Androgens play critical roles in epithelium proliferation in BPH not normal adjacent

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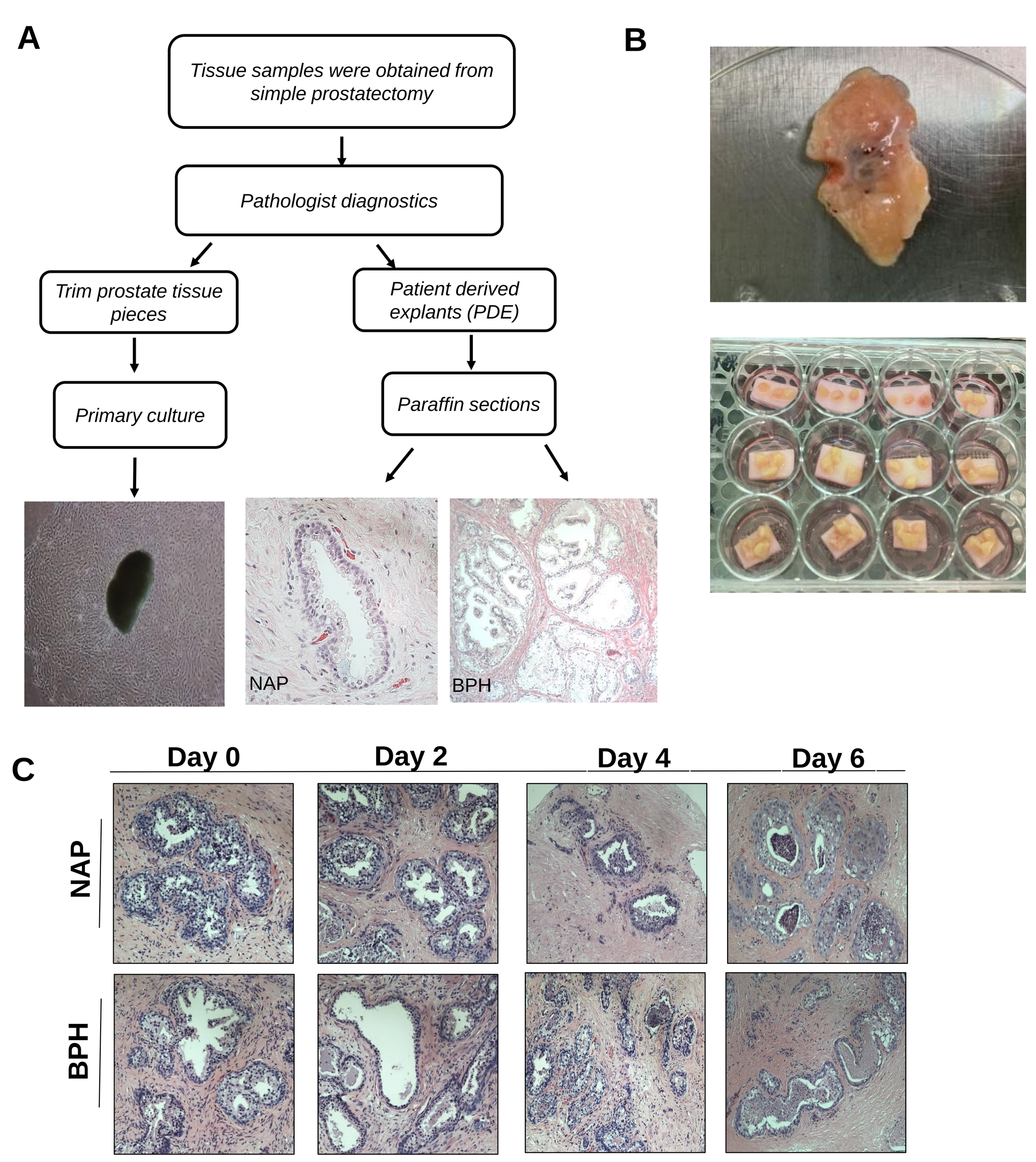
Background: The prostate is an androgen-sensitive organ composed of glandular cells embedded in a surrounding stroma, and stromal AR and androgens play critical roles in prostatic development and adult homeostasis. Our preliminary data showed the AR signaling are playing essential role in the proliferation of epithelial cells in BPH, but not stromal cells from normal adjacent prostate (NAP). Furthermore, androgens significantly increased the proliferation of prostatic epithelial cells in patient derived explant (PDE) of BPH but not the paired normal epithelial cells. However, the underlying mechanisms of this phenomenon are still unknown.

