Interactions between Androgen- and Hedgehog-Signaling maintain the cellular niche for pubertal prostate morphogenesis and growth

**Background:** The essential role of mesenchymal androgen signaling in the development of prostate epithelium has been known for over 30 years. However, the identity of the mesenchymal cells responsible for this paracrine regulation is unknown. In addition, the underlying mechanisms underlying stromal androgen action as the cell niche in facilitating prostatic stem/progenitor cells in pubertal prostatic epithelial morphogenesis and growth are largely unknown.

**Methods:** ROSA26R<sup>mTmG</sup>+/A<sup>LoxP/Y</sup>:Gli1<sup>CreERT2/+</sup> mice and ROSA26R<sup>mTmG</sup>+/Gli1<sup>CreERT2/+</sup> control littermates were administered Tamoxifen (TM) at postnatal day 14 (P14), and then systematically examined at P17, P35, and P56: before, during, and after puberty, respectively. Prostate tissues were dissected from AR-deficient and control mice at P35 following TM administration at P14, and prostatic cells were isolated for single cell RNA sequencing analyses.

**Results:** Selective deletion of androgen receptor (AR) in stromal Shh-responsive cells significantly impedes pubertal prostatic epithelial morphogenesis and growth. AR deletion increased Gli1 expression in prostatic stromal cells, elevated Shh expression in adjacent epithelial cells, and induced stark inhibition of prostate epithelial growth. Dysregulation of Shh and other developmental pathways revealed in both prostatic stromal and epithelial cells of AR-deficient mice at single-cell resolution. Trajectory analysis further revealed abnormal differentiation patterns of prostatic epithelia in mutant mice. Recombination of wild-type prostatic epithelial cells with AR-deficient stromal Gli1-expressing cells failed to develop normal prostatic epithelia.

**Conclusions:** These data provide novel, high-resolution insight into the regulatory mechanisms for androgen-Shh signaling in the cellular niche to control pubertal prostate morphogenesis and growth.
(A) Schematic for generating R26<sup>mTmG</sup>+/Gli1<sup>CreER</sup>+/ and R26<sup>mTmG</sup>+/Ar<sup>L/L</sup>/.Gli1<sup>CreER</sup>+/ mice.

(B) Representative images of prostates isolated from the indicated genotypes at day 35 and 56 postnatal. AP: anterior prostate; DLP: dorsolateral prostate; VP: ventral prostate.

(C) tSNE plot showing dimensional reduction of the distribution of 6871 individual cell transcriptomes from R26<sup>mTmG</sup>+/Gli1<sup>CreER</sup>+/ and 7148 individual cell transcriptomes from R26<sup>mTmG</sup>+/Ar<sup>L/L</sup>/.Gli1<sup>CreER</sup>+/.

(D) tSNE plot showing major clusters of epithelial and stromal lineages based on their transcription profiles.

(E) GSEA enrichment plots showing similar significant positive enrichment of Shh pathway in fibroblasts and smooth muscle.

(F) GSEA analysis with a p-value based, pre-ranked gene list comparing basal cells from ARKO versus control samples.

(G) Trajectory analysis of epithelial cells shows the differences in cell differentiation from R26<sup>mTmG</sup>+/Gli1<sup>CreER</sup>+/ and R26<sup>mTmG</sup>+/Ar<sup>L/L</sup>/.Gli1<sup>CreER</sup>+/.