

Bisphenol A Transcriptome Reveals Novel Effects on ERβ Expression that Correspond to AR Responsiveness in Prostate Cancer Cells

TOXICOGENOMICS

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Results: BPA and DHT elicited overlapping but distinct transcriptional signatures in prostate cancer cells expressing the BPA-responsive mutant AR-T877A. BPA dramatically attenuated estrogen receptor beta (ERβ) expression (-4.98 fold over vehicle control), and this finding was specific to prostate tumor cells wherein BPA induces cellular proliferation.

Figure 2

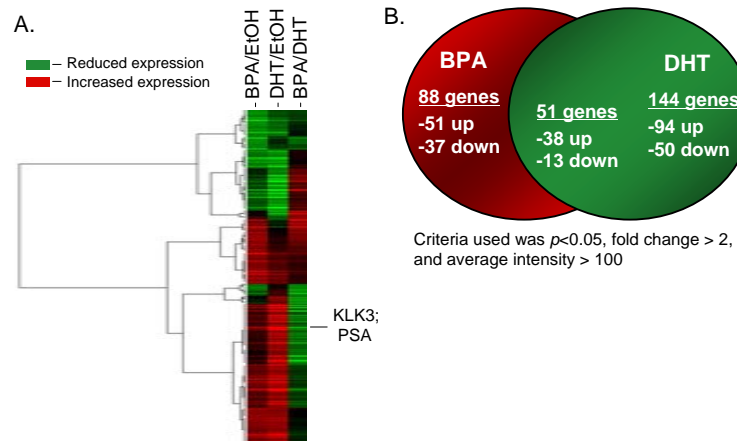


Fig. 2. BPA induces a unique transcriptome in prostate cancer cells expressing AR-T877A. Microarray analyses were performed using triplicate biological replicates and statistical analyses performed. Complete gene lists, slide preparation details and statistical analysis methods can be found at <http://microarray.uc.edu>. A) Heat map of relative gene expression, showing all genes with a statistically significant, greater than 2 fold, alteration over vehicle control. B) VENN diagram highlighting the disparity in DHT and BPA effects on gene regulation.

Figure 3

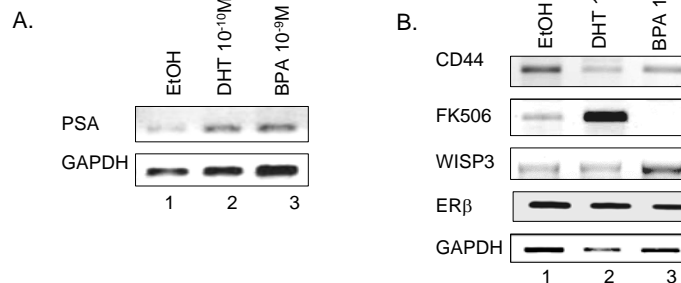


Fig. 3. Validation of selected targets. A) Validation of alterations in PSA expression were performed by RT-PCR. As expected, both DHT and BPA induced PSA expression in cells expressing AR-T877A. B) In agreement with the microarray, it was noted that BPA induces down-regulation of ERβ. In addition, marked induction of WISP3 was noted, and no significant effect was observed with FKBP5 or CD44. GAPDH was used as an internal control for RNA loading.

Figure 4

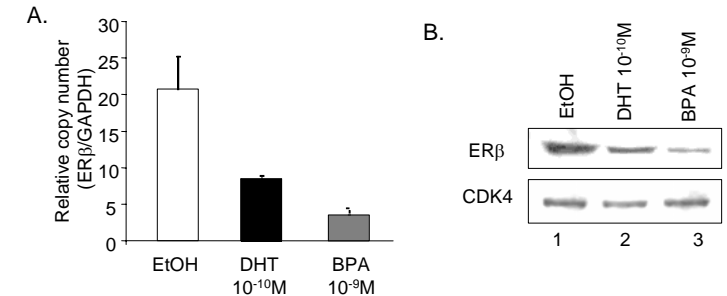


Fig. 4. BPA significantly down-regulates ERβ expression in cells expressing the BPA responsive AR-T877A mutant. A) The impact of BPA on ERβ was quantified by real-time PCR, and relative copy number was determined. B) Immunoblot of ERβ protein levels after BPA or DHT exposure. As shown, BPA exposure causes a marked reduction in ERβ protein expression.