Method: Tissue from human BPH specimens obtained through BPH patients undergoing simple prostatectomy for symptomatic BPH. PDE were utilized to evaluate the androgen-impact in BPH tissue and paired normal adjacent tissue. Proliferation, cytokines and androgen-responsive genes were validated in clinical BPH specimens and paired normal prostatic specimens via IHC and qPCR.

Results: The tissue could maintain original structure and AR signaling in PDE model at least 4 days. PDE models showed that androgen could impact epithelium proliferation in BPH tissue, but not NAP tissue Stromal cells of the BPH tissues secreted higher level of CCL family proteins (CCL8, CCL7, CCL11, CCL13 and CCL28), CXCL proteins (CXCL6, CXCL12), interleukins (IL6, IL7 and IL32), and growth factors than those from the paired normal adjacent tissues. RNA sequencing data showed that several cytokines and growth factors were up-regulated in BPH stromal cells after androgens stimulations but not in normal adjacent stromal cells, which are confirmed by qPCR.

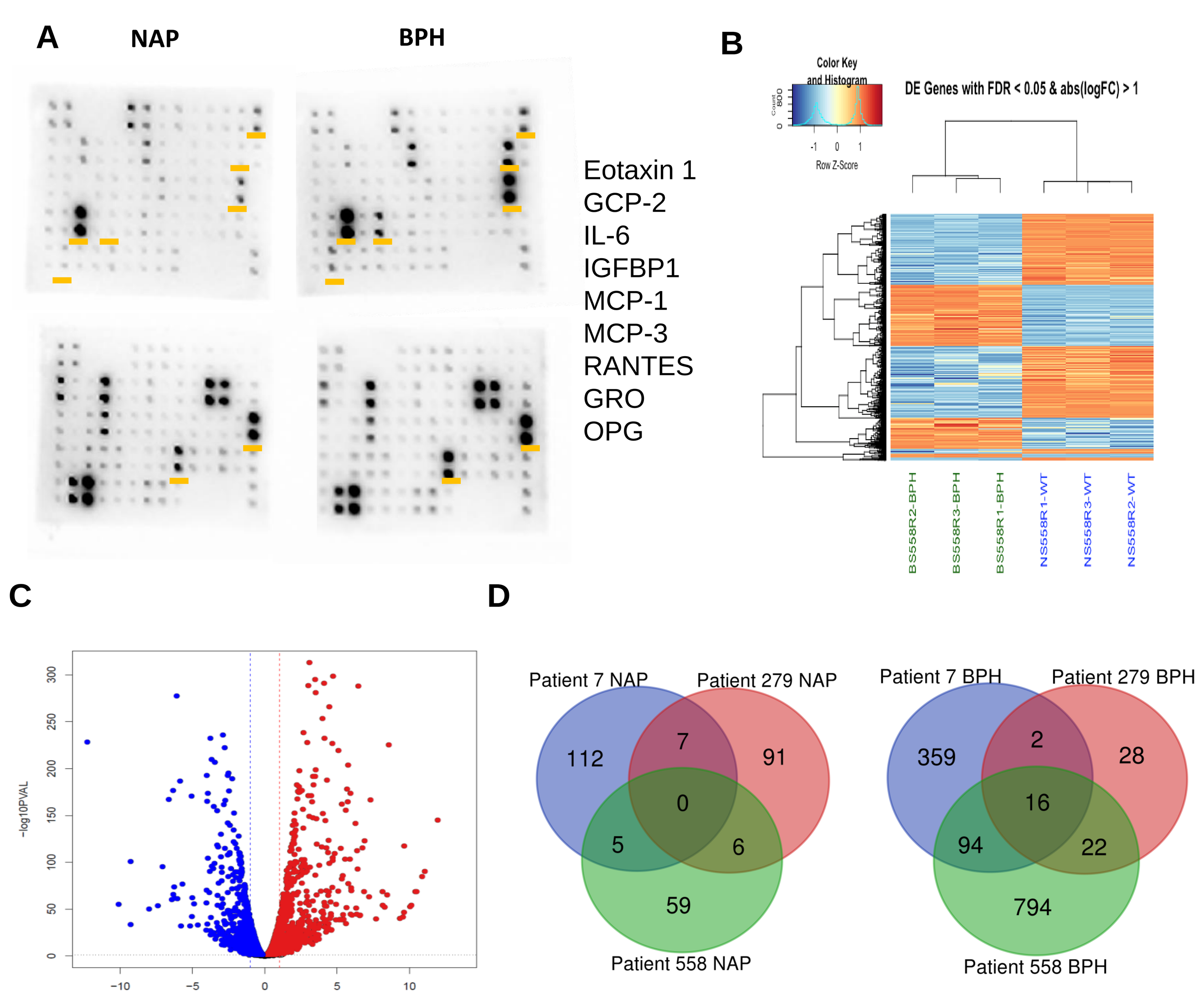
Conclusions: These findings suggested that PDE model were able to utilize as a predominant model to mimic the vitro environment. Androgens have been shown to increase the proliferation of epithelial cells in PDE model. Androgens have been shown to influence the expression of several genes including CXC and interleukins in BPH stromal cells, but not normal adjacent stromal cells. Our results suggest that androgen signaling in BPH stromal cells is dysregulated and could contribute to prostatic epithelial growth and provide a strong foundation to elucidate the mechanisms of androgen-dependent stromal regulation of epithelial cell growth in BPH.

