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Research article

Identification and verification of promising diagnostic genes in bisphenol A-associated breast cancer development via *in silico* analysis

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Abstract

Lifestyle patterns and exposure to toxic chemicals or environmental pollutants are the strongest risk factors for the chances of developing breast cancer, the leading and most lethal form of cancer in women. Bisphenol A (BPA), found in various consumer products, is known to deregulate multiple cellular signaling pathways, but its effect on cancer initiation and development in breast tissue has not yet been fully elucidated. Therefore, the identification of hub drivers is necessary to understand the molecular mechanisms underlying BPA-related malignancy and may help determine novel diagnosis and treatment strategies. This work aims at elucidating the molecular actors and mechanisms of action involved in BPA-induced breast cancer development using a bioinformatics analysis approach. A microarray dataset suitable for the study purposes was obtained from the publicly available Gene Expression Omnibus (GEO) repository, followed by DEG (differentially expressed genes) extraction, enrichment, and protein-protein interaction analyses to identify the hub genes. Expressional patterns, prognostic potentials, and immune infiltration levels of identified targets were tested and validated *in silico* using GEPIA2 and KM-plotter tools. According to PPI network results, *CCNA2* and *CCNB1* were identified as critical hub genes. Validation analyses clearly indicated that the identified genes are extremely critical in BPA-associated breast cancer processes. Findings from this study revealed that *CCNA2* and *CCNB1*, two cell cycle signaling-related hub genes that are overexpressed as a consequence of BPA exposure, are strongly associated with breast cancer.

Keywords: *Biomarker; BPA; breast cancer; cell cycle signaling; DEGs*

1. Introduction

Breast cancer is a lethal and common type of cancer in women and affects daily life in different ways. The disease emerges in the epithelium of the lobules or ducts (85%) in the glandular parts of the breast (Nardin et al., 2020). Initially, cancer has minimal potential for spread and generally causes no symptoms. However, cancer may progress over time and invade the surrounding breast tissue. Firstly, it spreads to the lymph nodes and then to other organs in the body (Zhou et al., 2021). World Health Organization (WHO) reports and GLOBOCAN cancer incidence and mortality estimates revealed that this cancer accounts for 11.7% of all cancer cases, with approximately 2.5 million cases in 2020 (Sung et al., 2021; WHO, 2023). Since it is a polygenic and multifactorial cancer, it includes complex mechanisms, signaling pathways, and cellular activities. The most critical issue in the treatment of the disease is diagnosing it at advanced stages due to the lack of sensitive biomarkers and effective treatment (Pei et al., 2020). Therefore, identifying the critical factors for this complicated type of cancer is very important, both to determine valuable diagnostics actors that can also be used in the treatment and to fully understand the molecular mechanism underlying the malignancy.

Bisphenol A (BPA) is an intermediate compound with a massive production volume worldwide, especially in

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polycarbonate and epoxy manufacture (Fauconnier et al., 2023). It is widely preferred in daily used storage containers, bottles, and other packaging items that are frequently used in daily life (Dehdashti et al., 2023). BPA can be released into the environment during the entire production and disposal processes of these products. The United States Environmental Protection Agency (EPA) statistics show annual environmental BPA leaks are almost 400,000 tons (EPA, 2023).

Preclinical studies have found that bisphenol exposure elevates breast cancer risk (Stillwater et al., 2020; Kwon, 2022). Novel research has shown that endocrine-disrupting chemicals (EDCs), including bisphenols, trigger breast cancer-associated genes and cell proliferation via binding to estrogen receptors (ERs) (Castillo-Sanchez et al., 2020). Impairments in the endocrine system in complex diseases such as birth defects and obesity caused by BPA have been partially clarified (Priyadarshini et al., 2023). However, it is unclear how BPA affects the onset and progression of cancer in the breast. Therefore, understanding the exact biological effects of endocrine disruptors is essential. This study aims to identify hub factors and pathways involved in BPA-associated breast cancer formation and progression via a bioinformatics approach.

2. Materials and methods

2.1. Collection and pre-processing of transcriptomic data

The mRNA expression dataset (GSE32158), which includes MCF-10F cells exposed to 10 μM BPA for two weeks and non-treated cells, was obtained from the GEO repository (Barrett et al., 2013) of NCBI. Next, DEGs between BPA-treated and control group cells were identified using the GEO2R web tool (GEO2R, 2023). Next, DEGs between BPA-treated and control cells were identified according to |logFC|>2.0 and p-value <0.05 criteria using the GEO2R web tool (Fig. 1).

2.2. Enrichment analyses of the DEGs

To identify the biological and molecular functional processes and significantly enriched pathways of the DEGs, the DAVID tool was used (Huang et al., 2009; DAVID, 2023).

