Development of a Human 3D Prostate Microtissue Assay for Anti-androgen Screening

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Altered androgen hormone biosynthesis and metabolism can modulate androgen levels, contributing to endocrine disruption that may result in impaired reproductive and sexual development. Steroid 5α-reductase isozymes are expressed in key peripheral tissues and catalyze the conversion of testosterone into the more potent androgen 5α - dihydrotestosterone (DHT). Activation of maximal androgen signaling requires conversion of testosterone to DHT to bind to the androgen receptor (AR) and induce transactivation of downstream gene signaling. The evaluation of chemical-mediated disruption of androgen signaling is typically performed through a combination of *in vitro* and *in vivo* tests that interrogate both receptor and non-receptor events including accessory sex organ (ASO) development. Chemical-mediated disruption of ASO development may occur via inhibition of 5α-reductase, or direct disruption of AR signaling. The objective here was to establish a higher-tier human-based, *in vitro* assay of ASO growth using a multiplexed 3D prostate epithelial cell microtissue model. The androgen-sensitive LNCaP cell line was seeded in a 96-well hanging drop culture model to evaluate microtissue growth over a period of 11 days. Prostate Specific Antigen (PSA) secretion, a biomarker for AR signaling, yielded a wide dynamic range with a Positive/Negative control (P/N) ratio of 3,326 and Z' of 0.78. Proliferation, measured by image analysis, resulted in microtissues with a mean area P/N ratio of 5.82 and a Z' of 0.69. LDH secretion and ATP levels were measured to establish viability thresholds. Microtissue growth challenged by a reference set of anti-androgens (bicalutamide and hydroxyflutamide) or 5α-reductase inhibitors (epristeride, finasteride, and dutasteride) yielded marked growth inhibition following treatment with bicalutamide, finasteride, and dutasteride, but not hydroxyflutamide or epristeride. The results demonstrate a multiplexed, medium-throughput 3D microtissue assay that holds promise as a higher-tier approach for evaluating the phenotypic effects of anti-androgen candidates generated from high-throughput screening assays.

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