Tissue derived from BPH and normal adjacent prostate for primary culture and explant culture



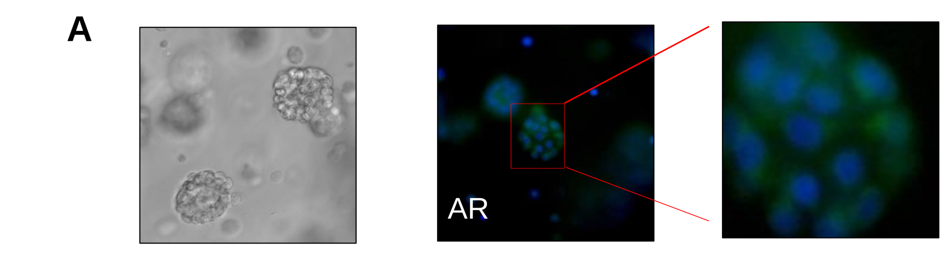
A. Flowchart of prostate tissue collection and processing. Primary cultures and explant culture were prepared directly undergoing simple prostatectomy for symptomatic BPH. BPH and normal adjacent were distinguished by a pathologist (Dr. Rajiv Dhir). **B.** PDE culture of tissue from symptomatic BPH. **C.** H&E staining of Day 0, 2, 4 and 6 of cultured tissue.

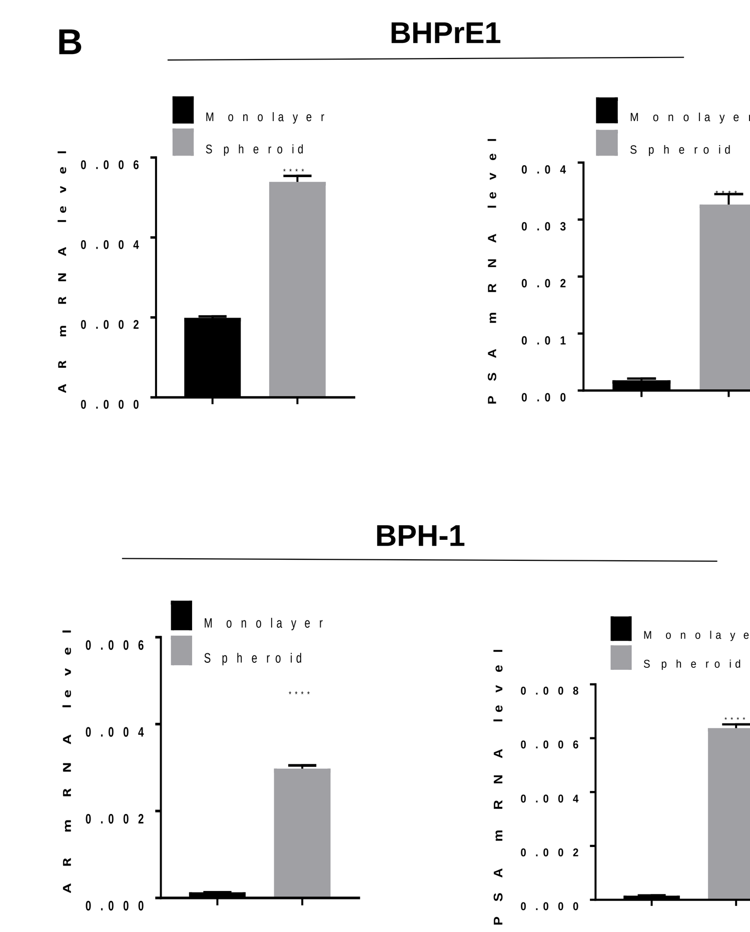
Stromal cells derived from BPH tissues expressed different cytokines and growth factors than normal adjacent