2.3. Protein-protein interaction (PPI) network analysis

Co-expression relationship between DEGs was determined through the PPI network created using STRING (Jensen et al., 2009; STRING, 2023). The confidence score cutoff value was set at 0.7. Then, the cytoHubba plug-in of Cytoscape (Shannon et al., 2003) was used to extract the hub genes using five widely preferred algorithms: Maximal Clique Centrality (MCC), Maximum Neighborhood Component (MNC), Degree, EcCentricity, and Closeness. The first fifteen genes of each algorithm result were ranked, and aligned genes were chosen as hub genes.

2.4. Data validation

The mRNA profiles of the core genes between tumor and healthy tissues in breast invasive carcinoma (BRCA) were verified using different sources and tools. Cumulative mRNA expressions of the hub genes in breast cancer patients and healthy individuals were analyzed using the GEPIA2 platform (Tang et al., 2019; GEPIA2, 2023) was used.

The association between the expression of hub genes and the clinical stage of the disease was analyzed using the UALCAN web portal (Chandrashekar et al., 2017; UALCAN, 2023) and the prognostic outputs of these genes on the overall survival of breast cancer patients were evaluated using the KM-plotter platform (Gyorffy, 2021; KM-plotter, 2023). The expression correlations of hub genes in breast cancer and the tumor infiltration level of immune cells were visualized by the TIMER2.0 database (Li et al., 2020; TIMER2.0, 2023). Finally, to determine whether the identified hub genes were among the genes interacting with BPA, the CTD database was used (Davis et al., 2021; CTD, 2023).

3. Results

3.1. DEGs

Based on the findings, a total of 1814 DEGs (976 up- and 838 down-regulated) were identified (|logFC| >2 and $p< 0.05$).

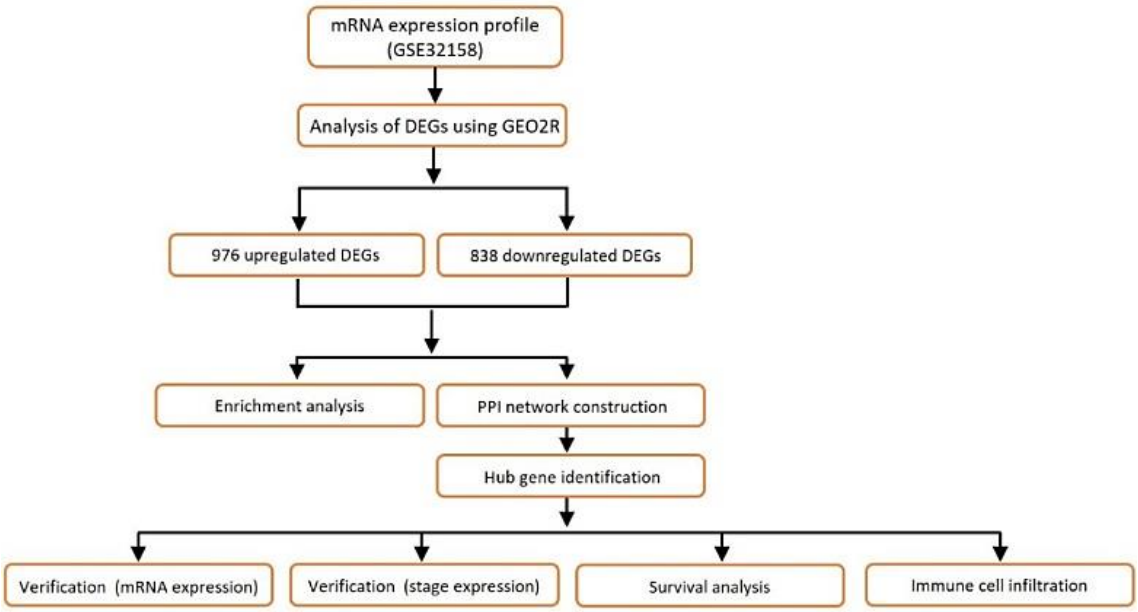

Fig. 1. The overall workflow of the study.

3.2. Functional characterization of DEGs

To further investigate the biological themes and pathways including the DEGs, GO and KEGG pathway enrichment analyses were performed. The first fifty down and upregulated DEGs were used for this analysis. Based on the DEGs annotation, it was found that the upregulated genes were significantly enriched in several GO terms, such as regulation of cell proliferation, cell surface, and DNA helicase activity. Downregulated genes were significantly enriched in transport, oxidation-reduction processes, and cytokine receptor activity. According to pathway enrichment results, while upregulated DEGs are importantly enriched in ECM-receptor interaction, downregulated DEGs are enriched in the complement and coagulation cascades (Supplementary Table 1 and 2).

3.3. Protein interaction network and hub gene detection

The PPI network constructed using the STRING (Fig. 2) was further analyzed with Cytoscape software. Next, network properties were analyzed by MCC, MNC, Degree, EcCentricity, and Closeness algorithms of the Cytohubba plug-in of Cytoscape. The genes that are the intersection of the first-ranked 15 genes with the highest score in these five algorithms were identified through the Venn diagram. Finally, *CCNA2* and *CCNB1* were identified as hub genes (Table 1 and Fig. 3).

