Figures and figure supplements

Differential requirements of androgen receptor in luminal progenitors during prostate regeneration and tumor initiation

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Figure 1. CARNs remain luminal after AR deletion. (A) Time course for lineage-marking of CARNs and inducible AR deletion using castrated and tamoxifen-treated control Nkx3.1^CreERT2/+, R26R-YFP/+ mice and Nkx3.1^CreERT2/+, Ar^lox/-, R26R-YFP/+ mice. (B) FACS analyses of lineage-marked YFP^+ cells in total EpCAM^+ epithelial cells. (C) Percentage of YFP^+ cells among total epithelial cells in castrated and tamoxifen-induced Nkx3.1^CreERT2/+, R26R-YFP/+ controls and Nkx3.1^CreERT2/+, Ar^lox/-, R26R-YFP/+ mice. Error bars represent one standard deviation; the difference between groups is not significant (p=0.51, independent t-test). (D) Expression of AR, luminal markers (CK8 and CK18), and basal markers (CK5 and p63) in lineage-marked CARNs (top) and AR-deleted CARNs (bottom). Note that all lineage-marked cells express luminal but not basal markers (arrows). Scale bars in D correspond to 50 μm.

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Figure 2. AR-deleted CARNs fail to generate lineage-marked cell clusters but remain bipotential during androgen-mediated regeneration. (A) Time course for lineage-marking and androgen-mediated regeneration. (B) Percentage of single YFP$^+$ cells or YFP$^+$ clusters of 2 cells, 3–4 cells, and >4 cells at 4, 7, 14, and 28 days of androgen-mediated regeneration. This analysis does not include YFP$^+$ AR$^+$ cells that fail to undergo AR deletion in the experimental mice; full quantitation of all cell populations is provided in Figure 2—source data 1. (C) YFP$^+$ cells (arrows) in prostates of mice with lineage-marked CARNs (top) and AR-deleted CARNs (bottom) at days 4, 7, 14 and 28 days during androgen-mediated regeneration. (D) Identification of basal YFP$^+$ cells (arrows) as progeny of CARNs (top) or AR-deleted CARNs (bottom). Scale bars in (C) and (D) correspond to 50 μm.

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Figure 3. AR-deleted CARNs and/or their progeny have defects in proliferation during regeneration and in renal grafts. 

(A,B) Time course of BrdU incorporation during androgen-mediated regeneration of castrated and tamoxifen-treated control Nkx3.1^{CreERT2/+}, R26R-YFP/+ mice and Nkx3.1^{CreERT2/+}, Ar^{flox/Y}, R26R-YFP/+ mice. BrdU injections were performed during either days 1 through 4 (A) or days 11 through 14 (B), followed by analysis at 28 days. 

(C) Identification of BrdU^{+}YFP^{+} cells (arrows) in control (top) and AR-deleted (bottom) prostate tissue after administration of BrdU during early stages of regeneration. 

(D) YFP-positive cells in control prostate tumors (top) can incorporate BrdU (arrow) but not in AR-deleted prostate tumors (bottom), after administration of BrdU during later stages of regeneration. 

(E,F) Percentage of BrdU^{+} and BrdU^{−} cells among total YFP^{+} cells after injection of BrdU from days 1 through 4 (E) or days 11 through 14 (F) of regeneration. Error bars represent one standard deviation; the difference in (E) is not statistically significant (p=0.34, independent t-test), but is significant in (F) (p=0.027, independent t-test). This analysis excludes YFP^{+}AR^{+} cells that fail to undergo AR deletion in the experimental mice; full quantitation of all cell populations is provided in Figure 3—source data 1. 

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Figure 3 continued

Schematic depiction of tissue recombination of lineage-marked CARNs with rat urogenital mesenchyme followed by renal grafting. (H) Analysis of grafts generated from lineage-marked CARNs (top) and AR-deleted CARNs (bottom); arrows in bottom panels indicate AR-expressing stromal cells surrounding the AR-negative prostate duct. Scale bars in C, D and H correspond to 50 μm.

