Environ Health Perspect

DOI: 10.1289/EHP8196

Supplemental Material

Examining the Developmental Trajectory of an *in Vitro* Model of Mouse Primordial Germ Cells following Exposure to Environmentally Relevant Bisphenol A Levels

Steen K.T. Ooi, Hui Jiang, Yanyuan Kang, and Patrick Allard

Table of Contents

Figure S1. Line graph showing ELISA standard curves generated using either kit-supplied or in-lab dissolved Bisphenol A (BPA) used in experiments. Data generated using BPA ELISA Kit, Detroit R & D, Inc. B indicates absorbance at 450nm observed at defined concentrations; B_0 indicates absorbance at 450nm observed in maximum binding wells (Sample Dilution Buffer only). For numerical values, see Excel Table S1.

Figure S2. Cell number analysis of following 48-hour Bisphenol A (BPA) exposure of ESCs. (A) Cartoon showing BPA exposure strategy.Illustration in part created with ©BioRender - biorender.com, as per the Biorender terms and conditions. Abbreviations are: Embryonic stem cell (ESC) and Leukmia inhibitory factor (LIF). (B) Scatter-bar plot indicating cell counts of *Blimp1-mVenus stella-ECFP* (BVSC) ESCs exposed to the different BPA doses indicated: error bars indicate mean +/- standard deviation. n = 4 for each condition. One-way ANOVA, $p > 0.05$. Calculated one-way ANOVA with Šidák-adjusted p values for comparison to water: $p > 0.05$ (vs. 1 nM); $p > 0.05$ (vs. 10 nM), $p > 0.05$ (vs. 100 nM); $p > 0.05$ (vs. 1 µM); and $p > 0.05$ (vs. 10 µM). For numerical values, see Excel Table S3.

Figure S3. Cell number analysis of two male (XY) epiblast-like cell (EpiLC) cell lines following Bisphenol A (BPA) exposure. Scatter-bar plot indicating cell counts of epiblast-like cells (EpiLCs) derived from either a wild-type Embryonic stem (ES) cell line (v6.5) or male *Blimp1-mVenus stella-ECFP* (BVSC) cell line (R8) exposed to the different Bisphenol A (BPA) doses indicated: error bars represent mean +/- standard deviation. n = 12 for each condition. One-way ANOVA *p*-values are > 0.05 for both (v6.5) and BVSC-R8). For numerical values, see Excel Table S3.

Figure S4. Cell number and transcriptome analysis of day 5 (d5) PGC-like cells (PGCLCs) derived from Bisphenol A (BPA)-exposed male (XY) epiblast-like cells (EpiLCs). A Scatter-bar plots showing absolute numbers of BVSC-R8 (male) cells in the different gated sub-populations indicated following exposure of EpiLCs to 100nM BPA. Error bars represent mean +/- standard deviation. n = 16 for each condition. Mann-Whitney Test p-values for R8 are > 0.05 for live cells; Blimp1-Stella-/DN (non-germ cells); Blimp1^{+}Stella-/SP (presumed transitioning germ cells); and Blimp1^{+}Stella^{+}/DP (PGCLCs). For numerical values, see Excel Table S4. **B** Principal Component Analysis (PCA) of transcriptome of d5 DP/BVSC cells, derived from untreated or 100 nM BPA-exposed EpiLCs. For full list of genes analysed and expression values, see Excel Table S6. **C** Expression level-ranked heatmap of germ cell markers indicated in RNA-seq data generated from d5 aggregate FACS-purified DP/PGCLCs, derived from BVSC-R8 EpiLCs exposed to control (untreated) or 100 nM BPA. Candidate germ cell marker list taken from Hayashi et al. (Hayashi et al, 2011). Wilcoxon matched-pairs signed rank test p-value > 0.05.

Figure S5. Control FACS density plots of **epiblast-like cells** (EpiLCs) treated with DNA damaging agent (etoposide, 10μM), apoptosis inducing agent (doxorubicin, 500nM) or both. Numbers indicate proportion of live-gated events in quadrants indicated.

Figure S6. Analysis of different d5 aggregate cell populations derived from epiblast-like cells (EpiLCs) exposed to Bisphenol A (BPA). Scatter-bar plots showing absolute numbers of cells in the different gated sub-populations indicated following exposure of EpiLCs to different BPA doses indicated. Plots show mean +/- standard deviation. n = 8. For one-way ANOVA with Šidák-adjusted p values for comparison to water, see Supplemental Table 2. For numerical values, see Excel Table S10.