3.4. Validation and analysis of hub genes

Firstly, GEPIA2 was used to confirm the mRNA expression patterns of two genes. It was determined that the mRNA levels of the two identified genes were notably elevated in tumor tissues compared to healthy tissues (Fig. 4a and Fig. 4d). Then, subgroup analysis was performed using UALCAN to uncover the expression changes of *CCNA2* and *CCNB1* genes in tumor stages. The results revealed that the relative expression of *CCNA2* and *CCNB1* increased significantly in advanced tumor stages (Fig. 4b and Fig. 4e). To further investigate the clinical importance and prognostic impacts of these hub genes in breast cancer, overall survival (OS) analysis was performed using the KM-plotter. The results showed that an increased *CCNA2* expression (HR 1.48 [1.34–1.64], P=1.9e-14) and *CCNB1* (HR 1.89 [1.71–2.1], P<1e-16) was significantly related to a poor OS in patients diagnosed with cancer (Fig. 4c and Fig. 4f).

The hub gene expression with immune infiltration level in breast cancer correlation was detected using the TIMER gene module. Results showed that there was a positive correlation between the expression of *CCNA2* and the infiltration of purity, B cells, CD4+ T cells, CD8+ T cells, dendritic cells, and neutrophils (Fig. 5a). In addition, there was a correlation between the *CCNB1* expression and the infiltration of purity, B cells, and neutrophils (Fig. 5b).

These findings clearly propose that *CCNA2* and *CCNB1*

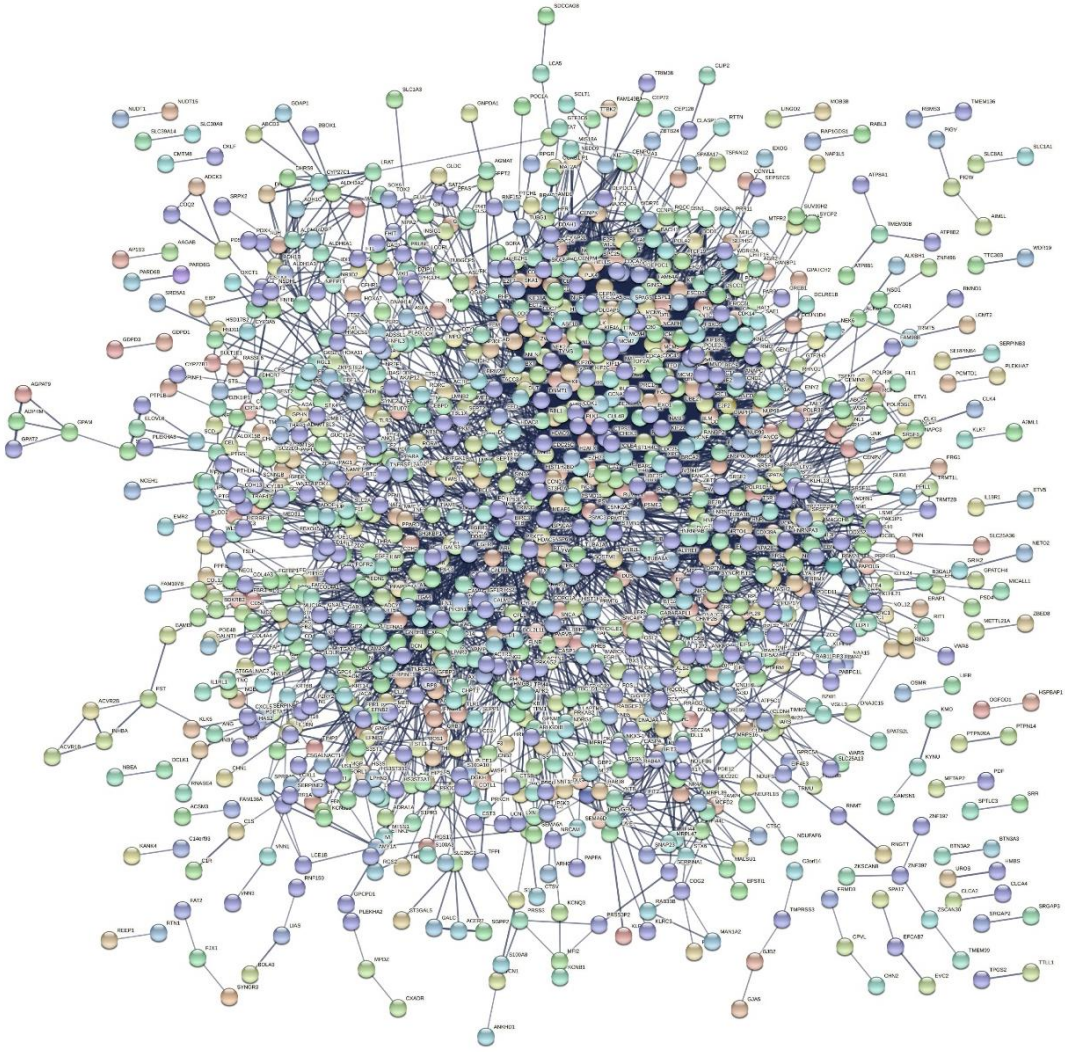

Fig. 2. PPI network of DEGs constructed by STRING database.

