isolated YFP-positive cells by flow cytometry for organoid culture (Fig. 3b). We found that the isolated CK5-trace cells were extremely inefficient at organoid formation (0.04% efficiency; Supplementary Table 1). Moreover, when organoids did not form in the presence of DHT, we performed quantitative real-time PCR analysis of expression of AR downstream genes in organoids cultured in the presence or absence of DHT. Results are from a single experiment representative of 2 independent experiments. All assays were performed using three technical replicates and normalized to GAPDH expression; Scale bars in e–j,n,o correspond to 50 μm, and in b–d,k–m to 100 μm.
Figure 3 Lineage-tracing shows that luminal cells are favoured for generation of prostate organoids. (a) Strategy for lineage-marking of basal and luminal epithelial cells for organoid culture. (b) Isolation of YFP-positive luminal cells from CK8-CreERT^2; R26R–YFP (CK8-trace) mice by flow cytometry. (c) CK5-trace organoid. (d) Many cells within a CK5-trace organoid are CK5 positive, including internal cells (arrowheads). (e) CK8-trace organoid. (f) CK18-trace organoid. (g) Efficiency of organoid formation from YFP-positive CK5-trace (n = 4 experiments), CK8-trace (n = 3 experiments) and CK18-trace (n = 2 experiments) epithelial cells. The differences in efficiency between CK5-trace and CK8-trace (P = 0.001) and between CK5-trace and CK18-trace (P = 0.00009) are statistically significant (*) using a two-tailed Student’s t-test; error bars correspond to one standard deviation. Source data are provided in Supplementary Table 1. (h,i) Expression of the basal marker CK5 (arrowheads) in a CK8-trace organoid, shown with (h) and without (i) a YFP overlay. (j,k) Expression of the basal marker p63 (arrowheads) in a CK8-trace organoid, shown with (j) and without (k) a YFP overlay. (l,m) Expression of the luminal marker CK18 in a CK8-trace organoid, shown with (l) and without (m) a YFP overlay. (n) Organoid generated from mixing of red CK18-trace cells and green CK5-trace cells shows green cells on the exterior, consistent with the localization of basal cells. (o) Serial passaging of CK18-trace organoids at passage 3. (p,q) CK18-trace organoids at passage 9 cultured in the presence (p) and absence (q) of DHT. (r,s) AR immunostaining is nuclear in CK18-trace organoids in the presence of DHT (r), but is weakly cytoplasmic in the absence of DHT (s). Scale bars in c-f, h-n correspond to 50 μm, and in o-s to 100 μm.

form, they were often heterogeneous, containing regions derived from non-YFP-expressing cells; for example, such organoids could arise from doublets containing a YFP-expressing and a non-expressing cell after flow sorting. The few homogeneously YFP-expressing CK5-trace organoids were small and contained both CK5-expressing and non-expressing cells (Fig. 3c,d).

In contrast, YFP-positive cells from tamoxifen-induced CK8-CreERT^2; R26R–YFP mice (CK8-trace) or CK18–CreERT^2; R26R–YFP mice (CK18-trace) gave rise to hollow organoids with large lumens (Fig. 3e,f), most of which were homogeneously YFP positive. Interestingly, the efficiency of organoid formation by luminal CK8-trace cells (0.22%) and CK18-trace cells (0.30%) was significantly higher than that of basal CK5-trace cells (Fig. 3g and Supplementary Table 1). In addition, the efficiency of organoid formation by CK8-trace or CK18-trace cells from castrated mice was similar (0.34%), consistent with the enhanced efficiency of CARNs relative to other
luminal cells in the regressed prostate (Supplementary Table 1). Thus, both basal and luminal cells can give rise to organoids, potentially explaining the heterogeneity of organoids from normal prostate epithelium (Fig. 2b,c), but luminal cells are favoured for organoid formation.

Notably, luminal cells could generate basal cells in organoid culture, as CK8-trace organoids with homogeneous YFP expression contained cells expressing basal markers (CK5, p63) (Fig. 3h–m). These basal cells were typically found on the outer layer of the organoids, as for normal organoids, but exhibited an irregular morphology that might suggest incomplete basal differentiation. To assess whether luminal cells would give rise to basal cells in the presence of normal basal cells, we mixed green CK5-trace cells from CK5–CreER<sup>T2</sup>; R26R–YFP mice with red CK8-trace cells isolated from CK18–CreER<sup>T2</sup>; R26R–Tomato mice. In the resulting cultures, we found organoids with an outer layer of green cells and inner red cells (Fig. 3n), suggesting that both basal and luminal cells are preferentially lineage-restricted, consistent with lineage-tracing analyses in vivo<sup>13,37,38</sup>.

We further investigated the properties of luminal-derived organoids generated from lineage-marked CK18–CreER<sup>T2</sup>; R26R–Tomato mice (CK18-trace). These organoids could be serially passaged at least 9 times without apparent loss of viability (Fig. 3o,p), suggesting that the normal luminal compartment contains a stem/progenitor population that can propagate organoids in culture. Moreover, following androgen deprivation after passaging, these luminal-derived organoids were decreased in size and lacked nuclear AR expression (Fig. 3q–s). Thus, lineage-marked luminal cells generate organoids that recapitulate key properties of organoids cultured from the bulk prostate epithelium.

