

Abstract

Estrogen (E_2) has many beneficial effects but paradoxically estrogen exposure increases cancer risk in the breast and in the uterus. Bisphenol A (BPA) and methylparaben (MP) are environmental estrogens that are known to influence the expression of estrogen sensitive genes. To further elucidate the mechanism of action of environmental estrogens in breast cancer cells, we treated T47D and MCF-7 cells with MP and BPA, respectively, and measured the expression of proteins in the E_2 signaling pathway utilizing Coomassie blue staining and Western Blotting.

Introduction

Estrogen (E_2) is an ovarian steroid that modulates reproductive function and governs a number of other physiological events. The genomic effects of E_2 are mediated through interactions with estrogen receptors α and β , ligand inducible transcription factors. Non-genomic effects of estrogen are mediated through membrane associated receptors coupled to second messenger systems or phosphorylation cascades. E_2 's effects are beneficial, but paradoxically, excessive estrogen exposure increases risk of developing cancer in breast and uterine tissue. Moreover, E_2 signaling is often dysregulated in estrogen dependent breast and uterine cancers.

Environmental estrogens (EEs) mimic the effects of ovarian estrogen and interact with the estrogen receptors. The precise effects of EEs depend on cell and promotor context. These compounds are ubiquitous. BPA is an EE found in many plastics and resins.

When treated with 10 nM BPA, mammospheres derived from MCF-7 cells exhibit increased size (Lillio et al.).

MP is commonly used in personal care products, food, and pharmaceutical and has been shown to increase tumor size of MCF-7 breast cancer cells (Lillio et al.).

We have measured the expression of ER α (involved in classic, genomic pathways) and MAPK (involved in rapid, non-genomic pathways) to generate a mechanistic model of EE action.

Methods & Materials

Cell Culture:

MCF-7 and T47D cells, are breast cancer cells that express endogenous estrogen receptors. They were obtained from Sigma Aldrich and cultured in T75 culture flasks with RPMI media supplemented with FBS at 37°C in 95% O₂ and 5% CO₂.

Exposure Time Trial:

Cells were plated at 2 million cells per well in 6 well plates with E_2 free RPMI culture media. There were treated with MP (1 μ M) for indicated periods of time. Controls included ethanol vehicle, and E_2 (10⁻⁸M). After treatments cells were collected using RIPA Lysis Buffer Protein concentration in cell lysates was quantified by a BCA assay.

Electrophoresis:

60 μ g of lysate was electrophoresed on 4-12% Bis-Tris gel at 200V.

Visualization:

The gels were stained with Coomassie Blue. ER α (66 KDa), ER β (59 KDa), and MAPK (42 KDa, 44 KDa) were identified by size relative to the protein marker (Magic Mark).

Densitometry was utilized to quantify expression of proteins.

Results and Conclusions

MCF-7 cells

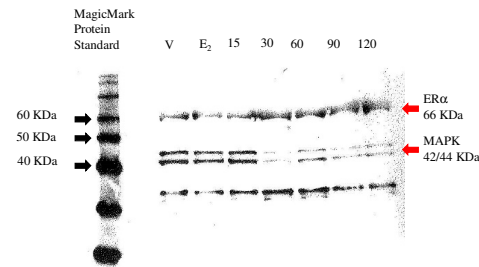


Figure 1. Bis-Tris gel containing proteins from the MCF-7 breast cancer cell line treated with BPA was visualized via a Western Blot. MCF-7 cells are a breast cancer cell line that expresses endogenous estrogen receptors. A MagicMark dye ladder was utilized to indicate the approximate protein length of the bands. ER α was observed at approximately 66 KDa for each of the time trails (15, 30, 60, 90, and 120 minutes). MAPK was visualized as a doublet at 42 KDa and 44 KDa at each of the time trails.

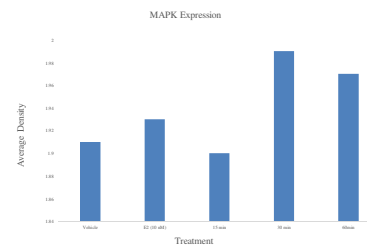


Figure 2. Average density of MAPK expression in MCF-7 cells treated with BPA for the vehicle, E_2 , 5, 30, and 60 minute increments.

T47D cells

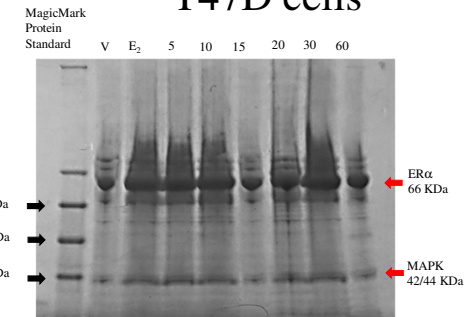


Figure 3. Bis-Tris gel containing proteins from the T47D breast cancer cell line treated with MP was visualized via a Coomassie Blue stain. T47D cells are a breast cancer cell line that expresses endogenous estrogen receptors. A MagicMark dye ladder was utilized to indicate the approximate protein length of the bands. ER α was observed at approximately 66 KDa for each of the time trails (5, 10, 15, 20, 30, and 60 minutes). MAPK was visualized as a doublet at 42 KDa and 44 KDa at each of the time trails.

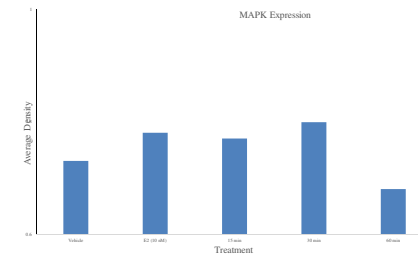


Figure 4. Average density of MAPK expression in T47D cells treated with MP for the vehicle, E_2 , 5, 30, and 60 minute increments (.85 to .77 to .84 to .79 and .68).

- 1) There was no change in ER α expression in either cell line over the time course with either the MP or the BPA treatment.
- 2) In the case of the T47D breast cancer cells there was no change in ER β expression and no change in ER α /ER β ratio over time with the MP treatment (data is not shown).
- 3) MAPK expression changes when breast cancer cells are challenged with MP and BPA. MAPK expression is increased with E_2 treatment (as compared to vehicle). MAPK expression decreased initially with EE (BPA and MP) treatment (as compared to E_2). MAPK increased after 30 minutes and then decreased at 60 minutes. *The time trials for the MCF-7 and T47D cells were different, but the patterns of expression at 15, 30, and 60 minutes is the same.*
- 4) The lack of effect on ER expression makes it unlikely that MP and BPA affect breast cancer cells via a genomic mechanism.
- 5) The activation and cycling of MAPK in both cases suggests that effects of environmental estrogens are nongenomic.
- 6) We continue to examine this phenomenon and want to observe precise effects environmental estrogens are having on the MCF-7 and T47D breast cancer cell lines. Future studies will determine if MAPK expression is correlated with proliferation of these cell lines.

References

Lillio MA et al. *Hum Cancer*. 2017 Apr;8(2):90-99.
Lillio et al. *Jour of App Toxi*. 2016;37(4), 417-425.

Acknowledgements

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