Table 1
Top 15 genes ranked by five different cytoHubba computational algorithms. The intersection genes are highlighted in bold.

Genes	MCC Score	Genes	MNC Score	Genes	Degree Score	Genes	EcCentricity Score	Genes	Closeness Score
CCNB1	9,22E+28	*CDK1*	181.0	*CDK1*	181.0	*EGFR*	0.15373	*CDK1*	516.93
CCNA2	9,22E+28	***CCNB1***	155.0	***CCNB1***	156.0	***CCNB1***	0.13177	***CCNB1***	491.55
AURKB	9,22E+28	***CCNA2***	154.0	***CCNA2***	154.0	***CCNA2***	0.13177	***CCNA2***	489.26
BUB1	9,22E+28	*BUB1*	149.0	*BUB1*	149.0	*BIRC5*	0.13177	*AURKB*	476.64
PLK1	9,22E+28	*BUB1B*	143.0	*NCAPG*	144.0	*CHEK1*	0.13177	*HSP90AA1*	473.56
BUB1B	9,22E+28	*AURKB*	142.0	*BUB1B*	143.0	*TYMS*	0.13177	*BUB1*	473.41
CHEK1	9,22E+28	*NCAPG*	142.0	*AURKB*	143.0	*BRCA1*	0.13177	*EGFR*	471.33
AURKA	9,22E+28	*TOP2A*	140.0	*KIF11*	142.0	*CDC25C*	0.13177	*ACTB*	470.02
TOP2A	9,22E+28	*KIF11*	140.0	*TOP2A*	140.0	*ACTB*	0.13177	*PLK1*	469.49
BRCA1	9,22E+28	*CCNB2*	136.0	*CCNB2*	136.0	*HSP90AA1*	0.13177	*BUB1B*	468.55
CCNB2	9,22E+28	*MAD2L1*	131.0	*MAD2L1*	131.0	*CCND1*	0.13177	*CHEK1*	467.38
NCAPG	9,22E+28	*CDC20*	129.0	*CDC20*	129.0	*PIK3R1*	0.13177	*AURKA*	467.30
RRM2	9,22E+28	*NDC80*	126.0	*NDC80*	126.0	*H2AFX*	0.13177	*TOP2A*	464.78
KIF11	9,22E+28	*TTK*	124.0	*PLK1*	125.0	*FN1*	0.13177	*BRCA1*	463.13
MAD2L1	9,22E+28	*ASPM*	123.0	*AURKA*	125.0	*H2AFV*	0.13177	*CCNB2*	462.39

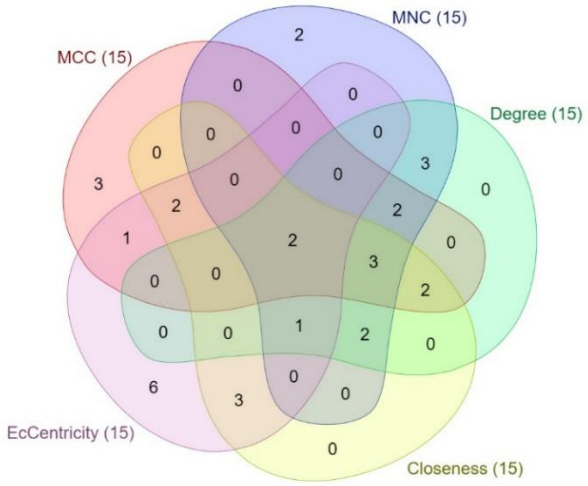

Fig. 3. Venn diagram of the intersecting genes derived using five algorithms.

may play an essential role in immune cell infiltration in patients with breast cancer. It is well that known genes with co-expression patterns are co-regulated or functionally related (Kustatscher et al., 2017). Therefore, to determine the mRNA expression correlation of hub genes in breast cancer the correlation module of TIMER was used. Consequently, it was found that *CCNA2* and *CCNB1* expressions were highly correlated with each other in BRCA (Fig. 6a). Finally, the CTD analysis tool MyGeneVenn was used to determine whether hub genes are among the genes regulated in response to BPA. Intersection analysis of a set of genes curated by CTD and hub genes from this study showed that *CCNA2* and *CCNB1* were predicted to be targeted by BPA (Fig. 6b).