A. Human cytokine array test on CM of stromal cells derived from NAP and BPH tissue. **B.** Differential gene expression of stromal cells derived from paired NAP and BPH tissue. **C.** Volcano chart shows the differential expression genes of stromal cells derived from NAP and BPH tissue. **D.** Genes altered in response to androgen in stromal cells derived from NAP compared to stromal cells derived from BPH tissue.

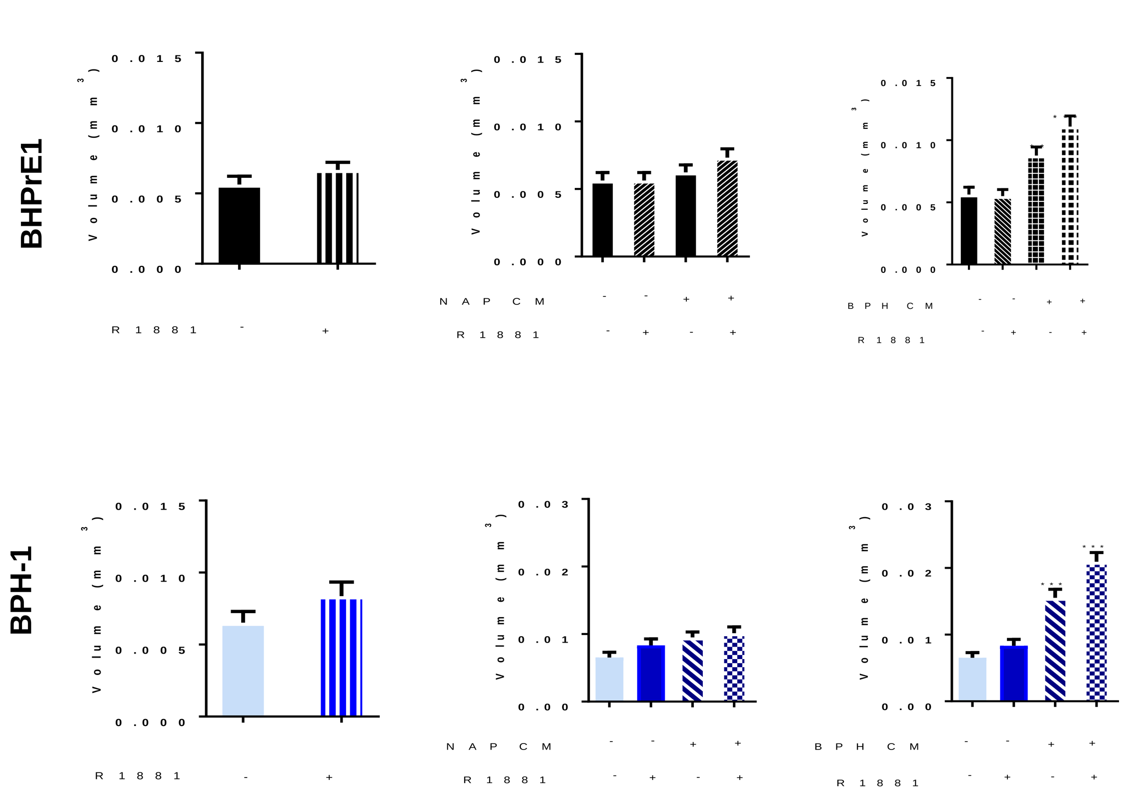
BPH-1 and BHPrE1 spheroids re-express AR





