Regulation of aryl hydrocarbon receptor function by Selective Estrogen Receptor Modulators

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Supplemental Figure 1

(A) MCF7 cells were transfected with either of two unique siRNA duplexes to ERα (siERα A and siERα C) or siRNA control (Mock). After 48 hours, the cells were treated for 4 hours with either vehicle, 100 nM 4OHT or 1 nM E2. PR mRNA was measured using qRT-PCR. (B) *CYP1A1* mRNA expression was analyzed by qRT-PCR in MCF7 cells treated with vehicle, 10 nM BNF, or 100 nM 4OHT for 1, 4, 8, or 24 hours. (C) MCF7 cells were transfected with either siRNA duplexes targeting ERα (left, 3 different siRNAs labeled A, B, C), AHR (right), or mock control. After 48 hours, whole cell extract was prepared and subjected to Western analysis, followed by immunoblotting for ERα, AHR, or Cytokeratin 18 (CYT18) as a loading control. (D) MCF7 cells were transfected with siRNA to AHR (siAHR) or siRNA control (Mock). After 48 hours, the cells were treated for 4 hours with either vehicle, 4OHT, or BNF. For A, B, and C, cDNA from RNA extracted from treated cells was analyzed by qRT-PCR. (E) MCF7 cells were treated for 75 minutes with vehicle, 100 nM 4OHT, or 100 nM BNF. Cells were harvested after cross-linking and subjected to immunoprecipitation with either control IgG or antibodies to AHR or RNA polymerase II (Pol II). After reversal of the cross-link, DNA was isolated and subjected to qPCR analysis. There was no significant recruitment of AHR to a distal region of the CYP1A1 promoter. (F) Differentiating RAW264.7 cells were treated with vehicle, 100 nM E2, 100 nM BNF, 100 nM 4OHT, or 100 nM RAL in the presence of either 1 μM ICI or 1 μM ANF. Total TRAP-positive multinucleated cells were counted after 8 days.

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