4. Discussion

The causes of polygenic diseases are blurry due to their complex nature, which complicates the detection of the contributing factors (Ceylan et al., 2019; Ritchie et al., 2021). Therefore, changes in the whole genome should be considered to elucidate the pathophysiology of multifactorial diseases such as breast cancer and thus determine effective therapy strategies (Ceylan, 2022). Identification of central genes through the bioinformatics-based transcriptome analysis approach is widely recognized in the scientific community and provides a molecular basis for related biological questions (Ceylan, 2021). The present study aimed to determine the carcinogenic properties of BPA in breast cancer and accurate targets for future therapy through the analysis of transcriptome data from BPA-treated human breast epithelial cells MCF-10F. Based on the integrated bioinformatics analysis, two hub genes (*CCNA2* and *CCNB1*) have been identified in this study. The mRNA expression of these two genes was dysregulated (over 12 and 32-fold changes, respectively) in the BPA-treated MCF-10F cells compared to non-treated cells.

The cell cycle is regulated tightly because perturbations in this operation can lead to crucial complications such as tumor development mediated by uncontrolled cell division and inappropriate cell proliferation (Pekarek et al., 2023). Dysregulation of the expression of related drivers that control the transitional stages of the cell cycle is well-documented in multiple cancers (García-Gutiérrez et al., 2019; Yesilkent and Ceylan, 2022). CDKs (cyclin-dependent kinases) are regulate cell cycle checkpoints in response to intracellular and extracellular signals (Niu et al., 2019). As a result, it is not surprising that abnormalities or impairment of CDKs and CDK-mediated pathways are characteristic of cancers and initiate a chain of events that prime cancer (Ding et al., 2020). Recent research has shown that these cyclins are raised in breast cancer and are related with worse clinical outcomes (Cai et al., 2023). CCNA2 (also known as cyclinA2) is a type of cyclin protein and is expressed in nearly all tissues (Zhang et al., 2023). Numerous reports have reported that *CCNA2* is overexpressed in different cancer types and is markedly related with bad prognosis (Dong et al., 2019). Similarly, unscheduled expression of CCNB1 (cyclin B1) has also been shown to be associated with uncontrolled cell-cycle progression in several cancers (Zuryn et al., 2019). Therefore, targeting these factors, especially in breast cancer, can be an ideal strategy to prevent tumor development and increase the effectiveness of chemotherapy.

Although the relationship of BPA with global health challenges such as diabetes and heart disorders has been largely proven, the mechanisms and key drivers of BPA-induced breast cancer are still not fully elucidated. Most EDCs stimulate

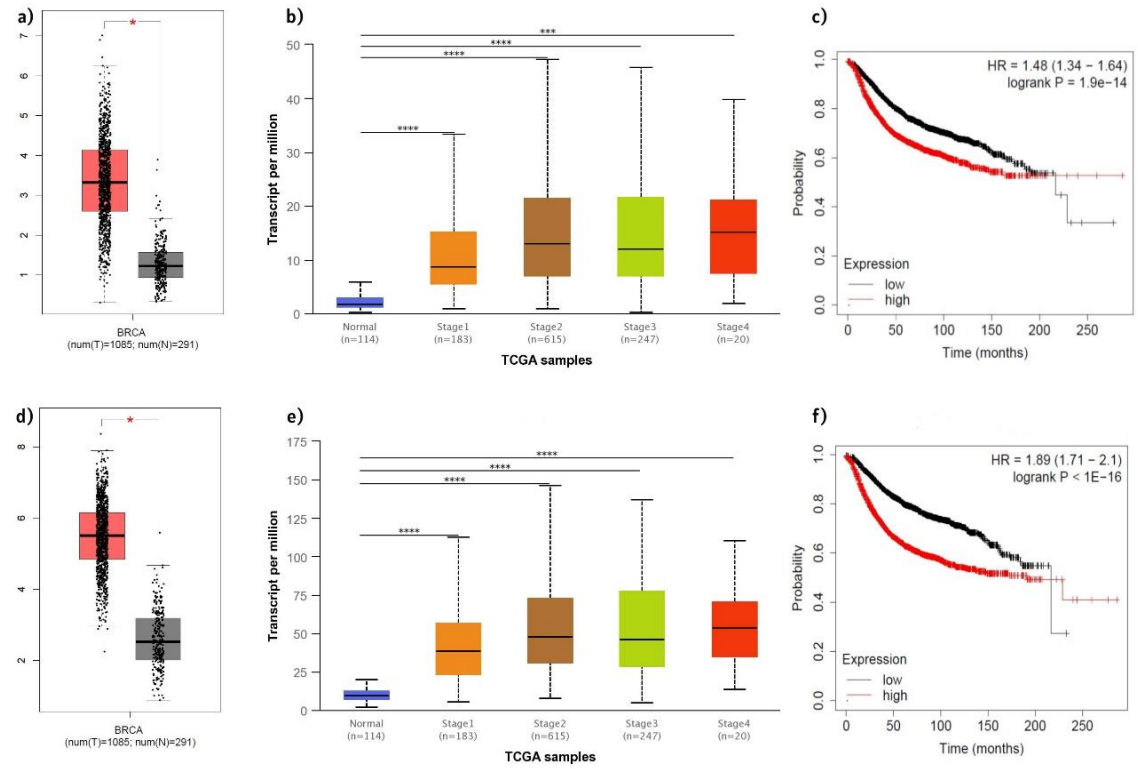

Fig. 4. The expression validation and analysis of hub genes. The expression profiles of *CCNA2* (a) and *CCNB1* (d) genes in breast cancer within GEPIA database. Red asterisk on top of the boxplot denotes the statistically significant difference. mRNA expression patterns of the hub genes in breast cancer patients with cancer stages (b-e). *p*-values < *0.05, **0.01, ***0.001, ****0.0001. Overall survival (OS) analysis of hub genes in BRCA patients (c-f).