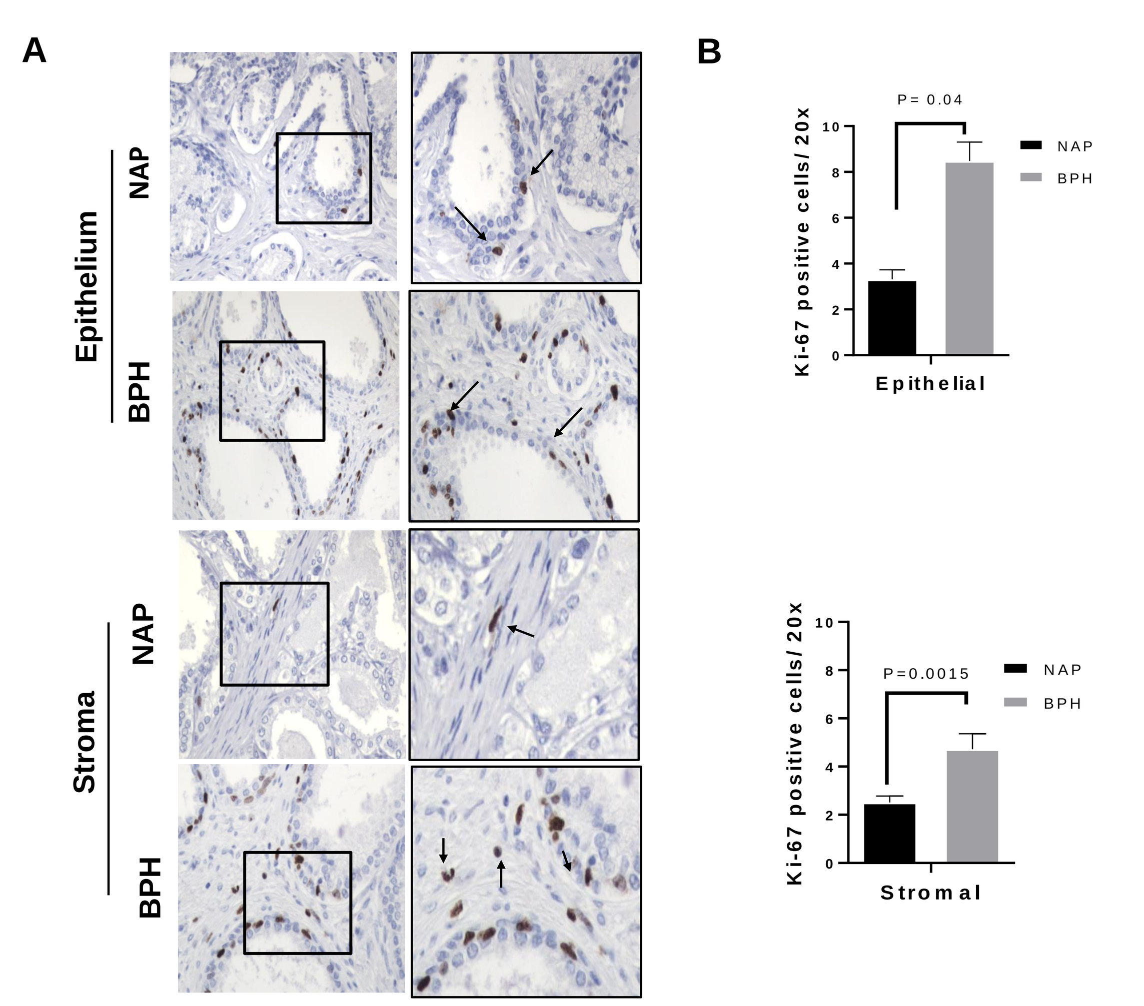
A. Immunofluorescent staining of AR in BPH-1 cell spheroids in 3D culture. **B.** Quantification of AR and PSA mRNA level of BPH-1 and BHPrE1 in organoid culture vs monolayer culture. (** p < 0.01, *** p < 0.001).

Androgens stimulate epithelial proliferation in the presence of conditioned medium from BPH stromal cells



