Prenatal exposure to BPA and BPS transgenerationally alters the transcriptome and epigenome in male germ cells

Mingxin Shi[1], Liang Zhao[1], Sarayut Winuthayanon[2], James A. MacLean II[1], Kanako Hayashi[1]

[1]School of Molecular Biosciences, Center for Reproductive Biology, Washington State University, Pullman, WA, USA

[2]Scientific Software Engineer, University of Missouri, Columbia, MO, USA

Prenatal exposure to bisphenol (BP) A and BPS leads to transgenerationally maintained lower sperm counts and motility in male mice. Rigorous prior research suggests that certain epimutations sustained in the germline can potentially be transmitted to subsequent generations. Thus, we examined the transcriptomic and epigenetic impacts of prenatal exposure to BPA and BPS on the transgenerational murine male germline using single-cell multiomics. Pregnant CD-1 females (F0) were administrated 50 µg/kg/day BPA or BPS orally from gestational day 7 to birth, and postnatal day 6 germ cells from F1, F2, and F3 males were collected for the multi-omics (scRNA-seq and scATAC-seq). In the F1 males, scRNA-seq analysis revealed that prenatal exposure to BPA and BPS induced upregulation of a large number of genes that are associated with the acceleration of spermatogonial stem cell differentiation and disrupt the balance of stemness in the neonatal germ cells. Pseudo-trajectory and Gene Ontology analysis further suggested that BP-induced accelerated stem cell differentiation was accompanied by metabolic changes. scATAC-seq analysis showed that differentially accessible chromatin sites were primarily in the promoter regions. Combined gene expression and transcription factor (TF) motif analysis, KLF/SP and DMRT family members were highly enriched and elevated motif activities by BP exposure. The candidate targets of top-active TFs were then predicted, and most genes showed a pattern of expression along with germ cell differentiation trajectory. In the F2 and F3 males, upregulated genes related to mitosis/meiosis and metabolic pathways were sustained in BP-exposed groups as in the F1 males. In contrast, fewer genes were explicitly identified in the F3 males. Interestingly, BPA exposure showed substantial effects on gene expression in the F1 offspring, whereas BPS exposure induced longer-lasting effects on the biological process associated with differentiation through F1 to F3 generations. Collectively, our findings provide the first systematic results for understanding the transgenerational transcriptomic and epigenetic alterations by prenatal exposure to BPA or BPS in male germ cells. Supported by NIH/NIEHS R21 ES031607.