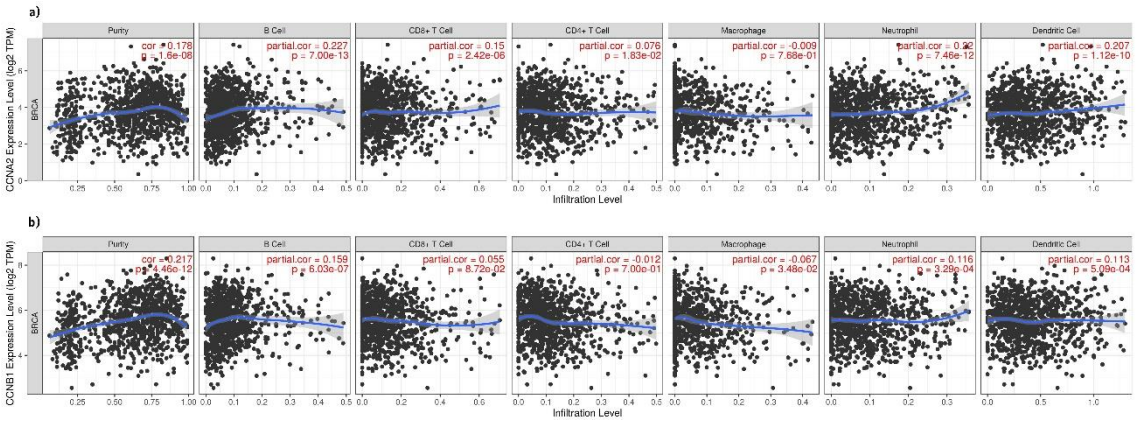

Fig. 5. *CCNA2* (a) and *CCNB1* (b) expressional correlation and abundance of tumor-infiltrating immune cells.

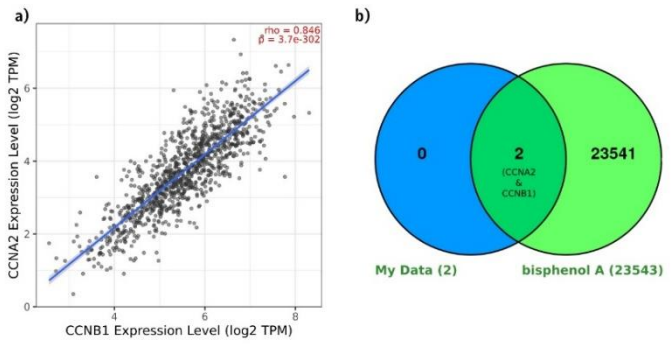

Fig. 6. Correlation of mRNA expression of *CCNA2* and *CCNB1* in patients with breast invasive carcinoma (a). Comparative toxicogenomic analysis and target verification in response to BPA (b).

estrogen-related responses by binding to estrogen receptors (ERs), resulting in gene expression remodeling in the target tissue (Bondar et al., 2009). Among EDCs, BPA, as an estrogenic compound that has shared structural characteristics with ERs, can stimulate cellular responses and cell function through its strong estrogenic activity and can cause very critical undesirable effects on the health (Almeida et al., 2018).

Several reports have revealed that due to its endocrine disrupting nature, BPA is associated with numerous principal non-communicable diseases (NCDs) such as obesity (Hong et al., 2023), infertility (Yadav et al., 2023), diabetes (Hwang et al., 2018), and cancer (Li et al., 2023a).

BPA's potential influence on cancer initiation and progression, especially in estrogen-related cases, is intriguing, stemming from research connecting BPA exposure to intricate molecular pathways in cancer development and spread (Ogawa et al., 2023). It has been demonstrated that normal ER signaling supports benign functions such as differentiation and

development in healthy breast tissue. However, numerous studies have proven that the abnormal activity of ER signaling triggers tumor formation in breast tissue (Xue et al., 2019). ER-alpha (α) and ER-beta (β) are two estrogen receptor subgroups that are thought to be the primary targets of BPA (Li et al., 2023b). A study reported that ERα moderates cell proliferation in breast cancer by modulating the cell cycle checkpoints (JavanMoghadam et al., 2016). Another report has indicated that the ERα agonistic activity of BPA triggers the development of ER-dependent cancer by activating the estrogen response element (ERE) promoter (Tillett, 2009). Taken together, tuning both ER signaling, and cell cycle control appear to be synchronized mechanisms underlying the biological effect exerted by BPA in breast cancer formation.