Establishment of tumour organoids from single transformed CARNs

As CARNs are a cell of origin for prostate cancer in vivo<sup>25</sup>, we investigated organoid formation from CARNs that had undergone oncogenic transformation in the context of a model of aggressive lethal prostate cancer<sup>39</sup>. In particular, Nkx3.1<sup>1CreERT2/+; Pten<sup>flox</sup>/flox; Kras<sup>G12D</sup>/flox; R26R–YFP/+</sup> mice (termed NPK) were castrated and induced with tamoxifen, so that combined <sup>Pten</sup> deletion, <sup>Kras</sup><sup>G12D</sup> activation and YFP expression occurred specifically in CARNs (Fig. 4a). Transformed lineage-marked CARNs were isolated by flow cytometry on the basis of their YFP expression, and used for organoid culture. The resulting NPK–CARN tumour organoids grew rapidly and exhibited extensive budding and branching (Fig. 4b). Notably, these NPK–CARN organoids exhibited histological phenotypes resembling PIN (Fig. 4c), and contained many proliferating cells (Fig. 4d). Immunostaining of NPK–CARN organoids showed membrane-localized phospho-Akt (pAkt; Fig. 4e), as well as patchy expression of phospho-Erk (pErk; Fig. 4f). Consistent with a tumour phenotype, the organoids exhibited strong luminal features, with relatively few cells expressing the basal markers p63 and CK5 (Fig. 4g,h); in addition, the NPK–CARN organoids showed nuclear Foxa1 expression (Fig. 4i). Importantly, the organoids exhibited nuclear AR in the presence of DHT; but mostly cytoplasmic AR in the absence of DHT (Fig. 4j,k). Furthermore, these tumour organoids could be frozen and thawed, and passaged at least 10 times without apparent loss of viability. Finally, these tumour organoids could be used to generate renal grafts that exhibited a high-grade PIN phenotype (Fig. 4l), and contained proliferating cells (Fig. 4m). These grafts exhibited membrane-localized phospho-Akt, patchy pErk expression and nuclear AR, and were uniformly YFP positive (Fig. 4n–p), indicating their phenotypic similarity to donor tumours in vivo<sup>39</sup>.

Given the tumour phenotype of NPK–CARN organoids, we investigated whether organoids could be derived from single transformed CARNs. We used flow-sorting to isolate transformed YFP-positive cells from Nkx3.1<sup>CreERT2/+; Pten<sup>flox</sup>/flox; Kras<sup>LSL–G12D/+; R26R–YFP/+</sup></sup> mice that were castrated and induced with tamoxifen, and mouth-pipetted individual cells into a 96-well plate. We found that 6/80 (7.5%) of single transformed CARNs could form organoids after ten days of culture (Fig. 4q). Thus, single NPK CARNs can initiate organoid formation at a frequency significantly higher than that of untransformed CARNs.

Tumour organoids can model prostate cancer phenotypes in culture

These findings suggest that organoid culture might represent a suitable system for modelling of tumour phenotypes and drug treatment responses. To test this idea, we first investigated whether tumour organoids could be generated from a range of well-studied mouse models of prostate cancer, namely: Nkx3.1<sup>−/−</sup> null mutants<sup>40,41</sup>; Nkx3.1<sup>1/+; Pten<sup>flox</sup>/flox</sup>; and tamoxifen-induced Nkx3.1<sup>1CreERT2/+; Pten<sup>flox</sup>/flox; Kras<sup>G12D</sup>/flox; (NP53)</sup> mice<sup>46</sup> (Fig. 5a–j). Interestingly, many of these organoids exhibited filled morphologies consistent with oncogenic transformation; in contrast, the Nkx3.1<sup>−/−</sup> organoids exhibited a more normal morphology, consistent with the low-grade PIN phenotype of Nkx3.1 mutant mice<sup>41,47</sup>. Moreover, all of these mouse models exhibited enhanced efficiencies of organoid formation relative to controls (Fig. 5k).

We also examined whether organoid culture could be used for the rapid induction of tumour phenotypes, using tamoxifen-inducible organoids from CK8–CreER<sup>T2</sup>; Pten<sup>flox</sup>/flox; Kras<sup>LSL–G12D/+; R26R–CAG–YFP</sup> mice (Fig. 5l). Although these organoids had normal phenotypes, they exhibited YFP expression and membrane-localized phospho-Akt after induction in culture with 4-hydroxy-tamoxifen (4-OHT; Fig. 5m,n). Following serial passaging in the absence of 4-OHT, the control organoids retained a hollow morphology without any detectable YFP expression. In contrast, in the presence of 4-OHT, the organoids were mostly YFP and pAkt positive, and exhibited PIN-like phenotypes (Fig. 5o–r).

We next determined whether tumour organoids could be used to assess drug response, using organoids from Nkx3.1<sup>1CreERT2/+; Pten<sup>flox</sup>/flox; R26R–YFP/+ (NP)</sup> mice, which were previously used to analyse therapeutic response in vivo<sup>48</sup>. Although NP mice initially form castration-sensitive prostate tumours, they eventually develop castration-resistant disease that is sensitive to combined treatment with the Akt inhibitor MK-2206 and the mTOR inhibitor MK-8669 (ridaforolimus)<sup>49</sup>. To assess therapeutic response, we isolated YFP-positive prostate cells from tamoxifen-induced NP mice for organoid culture, and subsequently dissociated organoids at the third passage to single-cell suspensions, followed by plating at 1,000 cells per well embedded within Matrigel/culture medium. Control cultures were established in the presence of DHT, and treatment cultures were
established without DHT. Treatment with the dimethylsulphoxide (DMSO) solvent control had no effect, as expected, and either the AR antagonist enzalutamide or MK-8669 had minimal effects on organoid formation (Fig. 6a–f). In contrast, combined treatment with enzalutamide and MK-8669 inhibited organoid formation (Fig. 6a,g,i), consistent with the known synergistic activities of AR and PI(3)K signalling in human prostate cancer. Interestingly, these effects were not simply due to inhibition of AR and PI(3)K pathway activities, as combined treatment with enzalutamide and MK-8669 could greatly reduce nuclear AR expression (Fig. 6j,k), but had no effect on phospho-Akt (Fig. 6l,m).