Figure S7. Expression analysis of genes in apoptosis and DNA damage Biological Process terms in epiblast-like cells (EpiLCs) exposed to 100 nM Bisphenol A (BPA). n indicates number of genes within each Gene Ontology GO term whose expression was detected in RNA-seq data set. For each plot, first set of data indicate 'untreated', followed by '100nM BPA-exposed'. For apoptosis, GO terms are as follows: GO:0008625 (extrinsic apoptotic signaling pathway via death domain receptors); GO:0012501(programmed cell death); GO:0043065 (positive regulation of apoptotic process); GO:0096915 (apoptotic process); GO:0097190 (apoptotic signaling pathway); GO:0097191 (extrinsic apoptotic signaling pathway); GO:1902255 (positive regulation of intrinsic apoptotic signaling pathway by p53 class mediator); GO:2001237 (negative regulation of extrinsic apoptotic signaling pathway); GO:2001244 (positive regulation of intrinsic apoptotic signaling pathway). For DNA damage, GO terms are as follows: GO:0000077 (DNA damage checkpoint); GO:0006974 (cellular response to DNA damage stimulus); GO:0030330 (DNA damage response, signal transduction by p53 class mediator); GO:0031572 (G2 DNA damage checkpoint); GO:0042770 (signal transduction in response to DNA damage); GO:0044773 (mitotic DNA damage checkpoint); GO:0072422 (signal transduction involved in DNA damage checkpoint); GO:0090734 (site of DNA damage); GO:1902402 (signal transduction involved in mitotic DNA damage checkpoint); GO:2001020 (regulation of response to DNA damage stimulus). Asterisks indicate results of Wilcoxon matched-pairs signed rank tests. For p-values, see Excel Table S13.

Figure S8. Circular Gene Ontology (CirGO) plot of enriched Cellular Component GO terms for genes upregulated in day 5 (d5) PGC-like cells (PGCLCs) derived from epiblast-like cells (EpiLCs) exposed to 100 nM Bisphenol A (BPA).

Figure S9. Candidate gene expression analysis in day 5 (d5) PGC-like cells (PGCLCs) derived from epiblast-like cells (EpiLCs) exposed to 100 nM Bisphenol A BPA. (A-C) Heatmap view of data presented in Figure 5. (D) Scatter-box plot showing expression level of Stra8. Mean +/- s.d., n = 3. Mann-Whitney p value = 0.2. (E) Heatmap view of expression levels of annotated nuclear hormone receptors indicated in PGCLCs derived from untreated or BPA-exposed EpiLCs. Asterisks indicate NHRs found to be differentially expressed. Estrogen and retinoic acid receptor genes indicated in bold. Benjamini-Hoechberg adjusted p-values: < 0.0001 (*Hnf4a*). For all heatmaps, first and second columns are untreated and 100nM BPA samples, respectively. Scale bars for all heat maps represent $\log_{10}$ cpm.

Figure S10. Heatmap view of data presented in Figure 5D-G, showing expression for meiosis-associated Gene Ontology (GO) gene sets indicated. First and second columns are untreated and 100 nM Bisphenol A (BPA) samples, respectively. Scale bars for all heat maps represent $\log_{10}$ cpm.

Figure S11. Expression analysis of genes in meiosis-associated Biological Process terms. n indicates number of genes within each Gene Ontology (GO) term whose expression was detected in RNA-seq data set. GO terms are as follows: GO:0007127 (meiosis I); GO:0045143 (homologous chromosome segregation); GO:0007144 (female meiosis I); GO:0000212 (meiotic spindle organization); GO:0000712 (resolution of meiotic recombination intermediates); GO:0007057 (spindle assembly involved in female meiosis I); GO:0007129 (synapsis); GO:0007131 (reciprocal meiotic recombination); GO:0007140 (male meiotic nuclear division); GO:0007143 (female meiotic nuclear division); GO:0016321 (female meiosis chromosome segregation); GO:0030893 (meiotic cohesin complex); GO:0034991 (nuclear meiotic cohesin complex); GO:0034993 (meiotic nuclear membrane microtubule tethering complex); GO:0040020 (regulation of meiotic nuclear division); GO:0040038 (polar body extrusion after meiotic divisions); GO:0044778 (meiotic DNA integrity checkpoint); GO:0045141 (meiotic telomere clustering); GO:0045835 (negative regulation of meiotic nuclear division); GO:0060903 (positive regulation of meiosis I); GO:0070197 (meiotic attachment of telomere to nuclear envelope); GO:0072687 (meiotic spindle); GO:1990918 (double-strand break repair involved in meiotic recombination); GO:0007135 (meiosis II); GO:0044771 (meiotic cell cycle phase transition); and GO:0045144 (meiotic sister chromatid segregation). For Wilcoxon matched-pairs signed rank test p-values, see Excel Table S20.

Figure S12. Expression analysis of factors involved in epigenetic processes in day 5 (d5) PGC-like cells (PGCLCs) derived from epiblast-like cells (EpiLCs). (A-E) Violin plots showing expression levels of different genes in the sets indicated. n indicates number of genes within each Gene Ontology (GO) term whose expression was detected in RNA-seq data set. Wilcoxon matched-pairs signed rank test p-values: < 0.01 (both GO: positive regulation of GO: protein maturation and negative regulation of transcription by RNA polymerase II); > 0.05 (GO: DNA methylation); > 0.05 (GO: histone methylation); and > 0.05 (GO: piRNA metabolic process).

Figure S13. Expression analysis of mammalian host factors involved in retrotransposon regulation. Heat map showing expression levels of factors indicated. Wilcoxon matched-pairs signed rank test p-values > 0.05 for Epigenetic/Nuclear Factor; KRAB-Zinc Finger; Transcription Factor; piRNA/RNAi Pathway; and Post-transcriptional. List of factors obtained from Goodier (Goodier, 2016).

Additional File- Excel Document