Environ Health Perspect

DOI: 10.1289/EHP5862

Supplemental Material

Role of TET Dioxygenases and DNA Hydroxymethylation in Bisphenols-Stimulated Proliferation of Breast Cancer Cells

Zhe Li, Cong Lyu, Yun Ren, and Hailin Wang

Table of Contents

Figure S2. Ten-eleven translocation (TETs) expression in MCF-7 cells treated with bisphenol A/S (BPA/BPS). (A) Quantitative reverse transcript-PCR (qRT-PCR) analysis of the expression of TET1, TET2 and TET3 in MCF-7 cells at 48 h of BPA and BPS treatment. (B) Western blot analysis of TET1, TET2 and TET3 proteins in MCF-7 cells after 48 h of BPA, BPS, or vehicle treatment. (C) Quantitative analysis of TET1, TET2 and TET3 proteins shown in (B) with Image J software. The relative intensity was calculated by the ratio relative to the GAPDH intensity. Data represent mean ± SD of three independent experiments. Statistical analysis was performed with Student's paired t-test, and asterisks indicated a significant difference from the control. *, $P < 0.05$.

Figure S3. Effects of bisphenol A (BPA) and bisphenol S (BPS) on the expression of ten-eleven translocation 2 (TET2) protein in MCF-7 cells transfected with shTET2 (A, B) and pcDNA3-FLAG-TET2 plasmids (C, D). Immunoblotting of TET2 protein in MCF-7 cells were carried out after 48 h of vehicle, BPA or BPS treatment. MCF-7 cells transfected with shNC and pcDNA3-FLAG plasmids were used as the control. Quantitative analysis of TET2 protein was carried out with Image J software. The relative intensity was calculated by the ratio relative to the GAPDH intensity. Data represent mean ± SD of three independent experiments. Statistical analysis was performed with Student's paired t-test. #, $P < 0.05$ vs control.

Figure S4. Cell proliferation and 5-hydroxymethylcytosine (5hmC) level of MCF-7 cells transfected with the second shRNA constructs for ten-eleven translocation 1 (TET1), TET2 and TET3 upon bisphenol A (BPA) or bisphenol S (BPS) exposure. (A) Identification of TET protein expression in the MCF7 cells transfected with the second shRNA using western blot. (B) Cell proliferation assay by MTS of MCF7 cells transfected with the second shRNA upon exposure to BPA and BPS. (C) Relative level of genomic 5hmC measured by ELISA assay of genomic DNA from MCF-7 cells transfected with the second shRNA upon exposure to BPA and BPS. Data represent mean ± SD of three independent experiments. Statistical analysis was performed with two-way ANOVA (Bonferroni posttest) (* for $P < 0.05$ and ** for $P < 0.01$ were indicated the differences relative to the control of NC).

Figure S5. Western blot assay of total and phosphorylated estrogen receptor α and β (ERα and ERβ) in MCF7 cells exposed to bisphenol A/S (BPA/BPS). (A) Changes of total and phosphorylated ERα proteins in MCF7 cells exposed to BPA and BPS. (B) Changes of total and phosphorylated ERβ proteins in MCF7 cells exposed to BPA and BPS. (C) Quantitative analysis of total and phosphorylated ERα proteins shown in (A) with Image J software. (D) Quantitative analysis of total and phosphorylated ERβ proteins shown in (B) with Image J software. The relative intensity was calculated by the ratio relative to the GAPDH intensity. The phosphorylated ratio was calculated by phosphor/total proteins. Data represent mean ± SD of three independent experiments. Statistical analysis was performed with two-way ANOVA (Bonferroni posttest): * for $P < 0.05$ and ** for $P < 0.01$ were indicated the differences relative to the control.

Figure S6. Verification of $ER\alpha^{-/-}$ and $ER\beta^{-/-}$ MCF-7 cells by sequencing (A) and western blot (B).