Culture of human prostate organoids

Finally, we examined whether organoids could be established from human prostate tissue and cell lines. Therefore, we obtained tissue samples from three radical prostatectomies, confirmed that they contained benign glands, and isolated epithelial cells by flow-sorting for EpCAM and E-cadherin. All three patient-derived samples could establish organoids over the course of three weeks and could be passaged successfully, with organoids exhibiting cystic morphologies and containing proliferative cells (Supplementary Fig. 3a–c). These organoids exhibited outer p63+ CK8+ double-positive intermediate cells, and inner CK8+ luminal cells (Supplementary Fig. 3d), with nearly all of the cells expressing AR and CK18 (Supplementary Fig. 3e). Thus, benign human organoids resemble normal mouse organoids, except that outer cells mostly co-express basal and luminal markers.

We also examined whether organoid culture could be used to propagate the VCaP human prostate cancer cell line, which has a luminal phenotype. VCaP organoids exhibited a relatively solid morphology, contained proliferating cells, and were successfully passaged (Supplementary Fig. 3f–h). Notably, VCaP organoids expressed luminal markers including AR, but not the basal marker p63 (Supplementary Fig. 3i). Consequently, our organoid culture conditions could readily maintain the VCaP luminal phenotype, and may therefore be suitable for studies of human prostate cancer.
Figure 5 Modelling tumour phenotypes in organoid culture. (a–j) Formation of organoids from mouse models of prostate cancer, shown in bright-field (a–e) and H&E-stained sections (f–j). (a,f) Organoids generated from TRAMP mice at 22 weeks of age. (b,g) Organoids from Nkx3.1<sup>CreER<sup>2</sup></sup>; Pten<sup>flox/flox</sup>; p53<sup>flox/flox</sup> (NPP53) mice induced with tamoxifen at two months of age and assayed at 10 months. (c,h) Organoids from Nkx3.1<sup>1/2</sup>; null mutant mice at 14 months of age. (d,i) Organoids from Hi–Myc transgenic mice at 9 months. (e,j) Organoids from Nkx3.1<sup>1/2</sup>; Pten<sup>+/–</sup> mice at 10 months. (k) Organoid formation efficiency from the indicated mouse models (data are from 10 technical replicates). (l–r) Induction of tumour phenotypes in culture by tamoxifen treatment of organoids derived from CK8–CreER<sup>2</sup>; Pten<sup>flox/flox</sup>; Kras<sup>G12D</sup>; R26R–CAG–YFP mice. (l) Time course of induction experiment. (m–p) Immunostaining for YFP and pAkt in control untreated organoids (m,o) or 4-hydroxy-tamoxifen (4-OHT)-treated organoids (n,p) at passage 1 (m,n) and at passage 4 (o,p). (q,r) H&E staining of control (q) and 4-OHT-treated organoids (r) at passage 4. Scale bars in a–j correspond to 100 μm, and in m–r to 50 μm.

DISCUSSION

The establishment of self-organizing organoids in ex vivo culture has become an emerging paradigm for the study of tissue stem cells. We have shown that organoids derived from normal mouse prostate can self-renew, generate differentiated basal and luminal cells, and exhibit long-term expansion of prostate epithelial progenitors for at least 13 passages. In addition, luminal cells are favoured for organoid formation, and at least some luminal cells exhibit bipotentiality in culture. Moreover, organoids can reconstitute either normal or transformed prostate tissue in renal grafts, depending on the starting material. These findings indicate that prostate organoids represent an excellent system for investigating prostate biology.

AR signalling represents a central theme in studies of prostate development and cancer. Although there have been many efforts to develop culture systems for prostate epithelium, functional AR activity has not been unambiguously demonstrated in previous studies. For instance, prostatic epithelial stem cells exhibit low or absent AR expression in the presence of DHT (refs 6,9), and other spheroid culture methods show nuclear AR but not alterations of growth or AR-regulated gene expression after androgen withdrawal. In prostate organoids, however, AR protein is localized to the nucleus in the presence of DHT, and DHT withdrawal affects organoid growth, AR subcellular localization and expression of AR-regulated genes. These responses to androgen withdrawal suggest that molecular mechanisms of castration resistance in prostate tumours can be effectively investigated in organoid culture.