5. Conclusion

In conclusion, the results of this study suggest that BPA, even at very low concentrations, causes breast cancer development and progression by stimulating ER signaling pathways and cell cycle-related factors that promote cancer cell proliferation.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

References

Almeida, S., Raposo, A., Almeida-Gonzalez, M., & Carrascosa, C. (2018). Bisphenol A: Food exposure and impact on human health. *Comprehensive Reviews in Food Science and Food Safet*, *17*(6), 1503-1517.

Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., ... & Soboleva, A. (2012). NCBI GEO: archive for functional genomics data sets-update. *Nucleic Acids Research*, *41*(D1), D991-D995.

Bondar, G., Kuo, J., Hamid, N., & Micevych, P. (2009). Estradiol-induced estrogen receptor-alpha trafficking. *Journal of Neuroscience*, *29*(48), 15323-15330.

Cai, Z., Wang, J., Li, Y., Shi, Q., Jin, L., Li, S., ... & Liu, Q. (2023). Overexpressed cyclin D1 and CDK4 proteins are responsible for the resistance to CDK4/6 inhibitor in breast cancer that can be reversed by PI3K/mTOR inhibitors. *Science China Life Sciences*, *66*(1), 94-109.

Castillo-Sanchez, R., Ramirez-Ricardo, J., Martinez-Baeza, E., Cortes-Reynosa, P., Candanedo-Gonzales, F., Gomez, R., & Salazar, E. P. (2020). Bisphenol A induces focal adhesions assembly and activation of FAK, Src and ERK2 via GPER in MDA-MB-231 breast cancer cells. *Toxicology In Vitro*, *66*, 104871.

Ceylan, H. (2021). Identification of hub genes associated with obesity-induced hepatocellular carcinoma risk based on integrated bioinformatics analysis. *Medical Oncology*, *38*(6), 63.

Ceylan, H. (2022). Integrated bioinformatics analysis to ıdentify alternative therapeutic targets for Alzheimer's disease: Insights from a synaptic machinery perspective. *Journal of Molecular Neuroscience*, *72*(2), 273-286.

Ceylan, H., Budak, H., Kocpinar, E. F., Baltaci, N. G., & Erdogan, O. (2019). Examining the link between dose-dependent dietary iron intake and Alzheimer's disease through oxidative stress in the rat cortex. *Journal of Trace Elements in Medicine and Biology*, *56*, 198-206.

Chandrashekar, D. S., Bashel, B., Balasubramanya, S. A. H., Creighton, C. J., Ponce-Rodriguez, I., Chakravarthi, B., & Varambally, S. (2017). UALCAN: A Portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia*, *19*(8), 649-658.

CTD, (2023). Official Website of The Comparative Toxicogenomics Database, https://ctdbase.org/, Last accessed on August 10, 2023.

DAVID, (2023). Official Website of The Database for Annotation, Visualization and Integrated Discovery, https://david.ncifcrf.gov/, Last accessed on August 10, 2023.

Davis, A. P., Grondin, C. J., Johnson, R. J., Sciaky, D., Wiegers, J., Wiegers, T. C., & Mattingly, C. J. (2021). Comparative Toxicogenomics Database (CTD): update 2021. *Nucleic Acids Research*, *49*(D1), D1138-D1143.

Dehdashti, B., Nikaeen, M., Amin, M. M., & Mohammadi, F. (2023). Health risk assessment of exposure to Bisphenol A in polymeric baby bottles. *Environmental Health Insights*, *17*, 11786302231151531.

Ding, L., Cao, J., Lin, W., Chen, H., Xiong, X., Ao, H., Yu, M., Lin, J., & Cui, Q. (2020). The roles of cyclin-dependent kinases in cell-cycle progression and therapeutic strategies in human breast cancer. *International Journal of Molecular Sciences*, *21*(6), 1-28.

Dong, S., Huang, F., Zhang, H., & Chen, Q. (2019). Overexpression of BUB1B, CCNA2, CDC20, and CDK1 in tumor tissues predicts poor survival in pancreatic ductal adenocarcinoma. *Bioscience Reports*, *39*(2), BSR20182306.

EPA. (2023). United States Environmental Protection Agency. https://www.epa.gov/. Last accessed on August 10, 2023.

Fauconnier, M. B., Albert, C., Tondreau, A., Maumy, L., Rouzier, R., & Bonneau, C. (2023). Bisphenol A and breast cancer: State of knowledge and meta-analysis. *Bulletin du Cancer*, *110*(2), 151-159.

García-Gutiérrez, L., Bretones, G., Molina, E., Arechaga, I., Symonds, C., Acosta, J. C., ... & León, J. (2019). Myc stimulates cell cycle progression through the activation of Cdk1 and phosphorylation of p27. *Scientific Reports*, *9*(1), 18693.

