Comparing the Effects of Bisphenol A and Bisphenol A Alternatives in Estrogenic and Non-Estrogenic Dependent Pathways

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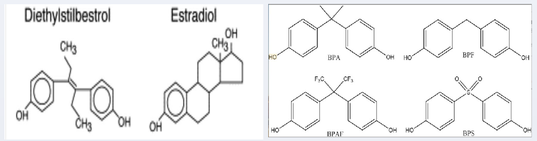
Abstract

Bisphenol-A is a synthetically produced chemical compound that is used in the manufacturing of various kinds of plastics and epoxy resins. Since it contains two hydroxyphenyl groups, its chemical structure is remarkably similar to the chemical compound estrogen, enabling it to easily bind to estrogen receptors such as ERα and ERβ.



The purpose of this study is to analyze the impact of BPA and BPA alternatives on estrogen and non-estrogen dependent pathways and a transcriptome analysis using RNA-seq on human breast cancer cells called MCF-7 was performed to study the effect of chemical exposure against a control group of cells. It is equally important to analyze the effects of BPA on organisms that do not have estrogen and how it impacts non-estrogen dependent pathways. *Drosophila melanogaster* (fruit fly) cells are studied to examine how BPA dosages impact neurological development.

Background

Estrogen Dependent Pathways:

- Estrogen is a chemical compound that is responsible for secondary sex characteristics in most female vertebrates.
- BPA is a chemical compound that is heavily used in plastics for durability and resistance.
- Studies have shown that BPA shares structural resemblance to estrogen compounds.
- Accumulation of BPA led to cell proliferation, increasing chances of cancer development, especially in the breasts.

Non-Estrogen Dependent Pathways:

- Fruit Flies (*Drosophila melanogaster*) belong to the insect class and estrogen is absent in female fruit flies.
- A sample of female fruit flies were exposed to high doses of BPA.
- Results showed that exposure to BPA led to downregulation of genes that affect neurological development.

Motivation

What is the Need?

- For many years, BPA was the primary compound that was used in majority of plastics, until studies found that it binds to estrogen receptors, leading to cancer growth.
- It is extremely crucial to study the alternatives of BPA are that introduced into the market and analyze their safety, in order to prevent malignant cell growth.

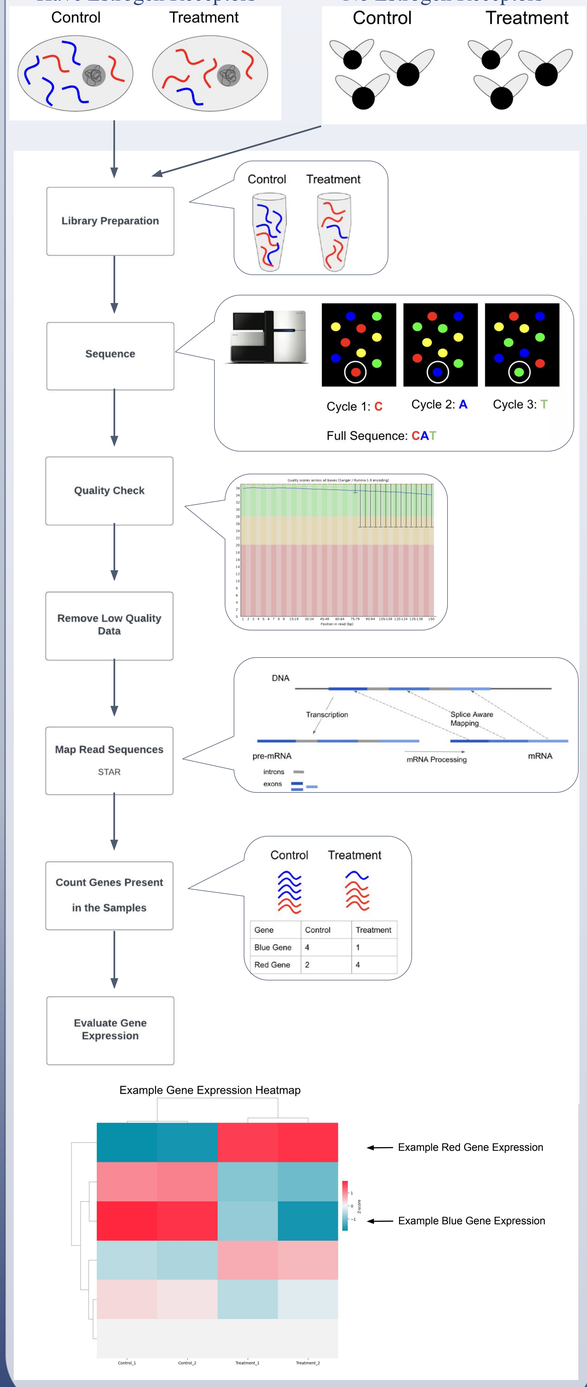
Approaches:

- **Analyzing gene expression differences between control and treated samples**
- **Perform Gene Set Enrichment Analysis (GSEA) to identify pathways perturbed by chemical exposure**

