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Organoid model shows effect of BPA on prostate development

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Abstract

Studies using rodent and adult human prostate stem-progenitor cell models indicate that exposure to bisphenol A (BPA) during development increases susceptibility of the prostate gland to premalignancy later in life. A novel human organoid model has now been generated to study the effects of BPA on embryonic human prostate development.

Bisphenol A (BPA), an environmental oestrogen, has been used for commercial plastic production since the 1950s. Biomonitoring studies indicate that BPA exposure is widespread and continuous in humans.¹ BPA is detectable in pregnant and nonpregnant women, and also in newborns,² suggesting that exposure to BPA might start at conception. In rats, exposure to low, environmentally relevant doses of BPA during development increases the susceptibility of the prostate gland to premalignancy later in life.³ Although high urinary levels of BPA have been associated with prostate cancer in humans,⁴ clinical evidence for the adverse effects of early life exposure to BPA on prostate health is still lacking. In a new study, Calderon-Gierszal and Prins⁵ have addressed this important issue using an *in vitro* model of *de novo* generation of prostate organoids through the directed differentiation of human embryonic stem cells (hESCs). They used this pioneering model to evaluate the effect of low-dose BPA on the development and maturation of prostate organoids starting from the hESC stage.

An important feature of this model is that human prostate organoids were generated entirely from embryonic stem cells, which are pluripotent and have a potential to differentiate into any of the three embryonic germ cell layers—endoderm, mesoderm and ectoderm. Prins *et al.*⁶ previously studied the direct effects of BPA on epithelial stem-like cells cultured from the prostates of young adult men. In contrast to hESCs, adult prostate stem-like cells are multipotent and able to differentiate into multiple lineages of epithelial cells—luminal, basal

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Competing interests

The authors declare no competing interests.

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and transit amplifying cells. The use of hESCs in the latest study widens the study window for investigating the effects of BPA to include the very early embryonic stages.

The study by Calderon-Gierszal and Prins⁵ provides for the first time a robust method by which human prostate organoids of entirely human origin can be generated. Directed differentiation of hESCs was induced by temporal exposure to defined growth factors and steroids known to be required in normal prostate development. The resulting hESC-derived prostate organoids were composed of epithelium and stroma purely of human origin. In previously established human prostate models,^{7–9} rodent mesenchyme was required for prostate induction, meaning that the resulting organoids were ‘hybrid combinants’ composed of human epithelium and rodent stroma. The progression into the use of pure human prostate organoids remarkably enhances the human relevance of this model for investigating endocrine disruption by BPA.

Although the *de novo* generation of three-dimensional prostate organoids was carried out entirely *in vitro*, hESCs were induced by growth factors and steroids to differentiate into specific developmental stages.⁵ Firstly, differentiation of hESCs into definitive endoderm was achieved using activin. Next, endoderm-differentiated cells were driven into prostatic fate determination using WNT-10b and fibroblast growth factor 10. Final prostate organoid maturation was achieved using a cocktail of R-spondin-1, noggin, pro-epidermal growth factor, retinoic acid and testosterone. This model recapitulates key stages of prostate development including epithelial budding and branching morphogenesis; it also models the cytodifferentiation and functional differentiation of epithelial and stromal cells into prostatic-like structures that are similar to adult human prostate. The *in vitro* induction of prostate organoids provides a robust model with a well-defined morphogenic environment that enables investigation of the effects of BPA or other endocrine-disrupting chemicals.

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BPA has been shown to have endocrine-disrupting effects at low, environmentally relevant doses in rodent prostates³ and human adult prostate stem-progenitor cells.⁶ Whether BPA affects hESCs in normal prostate development in humans has been unknown. In the present study, low levels of BPA exposure (1 nM and 10 nM), when maintained throughout prostate induction, affected early budding morphogenesis and disrupted stem cell homeostasis.⁵ Interestingly, biphasic effects of the two BPA doses were observed, with 1 nM BPA stimulating and 10 nM BPA repressing formation of epithelial buds. The stem-like cell population was increased by 10 nM BPA, as shown by the appearance of focal stem cell nests in mature organoids. This study provides evidence that BPA targets not only adult prostate stem-progenitor cells but also hESCs. Investigations using a broader range of BPA doses are needed to validate the nonmonotonic dose–response in the current human prostate organoid setting.

The present study establishes a robust model of *de novo* generation of pure human prostate organoids from hESCs without rodent mesenchymal induction. Low-dose BPA exposure targets hESCs and results in the perturbation of early prostate morphogenesis and stem cell homeostasis in mature organoids. These findings suggest that early embryonic stages might be susceptible to disruption by BPA exposure in the womb.

This model could be used to delineate the effects of BPA on the early embryonic stages that occur prior to formation of the urogenital sinus (prostate precursor), and to test whether a critical window of susceptibility to BPA exists early in fetal development. Given that human fetal prostates express oestrogen receptors¹⁰ and that developmental exposure to BPA induces epigenetic changes in rodent prostates,³ the current *in vitro* model could facilitate mechanistic studies of BPA-mediated oestrogen-receptor-dependent and oestrogen-receptor-

independent signalling pathways and epigenetic reprogramming in a human setting. The current model could also be used to detect developmental disruptors—including dietary, environmental and pathogenic agents—that might affect disease outcomes in the prostate.

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