Human iPSC-derived prostate organoids with germline *BRCA2* mutation undergo tumorigenic transformations

Bipul R. Acharya[1, 2#], George Lawless[1], Pablo Avalos[1], Prince Anand[2], Yesai Fstkchyan[2] , Shaughn Bell[1], Maria G. Otero[1], Samuel Guillemette[1], Zachary Myers[1], Michael Workman[1], William J. Catalona[4], Dan Theodorescu[2, 3#], Clive N. Svendsen[1#]

[1] Cedars-Sinai Board of Governors Regenerative Medicine Institute, Los Angeles, CA, USA
[2] Department of Urology, Cedars-Sinai Medical Center, Los Angeles, CA, USA
[3] University of Arizona Comprehensive Cancer Center, Tucson, AZ, USA
[4] Department of Urology, Northwestern University, Chicago, IL, USA
#Corresponding Authors

Correspondence to: Clive N. Svendsen PhD**,** Clive.Svendsen@cshs.org [Lead Contact], Bipul R. Acharya PhD, Bipul.Acharya@cshs.org, Dan Theodorescu MD PhD, theodorescu@arizona.edu

Background: The lack of physiologically relevant *in vitro* prostate models has hindered studies of prostate development and tumorigenesis. To address this, we reprogrammed peripheral blood mononuclear cells (PBMCs) from individuals with and without pathogenic germline BRCA2 mutations (MUT_BRCA2 and CON_BRCA2, respectively) into induced pluripotent stem cells (iPSCs). These iPSCs were then differentiated into prostate organoids, termed iPROS. Unlike earlier models that required rodent urogenital mesenchyme (UGM) for prostate specification, limiting clinical relevance, our system employs a chemically defined, UGM-free differentiation protocol. Additionally, we modeled tumor initiation in iPROS using genotoxic stress, providing a platform for studying early prostate tumorigenesis and enabling personalized investigations of cancer initiation and therapeutic response.

Methods: We developed an 8-week stepwise protocol to generate patient-specific iPSC-derived prostate organoids (iPROS). Differentiation stages included iPSC to Definitive Endoderm (DE), DE to Hindgut Endoderm (HE), HE to Prostate Progenitor (PP), and PP to mature iPROS. Tumorigenesis was induced in iPROS using dietary carcinogens PhIP and MNU, known inducers of prostate cancer (PCa) in rodent models. Human prostate morphology and PCa-associated features were evaluated in iPROS by chromogenic immunohistochemistry (IHC) and immunofluorescence (IF). Cell proliferation and DNA damage were assessed via Ki67 and γH2AX using both flow cytometry and IF. Prostate-specific and cancer-related gene expression was analyzed by qPCR and bulk RNA sequencing. Prostate-specific antigen (PSA) secretion was quantified by ELISA. For *in vivo* assessment, iPROS were transplanted either subcutaneously or under the renal capsule of NOD scid gamma (NSG) mice to evaluate tumor formation.

Results: Reprogramming of PBMCs from both MUT_BRCA2 and CON_BRCA2 individuals produced iPSCs with comparable morphology, proliferation rates, and expression of pluripotency markers. Differentiation of MUT_BRCA2 iPSCs into iPROS led to disrupted epithelial morphology, loss of polarity, increased proliferation, and elevated PSA secretion compared to CON_BRCA2-derived iPROS. Transcriptomic profiling revealed early prostate cancer gene expression signatures in MUT_BRCA2 iPROS. Upon carcinogen exposure, MUT_BRCA2 iPROS showed further increases in PSA levels and proliferation, along with upregulation of AMACR and downregulation of p63—hallmarks of aggressive PCa. *In vivo*, only the MUT_BRCA2 iPROS formed tumors in NSG mice within 12 weeks, both subcutaneously and in sub-renal capsule injections.

Conclusions: The iPROS platform accurately recapitulates human prostate tissue architecture and function, providing a scalable and ethical model for studying prostate development and tumorigenesis. It enables early detection of tumorigenic events, evaluation of therapeutic responses, and the study of genetic predisposition such as BRCA2 mutations. This system holds promise for advancing personalized medicine in prostate cancer research.

Funding Acknowledgements: This work was supported by an award from the Urological Research Foundation to CNS and DT, and institutional support to BRA (Donna and Jesse Garber Award for Cancer Research, 2025) and CNS from Cedars Sinai Medical Centre.

Conflicts of Interest Disclosure Statement: Authors declare a patent application filling related to this work.