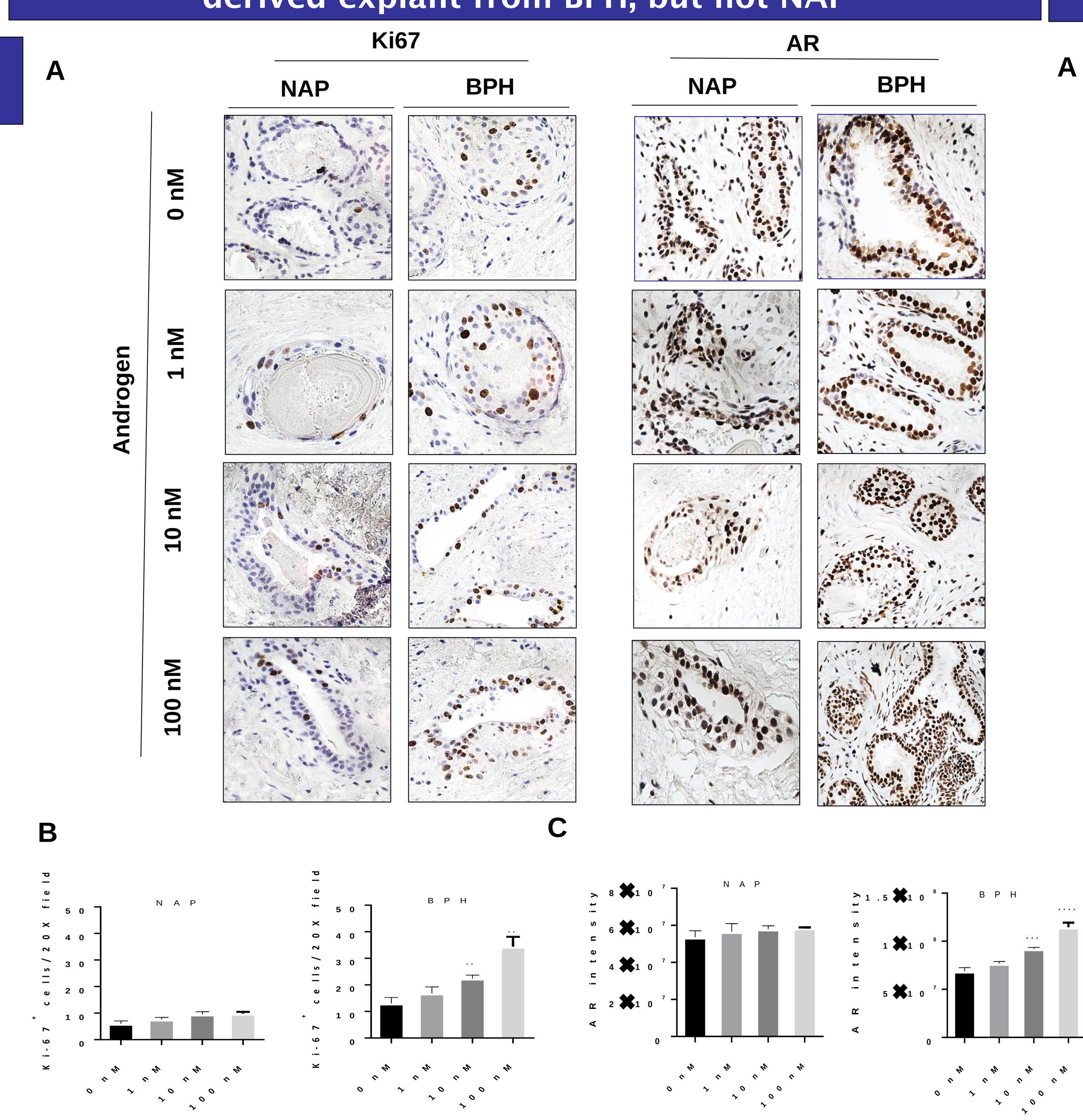
Quantification of BHPrE1 and BPH-1spheroid volume when cultured with and without CM from stromal cells derived from NAP and BPH tissues. The stromal cells derived from NAP and BPH were treated in the absence or presence of 1 nM R1881. After 24 hrs treatment, CM was used to culture the BHPrE1 cells in 3D for 6 days.

The proliferation in epithelial cells and stromal cells in human prostate tissue in BPH and normal adjacent



