BPA EXPOSURE: NETWORK ANALYSIS REVEALS NOVEL TRANSCRIPTION FACTORS ASSOCIATED WITH BISPHENOL A DOSE-RESPONSE

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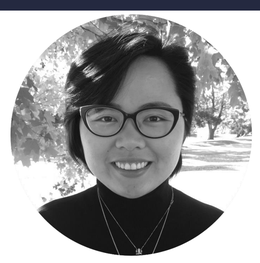

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INTRODUCTION

- Bisphenol A (BPA) is an industrial chemical used widely to harden plastics. Among everyday products, BPA can be found in:

plastic bottles **canned food** **medical devices**

- In Vietnam, **94%** of the general population is exposed to BPA.[1]
- BPA affects the reproductive systems in laboratory animals. However, human health effects from BPA at low environmental exposures are unknown.
- BPA was presumed to have potentially estrogenic activity, based on its structural similarity to DES (diethylstilbestrol) and other synthetic estrogens.

Study goal: **We compared the gene networks between BPA and estrogen to determine whether BPA and estrogen share similar transcriptional factors.**

METHODOLOGY

DATASET

W e used a publicly available microarray dataset **GSE50705** of MCF7 cell line from the Gene Expression Omnibus database. We generate several subsets:
- Estrogen-treated samples (n = 36)
- BPA-treated samples (n = 44)
- BPA low dose samples (n = 33)

NETWORK ANALYSIS

We used **Weighted Co-expression Network Analysis (WGCNA)**[2], which takes advantage of a graph theory to group genes into modules that typically have coordinated biological functions and regulatory mechanisms.

ENRICHMENT ANALYSIS

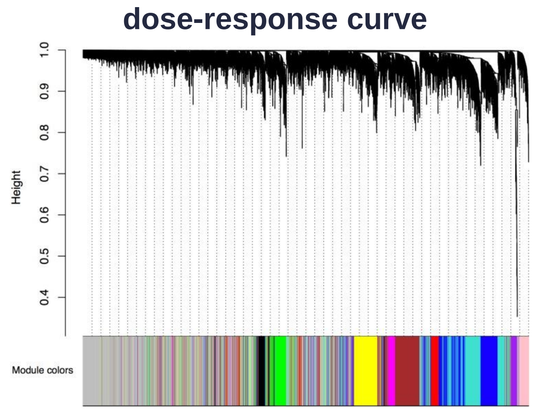
All statistically significant gene modules were analyzed in the EnrichR database for transcriptional factors restricted to MCF-7/10 cells. An adjusted *p*-value less than 0.01 based on Fisher's exact test is considered significant.

RESULTS

1 Consensus network analysis indicates minimal overlap between estrogen and BPA

We began by analyzing the dose-response curve of the BPA and estrogen dataset combined using WGCNA to look for a "consensus network"-a common pattern of genes that are correlated in all conditions.

Figure 1. Consensus network from BPA and estrogen dose-response curve



The majority of modules in the consensus analysis when analyzed for correlation with both BPA and estrogen showed virtually no similarity (**Table 1**), indicating that while there may be some overlap in genetic signatures, from a network topology perspective there is minimal conservation. Then we analyzed for transcription factors in EnrichR, the common module was enriched for E2F1, ZNF217, and RACK7, but not ESR1 or ESR2 (data shown in our paper).[3]

Table 1. Consensus network modules associated with BPA and estrogen

Module	Correlation	*p*-value
Red	-0.00025	1
Green	-0.092	0.4
Purple	NA	NA
Black	NA	NA
Brown	NA	NA
Magenta	NA	NA
Blue	NA	NA
Yellow	0.24	0.03
Pink	0.18	0.1
Gray	NA	NA

2 Low-Dose BPA network shows no enrichment of ESR1 or ESR2 genes

It has been speculated that BPA at low doses has fundamentally different effects than at high doses. Therefore, we restricted the BPA network to doses below 12.5 µM and calculated a network specific for this lower dose range. Despite the smaller sample size, the network still produced several modules that were significantly correlated with dose(**Table 2**).