From Biological Samples to Data

MCF-7 Cell Line
Have Estrogen Receptors

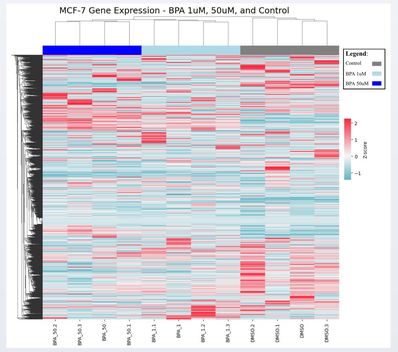
Drosophila melanogaster
No Estrogen Receptors



Results

Estrogen-dependent

Gene Expression in MCF-7 cells treated with 1uM, 50uM of BPA cluster according to treatment



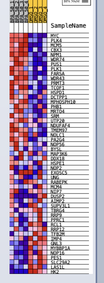
Above : The Gene Expression Heatmap for the MCF-7 cells. The samples are grouped into the control, 1uM BPA and 50uM BPA samples.

Similar Hallmark Gene Set Regulation seen in MCF-7 Cells (Estrogen Receptor +) after treatment with 50uM of BPA or related compounds

A

Enriched Gene Sets	BPA	BPS	4,4-BPF	2,4-BPF
HALLMARK_ESTROGEN_RESPONSE_EARLY	X	X	X	X
HALLMARK_ESTROGEN_RESPONSE_LATE	X	X	X	X
HALLMARK_MYC_TARGETS_V1	X	X	X	X
HALLMARK_MYC_TARGETS_V2	X	X	X	X
HALLMARK_MTORC1_SIGNALING	X	X	X	X
HALLMARK_MITOTIC_SPINDLE	X	X	X	X
HALLMARK_G2M_CHECKPOINT	X	X	X	X
HALLMARK_GLYCOLYSIS	X	X	X	X
HALLMARK_SPERMATOGENESIS	X	X	X	X
HALLMARK_E2F_TARGETS	X	X	X	X
HALLMARK_DNA_REPAIR	X		X	X
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION		X	X	X
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY		X	X	X
HALLMARK_UNFOLDED_PROTEIN_RESPONSE		X	X	X
HALLMARK_WNT_BETA_CATENIN_SIGNALING	X			X
HALLMARK_INFLAMMATORY_RESPONSE		X		X
HALLMARK_TNFA_SIGNALING_VIA_NFKB		X		X
HALLMARK_PROTEIN_SECRETION			X	X
HALLMARK_HYPOXIA				X
HALLMARK_PI3K_AKT_MTOR_SIGNALING				X
HALLMARK_IL2_STAT5_SIGNALING				X
HALLMARK_UV_RESPONSE_UP				X
HALLMARK_P53_PATHWAY				X

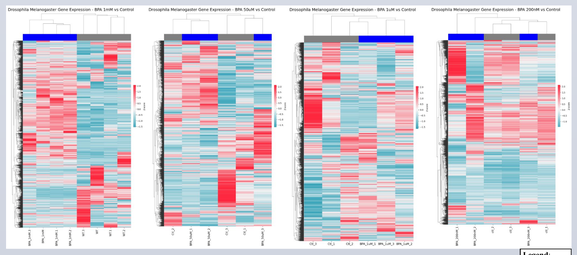
B



Above : (A) Significantly Enriched Gene Sets are marked with an X for each treatment compound. Gene sets are considered significantly enriched if they have a False Discovery Rate < 25%.
(B) Gene Set Heatmap for the Hallmark Gene Set: MYC Targets V2. 50uM BPA treated samples compared to the control DMSO treated samples. These Gene Sets can contain 10's to 100's of genes. The overall expression of the gene set is measured as a way to understand how a given pathway or cellular function is working.

Non-estrogen-dependent

In *Drosophila melanogaster*, higher doses of BPA are needed to group exclusively by treated and non-treated samples.



Above : The Gene Expression Heatmaps for *Drosophila melanogaster* compared to the control. From left to right, the BPA dosages are 1mM, 50uM, 1uM, and 200nM. At the 1mM concentration, BPA-treated samples cluster separately from the control samples.

Conclusions

In the cell line with an estrogen receptor, MCF-7, we see robust responses to BPA, BPS, 2,4-BPF, and 4,4-BPF at 50uM concentrations. Additionally, the affected pathways in the Hallmark Gene Set are relatively conserved between treatments.

In *Drosophila melanogaster*, which lacks an estrogen receptor, treatment with BPA can alter gene expression, but the effects are primarily seen at higher concentrations (1mM).

In both models, as the treatment compound concentration increases, the number of differentially expressed genes increase.

Future Work

Additional experiments should be done to determine BPA and similar compounds' effect on neurodevelopment pathways. These compounds are pervasive and we need to determine if stronger regulations should be put in place to protect consumers from ingesting these compounds. We need more information about dose-dependent effects as that could help consumers make informed decisions about what types of food or drink containers they choose to use.

Acknowledgment

We would like to thank Dr. Kimberly Mulligan and her students for their work to provide *Drosophila melanogaster* RNA-seq data. Additionally we would like to thank Eden Johnson and An Nguyen for their previous work studying BPA's effects on *Drosophila melanogaster* gene expression.
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