Interestingly, despite the importance of epithelial–mesenchymal interactions in prostate organogenesis and regeneration, much of the stromal requirement for prostate epithelial self-renewal and differentiation can apparently be replaced by soluble factors in the presence of extracellular matrix components found in Matrigel, such as collagen IV and laminin. Furthermore, prostate organoids generated from normal tissue seem to have unlimited potential for expansion of epithelial progenitors, similar to organoids established from other tissue types. As prostate epithelium in vivo is generally quiescent, our organoid culture conditions may contain potent...
proliferative signals and/or lack anti-proliferative signals derived from the adult stroma in vivo. In this regard, we note that our culture conditions are distinct from conditions employed in other organoid studies, use defined media containing EGF, the BMP inhibitor Noggin, and the canonical Wnt pathway activator R-spondin, in contrast with our serum-containing media. Future analyses may yield insights into improved culture protocols, and optimization through approaches such as epithelial–stromal co-culture.

Our studies provide important insights into luminal progenitors in the prostate epithelium. Although lineage-tracing studies have reported that luminal cells in the hormonally intact prostate epithelium do not exhibit bipotentiality in vivo, luminal cells can generate basal cells in organoid culture. This bipotentiality resembles that of CARNs in the regressed (androgen-deprived) epithelium during prostate regeneration. Notably, CARNs have a sixfold higher efficiency of organoid formation than that of non-CARNs. However, as CARNs represent less than 1% of epithelial cells in the regressed prostate, a substantial proportion of organoid-forming ability within the regressed epithelium seems to arise from cells that are not CARNs. One likely interpretation is that luminal progenitors distinct from CARNs exist within the regressed prostate epithelium, and perhaps in the hormonally intact epithelium as well. Another, non-mutually exclusive possibility is that some prostate luminal progenitors are lineage-restricted in vivo, but can exhibit plasticity in culture, similar to prostate basal cells.

As luminal cells are favoured for organoid formation, our culture conditions should be suitable for analyses of prostate tumour initiation and progression, as prostate adenocarcinoma has a luminal phenotype. Indeed, prostate tumour organoids can be established from genetically engineered mouse models ranging from relatively indolent (Nkx3.1 null) to highly aggressive (Hi-Myc, NPP53). Moreover, tumour phenotypes can be experimentally induced in phenotypically normal organoids in culture, indicating that stromal cells are not required for oncogenic transformation. The ability to passage organoids as single cells suggests that manipulations such as lentiviral infection and CRISPR/Cas9 targeting should be feasible for genetic engineering of tumour phenotypes in vivo, as shown for intestinal organoids. Furthermore, the ability to recapitulate treatment responses observed in human prostate cancer suggests that organoid culture can be used for drug screens and mechanistic studies of therapeutic response and resistance.

Finally, we have shown that organoids can be established from benign human prostate tissue as well as a luminal human prostate cancer cell line. Given the presence of intermediate cells in the benign human organoids, further optimization of our culture conditions may be advantageous. Nonetheless, a logical next step is to establish organoid cultures from human prostate tumour samples. Organoid culture may provide an alternative to tissue slice cultures, which are short-lived and exhibit varying androgen responsiveness, and to patient-derived xenographs, which are laborious and require large numbers of immunodeficient mice. In particular, patient-derived organoids established from primary tumours or metastases might be suitable for generation of a cryopreserved tumour organoid bank, and could potentially be used for prospective drug screening. Therefore, the continuing development of organoid culture systems may ultimately lead to advances in personalized medicine.

Figure 6 Modelling drug treatment response in organoid culture. (a) Efficiency of organoid formation using organoids from Nkx3.1CreERT2; Ptenfllox/fllox; R26R-YFP/+ (NP) mice. Passaged organoids were treated with the indicated compounds (n = 3 samples analysed per treatment condition); source data are provided in Supplementary Table 1. (b–g) Bright-field images of treated NP organoids. (h,i) H&E sections from control +DHT organoids (h) and enzalutamide + MK-8669-treated organoids (i). (j,k) AR expression in control +DHT organoids (arrows, j) and enzalutamide-treated organoids (k). (l–m) pAkt expression in control +DHT organoids (arrow, l) and enzalutamide + MK-8669-treated organoids (m). Scale bars in h–m correspond to 50 μm, and in b–g correspond to 100 μm. Error bars represent one standard deviation; **P < 0.01.
et al. 16. Sato, T.

et al. 14. Lancaster, M. A. & Knoblich, J. A. Organogenesis in a dish: modeling development

et al. 10. Guo, C., Zhang, B. & Garraway, I. P. Isolation and characterization of human prostate

et al. 9. Lukacs, R. U., Goldstein, A. S., Lawson, D. A., Cheng, D. & Witte, O. N. Isolation,

et al. 8. Garraway, I. P. et al.

et al. 7. Shi, X., Gipp, J. & Bushman, W. Anchorage-independent culture maintains prostate

et al. 2. Shen, M. M. & Abate-Shen, C. Molecular genetics of prostate cancer: new prospects

et al. 1. Peehl, D. M. Primary cell cultures as models of prostate cancer development. Endocr. Relat. Cancer 12, 19–47 (2005).

et al. 1, 23. Huch, M. et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat. Neurosci. 13, 133–140 (2010).

et al. 21. Barker, N. et al. Cross-species analysis of genome-wide regulatory networks identifies signatures of cell-of-origin in human prostate cancer. Cell 158, 1515–1529 (2014).

et al. 24. Greggio, C. et al. Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proc. Natl Acad. Sci. USA 107, 8129–8134 (2010).

et al. 25, 36. Madisen, L. et al. Multipotent and unipotent progenitors contribute to prostate postnatal development. Nat. Cell Biol. 14, 1131–1138 (2012).