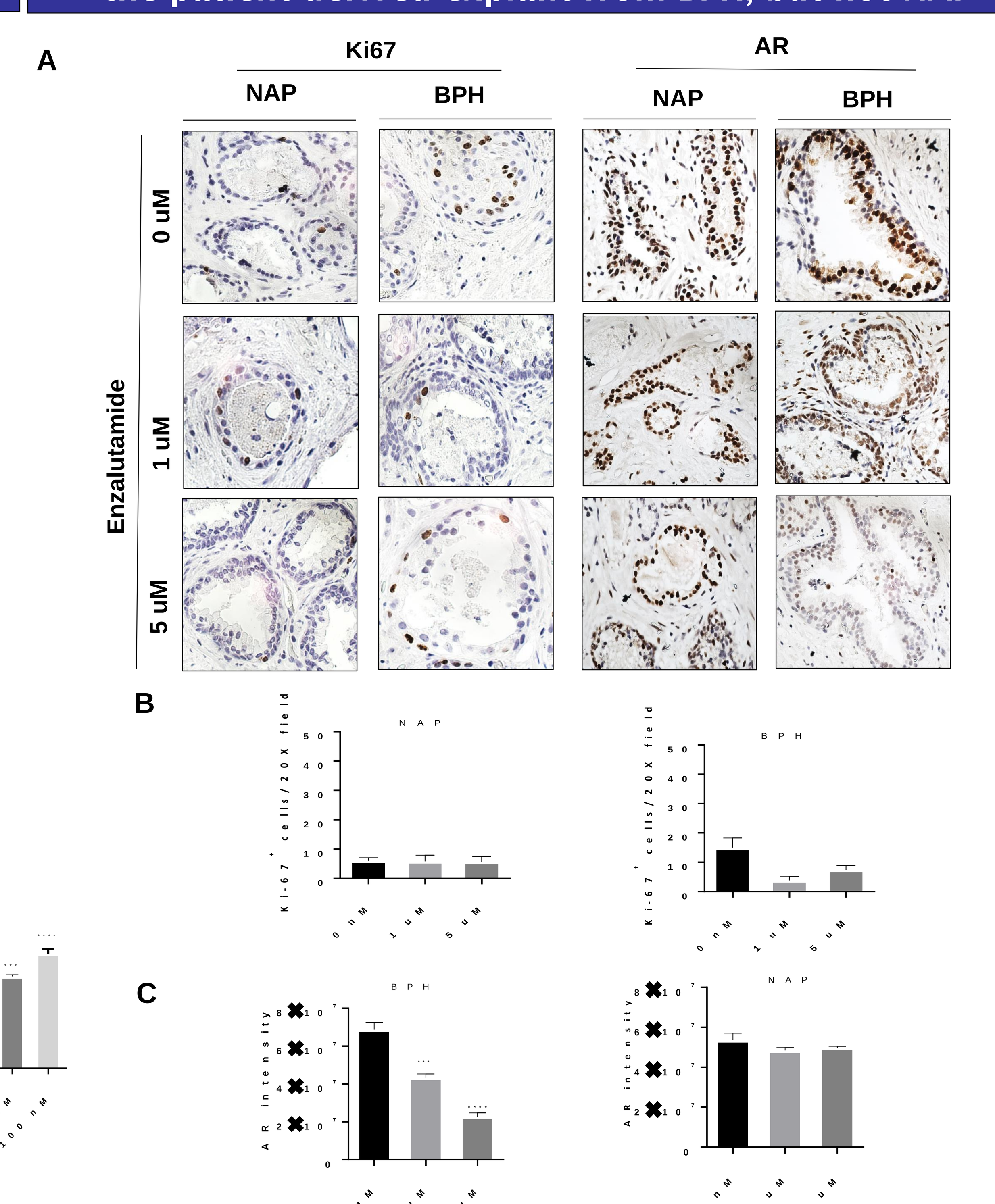
A. Histology of human prostate from BPH tissue and NAP. **B.** Quantification of Ki-67⁺ epithelial and stromal cells in human prostate with BPH (10 patients) and NAP (8 patients) tissue. Original magnification, 20x.

Androgens stimulate epithelial proliferation in the patient derived explant from BPH, but not NAP



A. Histology of human prostate from BPH tissue and NAP. **B.** Quantification of Ki-67⁺ epithelial in PDE of human prostate with BPH and NAP tissue. Original magnification, 20x. **C.** Quantification of epithelial AR in PDE of human prostate with BPH and NAP tissue. Original magnification, 20x.

Androgens inhibitor suppress epithelial proliferation in the patient derived explant from BPH, but not NAP



A. Histology of human prostate from BPH tissue and NAP. **B.** Quantification of Ki-67⁺ epithelial in PDE of human prostate with BPH and NAP tissue. Original magnification, 20x. **C.** Quantification of epithelial AR in PDE of human prostate with BPH and NAP tissue. Original magnification, 20x.

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