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Figure 4. Properties of cell lines established from CARNs and AR-deleted CARNs. (A) Morphology and marker expression of cell lines derived from single YFP^+ cells from castrated and tamoxifen-treated control Nkx3.1^CreERT2/+; R26R-YFP/+ mice and Nkx3.1^CreERT2/+; Ar^flx/Y; R26R-YFP/+ mice. The APCA lines (top) and ADCA lines (bottom) show similar bright-field morphology, expression of YFP, Foxa1, and Ki67, as well as co-expression of CK8 and CK5, but differ in expression of AR. (B) APCA and ADCA cell lines display similar cell growth at days 1, 2, 4, and 6 after plating in the absence or presence of DHT, as assessed by CellTiter-Glo assay. Results shown are from a single experiment with five technical replicates and are representative of two biological replicates after normalization with day 0 luminescent signal. (C) Colony formation by APCA and ADCA cell lines in the absence or presence of DHT. Results are from a single experiment with three technical replicates and are representative of two biological replicates. (D) Renal grafts generated from tissue recombinants of 100,000 APCA or ADCA cells with rat urogenital mesenchyme, and analyzed at 12 weeks. Bottom row shows APCA grafts treated with tamoxifen for 4 days at 7 weeks of growth to induce Ar deletion (bottom); arrows indicate cells that did not undergo Ar deletion after tamoxifen treatment. Scale bars in A) and D) correspond to 50 μm.

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Figure 4—figure supplement 1. Establishment of novel human prostate epithelial cell lines. (A) Flow-sorting strategy to eliminate EpCAM\(^{+}\)E-cadherin\(^{-}\) cells from dissociated benign human prostate epithelial cells obtained from radical prostatectomies. (B) Bright-field images of a human prostate epithelial cell line at passages 3 and 6. (C) HPE cells broadly express AR and both luminal (CK8) and basal (CK5) markers, and have more limited expression of PSA and Ki67. Scale bars in (B) correspond to 100 \(\mu\)m, and in (C) to 50 \(\mu\)m.

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Figure 5. Gene set enrichment analysis of the ADCA signature. (A) Selected biological pathways that are enriched in the ADCA versus APCA signature. (B) GSEA plot showing enrichment in the positive tail for a signature of AR-null mouse prostate epithelial cells. (C) Cross-species GSEA showing lack of enrichment with a signature based on isolated human prostate basal and luminal epithelial populations. (D–F) Cross-species GSEA comparing the ADCA expression signature with three independent expression signatures based on tumor samples from human patients. NES: normalized enrichment score; p-value is calculated using 1000 gene permutations.

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Figure 6. Deletion of AR alters the ability of CARNs to serve as a cell of origin for prostate cancer. (A) Prostate histology and marker expression in Nkx3.1^CreERT2/+, Pten^floxflox; R26R-YFP/+ (NP-CARN) and Nkx3.1^CreERT2/+, Pten^floxflox; Ar^floxflox; R26R-YFP/+ (NPA-CARN) mice that have been castrated and tamoxifen-treated, followed by androgen-mediated regeneration for 1 month. Shown are representative images for hematoxylin-eosin staining (H and E) and immunofluorescence for YFP, AR, phospho-Akt (pAkt), E-cadherin (Ecad), Ki67, and cleaved caspase-3 (CC3). Arrows indicate occurrence of cell death (YFP/AR in NPA-CARN), proliferation (Ecad/Ki67), and apoptosis (Ecad/CC3). (B) Quantitation of Ki67^+ and CC3^+ positive cells in total Ecad^+ epithelial cells in NP-CARN and NPA-CARN prostates. Error bars represent one standard deviation; differences between groups are statistically significant as determined by independent t-test. (C) Prostate tumor histology and marker expression in Nkx3.1^CreERT2/+, Pten^floxflox; Kras^LSL-G12D/+; R26R-YFP/+ (NPK-CARN) and Nkx3.1^CreERT2/+, Pten^floxflox; Kras^LSL-G12D/+; Ar^floxflox; R26R-YFP/+ (NPKA-CARN) mice that have been castrated and tamoxifen-treated, followed by androgen-mediated regeneration for 1 month. Arrows indicate cells undergoing proliferation (Ecad/Ki67) and apoptosis (Ecad/CC3). (D) Quantitation of Ki67^+ and CC3^+ positive cells in total Ecad^+ epithelial cells in NPK-CARN and NPKA-CARN prostates. Differences between groups are not statistically significant as determined by independent t-test (Ki67, p=0.724; CC3, p=0.507). (E) Focal neuroendocrine differentiation in NPKA-CARN tumors. Shown are H and E and immunohistochemical staining (IHC) of serial sections for Synaptophysin (Syn) and Aurora kinase A (Aurka), IHC for Foxa2 and Chromogranin A (ChrA), as well as immunofluorescence for YFP and Syn shown as an overlay and as individual channels; arrows indicate positive cells. (F) Quantitation of Syn^+ cells in total epithelial cells in NPK-CARN and NPKA-CARN tumors. Scale bars for H and E and IHC in A, C, and E correspond to 100 μm, and in other panels to 50 μm.

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