et al. 27. Lang, Z. et al. ROCK inhibitor Y-27632 suppresses dissociation-induced apoptosis of murine prostate stem/progenitor cells and increases their cloning efficiency. PLoS One 6, e18271 (2011).

et al. 29. Liu, X. et al. A probasin-large T antigen transgenic mouse line develops prostate cancer in a transgenic mouse. Proc. Natl Acad. Sci. USA 102, 979–985 (2013).

et al. 30. Van Keymeulen, A. et al. Distinct stem cells contribute to mammary gland tumorigenesis and maintenance. Nature 479, 189–193 (2011).

et al. 31. Aytes, A. et al. Forkhead box A1 regulates prostate ductal morphogenesis and promotes epithelial cell maturation. Development 133, 3431–3443 (2006).

et al. 32. Srinivas, S. et al. Cre reporter strains produced by targeted insertion of EyFP and ECFP into the ROSA26 locus. BMC Dev. Biol. 1, 4 (2001).

et al. 33. Srinivas, S. et al. A probasin-large T antigen transgenic mouse line develops prostate cancer in a transgenic mouse. Proc. Natl Acad. Sci. USA 102, 979–985 (2013).

et al. 34. Van Keymeulen, A. et al. Distinct stem cells contribute to mammary gland tumorigenesis and maintenance. Nature 479, 189–193 (2011).

et al. 35. Ousset, M. et al. Multipotent and unipotent progenitors contribute to prostate postnatal development. Nat. Cell Biol. 14, 1131–1138 (2012).

et al. 36. Madisen, L. et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat. Neurosci. 13, 133–140 (2010).

et al. 37. Choi, N., Zhang, B., Zhang, L., Ittmann, M. & Xin, L. Adult murine prostate basal and luminal cells are self-sustained lineages that can both serve as targets for prostate cancer initiation. Cancer Cell 21, 253–265 (2012).

et al. 38. Lu, T. L. et al. Conditionally ablated Plen in prostate basal cells promotes basal-to-luminal differentiation and causes invasive prostate cancer in mice. Am. J. Pathol. 182, 975–991 (2013).

et al. 39. Aytes, A. et al. ETV4 promotes metastasis in response to activation of PI3-kinase and Ras signaling in a mouse model of advanced prostate cancer. Proc. Natl Acad. Sci. USA 110, E3506–E3515 (2013).

et al. 40. Masumori, N. et al. Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proc. Natl Acad. Sci. USA 107, 8129–8134 (2010).

et al. 41. Aytes, A. et al. Forkhead box A1 regulates prostate ductal morphogenesis and promotes epithelial cell maturation. Development 133, 3431–3443 (2006).

et al. 42. Srinivas, S. et al. Cre reporter strains produced by targeted insertion of EyFP and ECFP into the ROSA26 locus. BMC Dev. Biol. 1, 4 (2001).

et al. 43. Van Keymeulen, A. et al. Distinct stem cells contribute to mammary gland tumorigenesis and maintenance. Nature 479, 189–193 (2011).

et al. 44. Masumori, N. et al. Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proc. Natl Acad. Sci. USA 107, 8129–8134 (2010).

et al. 45. Van Keymeulen, A. et al. Distinct stem cells contribute to mammary gland tumorigenesis and maintenance. Nature 479, 189–193 (2011).

et al. 46. Madisen, L. et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat. Neurosci. 13, 133–140 (2010).

et al. 47. Choi, N., Zhang, B., Zhang, L., Ittmann, M. & Xin, L. Adult murine prostate basal and luminal cells are self-sustained lineages that can both serve as targets for prostate cancer initiation. Cancer Cell 21, 253–265 (2012).

et al. 48. Lu, T. L. et al. Conditionally ablated Plen in prostate basal cells promotes basal-to-luminal differentiation and causes invasive prostate cancer in mice. Am. J. Pathol. 182, 975–991 (2013).
52. McKeehan, W. L., Adams, P. S. & Rosser, M. P. Direct mitogenic effects of insulin, epidermal growth factor, glucocorticoid, cholera toxin, unknown pituitary factors and possibly prolactin, but not androgen, on normal rat prostate epithelial cells in serum-free, primary cell culture. Cancer Res. 44, 1998–2010 (1984).

53. Lang, S. H. et al. Differentiation of prostate epithelial cell cultures by matrigel/stromal cell glandular reconstruction. In Vitro Cell. Dev. Biol. Anim. 42, 273–280 (2006).

54. Lamb, L. E., Knudsen, B. S. & Miranti, C. K. E-cadherin-mediated survival of androgen-receptor-expressing secretory prostate epithelial cells derived from a stratified in vitro differentiation model. J. Cell Sci. 123, 266–276 (2010).

55. Marker, P. C., Donjacour, A. A., Dahiya, R. & Cunha, G. R. Hormonal, cellular, and molecular control of prostatic development. Dev. Biol. 253, 165–174 (2003).

56. Cunha, G. R. Mesenchymal-epithelial interactions: past, present, and future. Differentiation 76, 578–586 (2008).

57. Stange, D. E. et al. Differentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. Cell 155, 357–368 (2013).

58. Liu, J. et al. Regenerated luminal epithelial cells are derived from preexisting luminal epithelial cells in adult mouse prostate. Mol. Endocrinol. 25, 1849–1857 (2011).