Table 2. Low dose BPA module associated with dose

Module	Correlation	*p*-value
Turquoise	0.71054	5.21E-06
Dark Green	0.66763	2.99E-05
Dark Red	0.54873	1.15E-03
Light Yellow	0.47024	6.61E-03
Brown	0.45995	8.08E-03
Salmon	0.44326	1.11E-02
Grey60	-0.4753	5.98E-03
Blue	-0.539	1.46E-03
Yellow	-0.5535	1.02E-03
Black	-0.6395	8.13E-05
Midnight Blue	-0.736	1.58E-06

This low-dose BPA network also showed consistent transcription factors (ZNF217, TFAP2C, RACK7/ZMYND8, and PADI4) with the larger BPA network as well as the estrogen network (data shown in our paper)[3], but no modules were enriched for genes with ESR1 or ESR2 with a *p*-value cut-off of < 0.01 (**Table 3**).

Table 3. Enriched transcription factors in low dose BPA network

Module	TF	Adjusted *p*-value
Black	RACK7	1.78E-07
	TFAP2C	0.00001339
	RUNX1	0.00004104
Blue	ELK1	0.00000437
	ZNF217	6.14E-07
	PADI4	0.000007639
	FOXM1	9.66E-08
	HIF1A	0.005915
	AHR	0.00208
	E2F1	0.002221
	ARNT	0.005915
	RUNX1	0.008124
	GATA3	0.008124
Brown	E2F1	0.002459
	PADI4	0.006908
Turquoise	ZNF217	0.00000179
	RACK7	0.00001912
	GATA3	0.00002574
	PADI4	0.0001018
	FOXM1	0.0001703
	RUNX1	0.0004958
	E2F1	0.001544
Yellow	E2F1	6.34E-18
	PADI4	0.003362
	RACK7	0.00334
	FOXM1	0.009047

3 Low-dose BPA network had unique transcription factors not present in the estrogen dataset

To further her predict possible transcription factors unique to BPA signaling compared to estrogen, we examined the genes in all modules significantly associated with the low-dose BPA network that were not present in the estrogen network. To expand our search for transcription factors that may not have been studied in MCF-7 cells in the CHEA EnrichR dataset, we also analyzed the list of genes for enrichment against the ARCHS4 database (**Table 4**).

Table 4 . Low-dose BPA modules associated with dose

Of the top transcription factors identified as significantly correlated with the gene list, 18 were also present in the low-dose BPA network, highlighted in orange.

Transcription factor	Adjusted p-value
HSF1	4.54E-32
MBD3	4.72E-30
ZNF787	4.54E-32
HM320B	1.48E-29
REPIN1	4.76E-29
FIZ1	4.76E-29
SLC2A4RG	1.48E-29
SREBF1	2.14E-28
ZNF598	9.16E-28
THAP4	1.75E-26
SNAPC4	4.29E-27
ZNF768	1.75E-26
E4F1	6.78E-26
MRPL28	6.78E-26
TUT1	2.67E-25
ERF	2.67E-25
CENPB	6.78E-26
KLF16	2.67E-25
WIZ	1.98E-23
ANAPC2	1.17E-24
ZFP41	1.98E-23
DVL2	6.94E-23
ZNF512B	1.98E-23
SRF	1.98E-23
ELK1	6.94E-23
ZNF282	6.94E-23
AKAP8L	2.65E-22
ZBTB45	6.94E-23
NCOR2	6.94E-23
CIZ1	2.65E-22
TRMT1	2.65E-22
CIC	6.94E-23
NR2F6	1.01E-21
ZNF687	2.65E-22
MTA1	3.81E-21
RBM10	1.01E-21
GATAD2A	1.01E-21
ZNF653	1.01E-21
ZNF777	3.81E-21
EDF1	3.81E-21
PRR12	3.81E-21
SIX5	5.23E-20
TIGD5	5.23E-20
MTA2	1.43E-20
MAZ	1.43E-20
CCDC71	1.43E-20
MLLT1	1.43E-20
SF3A2	2.04E-19
GMEB2	5.23E-20

CONCLUSION

- We demonstrated that even at low doses there are unique transcription factors that appear to be driving the biology of BPA.
- Our study is consistent with other findings that the assumption that BPA works exclusively or even predominantly on canonical ESR1 or ESR2 gene regulation may be misleading or an oversimplification.

REFERENCES

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