Figure S7. Quantitative reverse transcript-PCR (qRT-PCR) analysis of the expression of ten-eleven translocation 1 (TET1), TET2 and TET3 in ERα$^{-/-}$ (A) and ERβ$^{-/-}$ (B) MCF-7 cells at 48 h after vehicle, bisphenol A (BPA) or bisphenol S (BPS) treatment. Data represent mean ± SD of five independent experiments. Statistical analysis was performed with Student's paired t-test: * for $P < 0.05$ was indicated the differences relative to the control.

Figure S8. Western blot results of ten-eleven translocation 1 (TET1), TET2 and TET3 proteins in ERα$^{-/-}$ and ERβ$^{-/-}$ MCF-7 cells at 48 h of bisphenol A (BPA) or bisphenol S (BPS) treatment.

Figure S9. Interaction of ERα with ten-eleven translocation 2 (TET2) protein in bisphenol A/S (BPA/BPS) exposed MCF7 cells, showing no detectable interaction of ERα and TET2. Immunoprecipitation was performed using anti-ERα antibody followed by western blotting with indicated antibodies. Sp1 protein, the co-factor of ERα, was used as the positive control.

Figure S10. ERα-mediated DNA methyltransferases (DNMTs) expression in MCF7 cells under bisphenol A/S (BPA/BPS) exposure. (A) Quantitative reverse transcript-PCR (qRT-PCR) analysis of the expression of DNMT1, DNMT3A and DNMT3B in MCF-7 cells after 48 h of vehicle, BPA, or BPS treatment. Data represent mean ± SD of five independent experiments. (B) Immunoblotting of DNMT1, DNMT3A and DNMT3B proteins in MCF-7 cells at 48 h after vehicle, BPA, or BPS treatment. (C) Quantitative analysis of DNMT1, DNMT3A and DNMT3B proteins shown in (B) with Image J software. The relative intensity was calculated by the ratio relative to the GAPDH intensity. Data represent mean ± SD of three independent experiments. (D) qRT-PCR analysis of the expression of DNMT1, DNMT3A and DNMT3B in ERα$^{-/-}$ MCF-7 cells at 48 h after vehicle, BPA, or BPS treatment. Data represent mean ± SD of five independent experiments. (E, F) Immunoblotting of DNMT1, DNMT3A and DNMT3B proteins in ERα$^{-/-}$ MCF-7 cells at 48 h after vehicle, BPA, or BPS treatment. The relative intensity was analyzed with Image J software and calculated by the ratio relative to the GAPDH intensity. Data represent mean ± SD of three independent experiments. Statistical analysis was performed with two-way ANOVA (Bonferroni posttest). * for $P < 0.05$ and ** for $P < 0.01$.

Figure S11. Cell proliferation, ten-eleven translocation 2 (TET2) expression and promoter methylation of MCF-7 cells transfected with the second shRNA constructs for DNA methyltransferase 1 (DNMT1), DNMT3A and DNMT3B upon bisphenol A (BPA) or bisphenol S (BPS) exposure. (A) Identification of the MCF7 cells transfected with the second shRNA using western blot. (B) Cell proliferation assay by MTS of MCF7 cells transfected with the second shRNA upon exposure to BPA or BPS. (C) Quantitative reverse transcript-PCR (qRT-PCR) analysis of the expression of TET2 in MCF-7 cells transfected with the second shRNA at 48 h of BPA or BPS treatment. (D, E) Immunoprecipitation of TET2 protein in MCF7 cells transfected with the second shRNA-DNMTs at 48 h of BPA or BPS treatment. The relative intensity was analyzed with Image J software and calculated by the ratio relative to the GAPDH intensity. Data represent mean ± SD of three independent experiments. (F) Quantitative DNA sequencing analysis of methylation specific PCR product of TET2 promoter in MCF7 cells transfected with the second shRNA for DNMTs at 48 h of BPA/BPS treatment. Data represent mean ± SD of five independent experiments. Statistical significance was evaluated by two-way ANOVA (Bonferroni posttest). *, $P < 0.05$ or **, $P < 0.01$ vs control of NC.

Additional File- Excel Document