59. Koo, B. K. et al. Controlled gene expression in primary Lgr5 organoid cultures. Nat. Methods 9, 81–83 (2012).

60. Schwank, G. et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13, 653–658 (2013).

61. Sachs, N. & Clevers, H. Organoid cultures for the analysis of cancer phenotypes. Curr. Opin. Genet. Dev. 24, 68–73 (2014).

62. Ranga, A., Gjorevski, N. & Lutolf, M. P. Drug discovery through stem cell-based organoid models. Adv. Drug Deliv. Rev. 69-70, 19–28 (2014).

63. Centenera, M. M., Raj, G. V., Knudsen, K. E., Tilley, W. D. & Butler, L. M. Ex vivo culture of human prostate tissue and drug development. Nat. Rev. Urol. 10, 483–487 (2013).

64. Toivonen, R. et al. A preclinical xenograft model identifies castration-tolerant cancer-repopulating cells in localized prostate tumors. Sci. Transl. Med. 5, 187ra171 (2013).

65. Lin, D. et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res. 74, 1272–1283 (2014).
Mouse strains and genotyping. The Nkx3.1<sup>CreERT<sup>2</sup></sup> allele (Nkx3.1<sup>CreERT2;+/-</sup>) has been previously described<sup>46</sup>. The CK8–CreER<sup>T2</sup> (TgKrt8Cre/CreERT2)17Blpn/J, no. 007930; ref. 36), R26R–CAG–YPF (B6;129S6-Gt(ROSA)26Sor<sup>Cre</sup>/J, no. 007998; ref. 36), and conditional Pten<sup>flx</sup> (B6;129S4-Pten<sup>flx</sup>/J, no. 006440; ref. 67) strains were obtained from the Jackson Laboratory Induced Mutant Resource. The inducible Kras<sup>G12D</sup>–G220V;Cre<sup>ERT2</sup> (B6;129S4-Pten<sup>flx</sup>/J, no. 006148; ref. 33), R26R–CAG–YPF (B6;129S6-Gt(ROSA)26Sor<sup>Cre</sup>/J, no. 007930; ref. 36), and conditional Pten<sup>flx</sup> (B6;129S4-Pten<sup>flx</sup>/J, no. 006440; ref. 67) strains were obtained from the National Cancer Institute Mouse Institute of Human Cancer Consortium Repository: CK5–CreER<sup>T2</sup> (Krt5<sup>CreERT2</sup>; B6.129X1-Gt(ROSA)26Sor<sup>Cre</sup>/J, no. 006148; ref. 33). Animals were maintained on a congenic C57BL/6N background. Genotyping was performed using the primers listed in Supplementary Table 3.

For lineage-marking of CARNs, Nkx3.1<sup>CreERT2;+/-</sup>; R26R–YPF/+ males were castrated after 8 weeks of age and allowed to regress for 4 weeks, then treated with tamoxifen (Sigma) (9 mg per 40 g body weight in corn oil) by daily oral gavage for 4 consecutive days, followed by a chase period as previously described<sup>26</sup>. For lineage-marking with the CK5–CreER<sup>T2</sup>, CK8–CreER<sup>T2</sup>, and CK18–CreER<sup>T2</sup> drivers, 8-week-old hormonally intact males were treated with tamoxifen as previously described<sup>26</sup>. To generate androgen-deprived males with lineage-marked cells using CK8–CreER<sup>T2</sup> and CK18–CreER<sup>T2</sup> drivers, tamoxifen-treated animals were castrated and allowed to regress for 2–3 weeks. Lineage-marking efficiencies were calculated using sections from anterior prostate lobes. No statistical methods were used to pre-determine sample size, and experiments were not randomized; investigators were not blinded to allocation during experiments and outcome assessment. All animal experiments received approval from the Institutional Animal Care and Use Committee at Columbia University Medical Center.

Sample acquisition for human organoids. Benign human organoids were derived from radical prostatectomy samples obtained from patients undergoing surgery at Columbia University Medical Center. All patients gave informed consent under the auspices of an Institutional Review Board-approved protocol. Candidate benign regions were dissected and transported to the laboratory in DMEM/F12 (Gibco no. 10565) supplemented with 5% fetal bovine serum (FBS) for tissue dissociation. Benign pathology was initially confirmed by H&E analyses of adjacent rapid frozen sections, and was further confirmed by immunostaining of paraffin sections from the primary sample used for organoid establishment for p63, high-molecular-weight cytokeratin, and α-methyl acyl coenzyme A racemase (AMACR) using the PIN-4 cocktail<sup>28</sup> (Biocare Medical no. PPM 225 DSA). The VCaP cell line was purchased from the American Type Culture Collection (CRL-2876).

For serial passaging experiments, organoids were passaged at a 1:4 dilution every 1–2 weeks with 0.25% trypsin for 5 min at 37 °C, followed by mechanical dissociation to nearly single-cell suspensions. Organoids were frozen in complete media with 50% FBS and 10% DMSO. Efficiency of organoid formation was calculated by averaging the number of organoids visible in each well after 7 days of growth using a ×10 objective. For statistical analyses, efficiency percentages were arcsin converted to perform unpaired two-tailed Student’s t-tests.