Figure 5

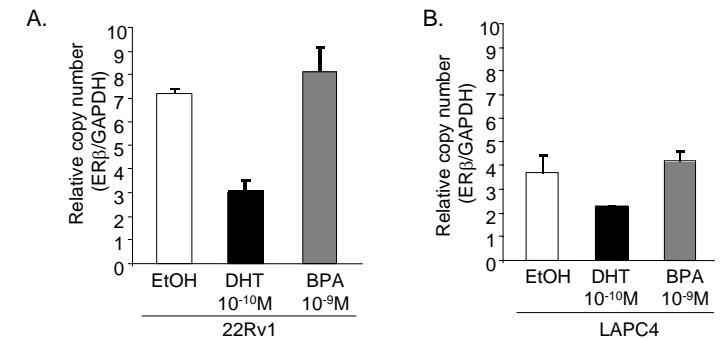


Fig. 5. Specificity of BPA-mediated modulation of ERβ. ERβ expression was monitored and quantified by real-time PCR in cells expressing AR-H874Y (22Rv1, panel A) or wild-type AR (LAPC4, panel B). Although DHT facilitated modest decrease in ERβ, BPA had no effect in either cell type.

Conclusions: BPA induces a unique gene expression signature in PCa cells expressing select somatic AR mutation. A molecular consequence of BPA exposure is down-regulation of ERβ. ERβ functions to antagonize AR function and AR-dependent proliferation, revealing a novel mechanism by which BPA may regulate cellular proliferation.

Background:

There is increasing concern that exposure to low levels of bisphenol A (BPA) may have adverse effects on human health. Recent evidence has demonstrated that BPA exposure adversely affects prostate cancer progression and treatment. Prostate cancer cells are dependent on androgen receptor (AR) activity for growth and progression, and therapy for disseminated disease depends on ablation of AR activity. While initially effective, recurrent tumors ultimately arise wherein AR has been reactivated. A major mechanism of AR restoration is via somatic mutation of the receptor, wherein mutant receptors become susceptible to activation by alternate ligands, including BPA. In tumors with specific AR mutations, BPA can restore AR activity and facilitate proliferation of prostate cancer cells, thereby promoting therapeutic relapse.

Objective and Methods:

The objective of this study was to determine the molecular mechanism of BPA action in cancer cells carrying BPA-responsive AR mutants. The transcriptional signature of BPA in prostate cancer cells was delineated and compared to that of the canonical AR ligand, dihydrotestosterone (DHT).

Figure 1

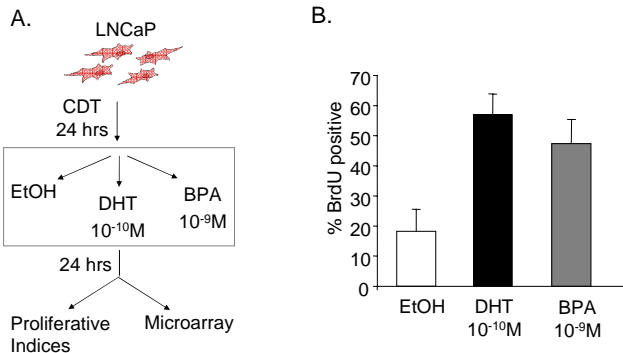


Fig. 1. BPA induces androgen-independent cellular proliferation in cells expressing AR-T877A. A) LNCaP cells were cultured under conditions of steroid hormone depletion (5% charcoal dextran treated serum, CDT) and subsequently treated with either DHT, BPA or vehicle control (0.1% ethanol). B) Cells cultured as in panel A were pulse labeled with BrdU for the last 16 hrs of treatment, fixed, and BrdU incorporation quantified by indirect immunofluorescence.