For drug treatments, organoids were dissociated by digestion with 0.25% trypsin–EDTA (STEMCELL Technologies no. 07901) and passed through a 40 μm cell strainer. Forty microliters of the resulting cell suspension containing 500–3,000 dissociated cells was mixed with 60 μl of Matrigel, and the mixture was pipetted around the rim of wells in a 24-well plate. The mixture was allowed to solidify for 10 min at 37 °C. Twenty-four hours after plating, organoids were fixed with 4% paraformaldehyde in PBS at 4 °C for 1 h, washed with PBS, and embedded in OCT (Tissue-Tek). For paraffin sectioning, organoids were fixed in 10% formalin for 1 h and placed in Histogel (Thermo Scientific) before tissue processing and embedding.

Histology and immunostaining. Tissues were processed for cryosections or paraffin sectioning using standard protocols. For cryosections, organoids and tissues were floated overnight in 4% paraformaldehyde in PBS at 4 °C for 1 h, placed in 30% sucrose in PBS, and embedded in OCT (Tissue-Tek). For paraffin sectioning, organoids were fixed in 10% formalin for 1 h and placed in Histogel (Thermo Scientific) before tissue processing and embedding.

EpCAM or E-cadherin were used for compensation. Both side-scatter pulse width (SSC-W) versus area (SSC-A) and forward side-scatter pulse area (FSC-A) versus heights (FSC-H) were used to isolate single dissociated cells. For normal prostate epithelium, cells expressing either EpCAM and/or E-cadherin were isolated. For isolation of lineage-marked CARNs and transformed CARNs, as well as lineage-marked basal or luminal populations, cells were sorted on the basis of their YFP or Tomato expression; non-YFP-expressing cells were obtained by sorting EpCAM and/or E-cadherin-positive but YFP-negative cells. Sorted cells were plated in low-attachment 96-well plates at densities ranging from 100 (for CARNs) to 10,000 cells per well. For single-cell experiments, sorted YFP-positive cells were picked by mouth-pipetting using an inverted microscope, followed by re-plating in wells of 96-well low-attachment plates.

Organoid culture. We used two methods for three-dimensional culture of prostate organoids from isolated prostate epithelial cells, corresponding to flotation in low-percentage Matrigel or embedding within Matrigel; the embedding method was used for drug treatment experiments and is described below. For the floating method, prostate epithelial cells were resuspended in prostate organoid culture medium, consisting of: hepatocyte medium supplemented with 10 ng ml<sup>−1</sup> epidermal growth factor (EGF; Corning no. 355056), 10 μM Y-27632 (STEMCELL Technologies no. 07171), 1× Glutamax (Gibco no. 35050), 5% Matrigel (Corning no. 354234) and 5% charcoal-stripped FBS (Gibco no. 12676), which had been heat-inactivated at 55 °C for 1 h. After resuspension in prostate organoid medium, 100–1000 dissociated cells were plated into wells of ultralow-attachment 96-well plates (Corning no. 3474) in the presence of 100 nM DHT for mouse or 10 nM DHT for human (Sigma-Aldrich no. A-8380). One hundred microlitres of fresh organoid medium was added to the wells every four days, and the medium was changed every 12 days for up to one month.

For serial passaging experiments, organoids were passaged at a 1:4 dilution every 1–2 weeks with 0.25% trypsin for 5 min at 37 °C, followed by mechanical dissociation to nearly single-cell suspensions. Organoids were frozen in complete media with 50% FBS and 10% DMSO. Efficiency of organoid formation was calculated by averaging the number of organoids visible in each well after 7 days of growth using a ×10 objective. For statistical analyses, efficiency percentages were arcsin converted to perform unpaired two-tailed Student’s t-tests.

For analyses of androgen withdrawal, organoids were passaged and then cultured for 7–10 days in culture medium in the presence or absence of DHT. For induction of Cre recombinase activity in culture, epithelial cells from un-induced CK8–CreER<sup>T2</sup>; Pten<sup>flx</sup>/J, Kras<sup>G12D</sup>–G220V;Cre<sup>ERT2</sup>; R26R–CAG–YPF mice were sorted on the basis of organoid and E-cadherin expression, and cultured until organoid formation was evident. The resulting organoids were passaged, followed by addition of 1 μM 4-OHT on the day after passaging to induce Cre recombination.

A detailed protocol for organoid establishment and culture will be provided on Nature Protocol Exchange immediately following publication.

Drug treatments. The embedding method was used to culture organoids for drug treatment experiments. Organoids were dissociated by digestion with 0.25% trypsin–EDTA (STEMCELL Technologies no. 07901) and passed through a 40 μm cell strainer. Forty microliters of the resulting cell suspension containing 500–3,000 dissociated cells was mixed with 60 μl of Matrigel, and the mixture was pipetted around the rim of wells in a 24-well plate. The mixture was allowed to solidify for 10 min at 37 °C. Twenty-four hours after plating, organoids were fixed with 4% paraformaldehyde in PBS at 4 °C for 1 h, washed with PBS, and embedded in OCT (Tissue-Tek). For paraffin sectioning, organoids were fixed in 10% formalin for 1 h and placed in Histogel (Thermo Scientific) before tissue processing and embedding.

EpCAM or E-cadherin were used for compensation. Both side-scatter pulse width (SSC-W) versus area (SSC-A) and forward side-scatter pulse area (FSC-A) versus heights (FSC-H) were used to isolate single dissociated cells. For normal prostate epithelium, cells expressing either EpCAM and/or E-cadherin were isolated. For isolation of lineage-marked CARNs and transformed CARNs, as well as lineage-marked basal or luminal populations, cells were sorted on the basis of their YFP or Tomato expression; non-YFP-expressing cells were obtained by sorting EpCAM and/or E-cadherin-positive but YFP-negative cells. Sorted cells were plated in low-attachment 96-well plates at densities ranging from 100 (for CARNs) to 10,000 cells per well. For single-cell experiments, sorted YFP-positive cells were picked by mouth-pipetting using an inverted microscope, followed by re-plating in wells of 96-well low-attachment plates.
For immunostaining, sections underwent antigen-retrieval by boiling in citrate acid-based antigen unmasking solution (Vector Labs) for 10 min. Primary antibodies were applied to sections and incubated at 4°C overnight in a humidified chamber. Alexa Fluors (Life Technologies) were used for secondary antibodies. Tyramide amplification (Life Technologies) or ABC Elite (Vector Labs) kits were used for signal detection. For lineage-tracing experiments, analysis of marked basal or luminal cells was performed by manual counting of cells from confocal images taken with a ×40 objective. Details on the antibodies used are provided in Supplementary Table 4.

Quantitative real-time PCR analysis. For RNA extraction, 4–6 wells of organoids were pooled, pelleted and dissolved in Trizol reagent before processing by the MagMAX 96 Total RNA Isolation Kit (Ambion, Life Technologies). Three hundred to five hundred nanograms of RNA was used for cDNA synthesis using the Superscript First Strand Synthesis System (Invitrogen). Quantitative real-time PCR was carried out using SYBR green master mix reagent (QIAGEN) in the Realplex2 instrument (Eppendorf). cDNA samples were diluted 1:5–1:10 for all analyses, which were performed in triplicate. Expression values were obtained using the ΔΔCT method and normalized to GAPDH expression; average values are shown. Primer sequences are provided in Supplementary Table 3.

Repeatability of experiments. For histological and immunofluorescence analyses of organoids and tissue recombination experiments, representative staining patterns were confirmed in at least 3 samples from at least 2 independent experiments. All DHT-withdrawal experiments were repeated at least twice. Data shown for quantitative real-time PCR analysis are from a single experiment that was representative of 2 independent experiments. The drug treatment experiment was repeated at a different passage and gave similar results and statistical significance.

66. Van Keymeulen, A. et al. Epidermal progenitors give rise to Merkel cells during embryonic development and adult homeostasis. J. Cell. Biol. 187, 91–100 (2009).
67. Lesche, R. et al. Cre/loxP-mediated inactivation of the murine Pten tumor suppressor gene. Genesis 32, 148–149 (2002).
68. Jackson, E. L. et al. Analysis of lung tumor initiation and progression using conditional expression of oncopgenic K-ras. Genes Dev. 15, 3243–3248 (2001).
69. Rock, J. R. et al. Basal cells as stem cells of the mouse trachea and human airway epithelium. Proc. Natl Acad. Sci. USA 106, 12771–12775 (2009).
70. Giannico, G. A., Ross, H. M., Lotan, T. & Epstein, J. I. Aberrant expression of p63 in adenocarcinoma of the prostate: a radical prostatectomy study. Am. J. Surg. Pathol. 37, 1401–1406 (2013).
Supplementary Figure 1  Analysis of normal organoids during serial passaging.  (a-c) Bright-field images of organoids from normal prostate epithelium at passage 1 (a), passage 6 (b), and passage 13 (c). (d,e) Analysis of organoids at passage 3 shows normal expression of the basal marker p63 (arrowheads, d) and the luminal marker CK8 (d,e), as well as nuclear expression of AR (arrows, e); yellow arrow in d indicates intermediate cell that co-expresses p63 and CK8. Scale bars in d,e correspond to 50 microns and in a-c to 100 microns.
**Supplementary Figure 2** Specificity of luminal and basal lineage-marking in prostate epithelium *in vivo*. Sections from the anterior prostate of CK5-CreERT²; R26R-YFP mice (a), CK8-CreERT²; R26R-YFP mice (b), CK18-CreERT²; R26R-YFP mice (c), and CK18-CreERT²; R26R-Tomato mice (d) demonstrate specificity of basal marking (arrowheads, a) and luminal marking (arrows, b-d). Scale bars correspond to 100 microns.
Supplementary Figure 3 Generation of human prostate organoids. (a-e) Establishment of organoids from benign human prostate specimens. (f-j) Generation of organoids from the VCaP prostate cancer cell line. (a,f) Bright-field images. (b,g) H&E staining. (c,h) Ki67 immunostaining; arrows indicate proliferating cells. (d) Most exterior cells in benign human organoids are CK8\(^{+}\)p63\(^{+}\) cells (arrowheads), while many interior cells are CK8\(^{+}\) only (arrow). (e) Most cells in benign human organoids are AR\(^{+}\)CK18\(^{+}\) cells (arrows). (i) VCaP organoids display immunostaining for CK18, but not p63. (j) VCaP organoids are strongly positive for both AR and CK8. Scale bars in b-e,g-j correspond to 50 microns, and in a,f correspond to 100 microns.
Supplementary Table Legends

Supplementary Table 1. Efficiency of organoid formation. Shown are source data for Fig. 1n, 3g and 6a accompanied by mean and standard deviation.

Supplementary Table 2. Lineage-marking efficiencies in vivo. n=total number of cells examined from 2 or 3 independent mice, with mean values as indicated.

Supplementary Table 3. Primers used in this study.

Supplementary Table 4. Antibodies used in this study.