GEO2R, (2023). Gene Expression Omnibus, https://www.ncbi.nlm.nih.gov/geo/geo2r/, Last accessed on August 10, 2023.

GEPIA2, (2023). Gene Expression Profiling Interactive Analysis, http://gepia2.cancer-pku.cn/#index, Last accessed on August 10, 2023.

Gyorffy, B. (2021). Survival analysis across the entire transcriptome identifies biomarkers with the highest prognostic power in breast cancer. *Computational and Structural Biotechnology Journal*, *19*, 4101-4109.

Hong, X., Zhou, Y., Zhu, Z., Li, Y., Li, Z., Zhang, Y., ... & Shen, T. (2023). Environmental endocrine disruptor bisphenol A induces metabolic derailment and obesity via upregulating IL-17A in adipocytes. *Environment International*, *172*, 107759.

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, *4*(1), 44-57.

Hwang, S., Lim, J. E., Choi, Y., & Jee, S. H. (2018). Bisphenol A exposure and type 2 diabetes mellitus risk: a meta-analysis. *BMC Endocrine Disorders*, *18*(1), 81.

JavanMoghadam, S., Weihua, Z., Hunt, K. K., & Keyomarsi, K. (2016). Estrogen receptor alpha is cell cycle-regulated and regulates the cell cycle in a ligand-dependent fashion. *Cell Cycle*, *15*(12), 1579-1590.

Jensen, L. J., Kuhn, M., Stark, M., Chaffron, S., Creevey, C., Muller, J., ... & von Mering, C. (2009). STRING 8-a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Research*, *37*(suppl_1), D412-D416.

KM-plotter, (2023). Official Website of The Kaplan Meier plotter, https://kmplot.com/analysis/, Last accessed on August 10, 2023.

Kustatscher, G., Grabowski, P., & Rappsilber, J. (2017). Pervasive coexpression of spatially proximal genes is buffered at the protein level. *Molecular Systems Biology*, *13*(8), 937.

Kwon, Y. (2022). Potential pro-tumorigenic effect of bisphenol A in breast cancer via altering the tumor microenvironment. *Cancers*, *14*(12), 3021.

Li, T., Fu, J., Zeng, Z., Cohen, D., Li, J., Chen, Q., Li, B., & Liu, X. S. (2020). TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Research*, *48*(W1), W509-W514.

Li, L., Wang, M. Y., Jiang, H. B., Guo, C. R., Zhu, X. D., Yao, X. Q., Zeng, W. W., Zhao, Y., & Chi, L. K. (2023a). Bisphenol A induces testicular oxidative stress in mice leading to ferroptosis. *Asian Journal of Andrology*, *25*(3), 375-381.

Li, C., Sang, C., Zhang, S., Zhang, S., & Gao, H. (2023b). Effects of bisphenol A and bisphenol analogs on the nervous system. *Chinese*

Medical Journal, *136*(3), 295-304.

Nardin, S., Mora, E., Varughese, F. M., D'Avanzo, F., Vachanaram, A. R., Rossi, V., Saggia, C., Rubinelli, S., & Gennari, A. (2020). Breast cancer survivorship, quality of life, and late toxicities. *Frontiers in Oncology*, *10*, 864.

Niu, Y., Xu, J., & Sun, T. (2019). Cyclin-dependent kinases 4/6 ınhibitors in breast cancer: Current status, resistance, and combination strategies. *Journal of Cancer*, *10*(22), 5504-5517.

Ogawa, M., Kitamoto, J., Takeda, T., & Terada, M. (2023). Bisphenol A prevents MCF-7 breast cell apoptosis via the inhibition of progesterone receptor transactivation. *Journal of Biochemical and Molecular Toxicology*, e23367.

Pei, J., Wang, Y., & Li, Y. (2020). Identification of key genes controlling breast cancer stem cell characteristics via stemness indices analysis. *Journal of Translational Medicine*, *18*(1), 74.

Pekarek, L., Torres-Carranza, D., Fraile-Martinez, O., García-Montero, C., Pekarek, T., Saez, M. A., ... & Ortega, M. A. (2023). An Overview of the Role of MicroRNAs on Carcinogenesis: A Focus on Cell Cycle, Angiogenesis and Metastasis. *International Journal of Molecular Sciences*, *24*(8), 7268.

Priyadarshini, E., Parambil, A. M., Rajamani, P., Ponnusamy, V. K., & Chen, Y. H. (2023). Exposure, toxicological mechanism of endocrine disrupting compounds and future direction of identification using nano-architectonics. *Environmental Research*, *225*, 115577.

Ritchie, S. C., Lambert, S. A., Arnold, M., Teo, S. M., Lim, S., Scepanovic, P., ... & Inouye, M. (2021). Integrative analysis of the plasma proteome and polygenic risk of cardiometabolic diseases. *Nature Metabolism*, *3*(11), 1476-1483.

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research*, *13*(11), 2498-2504.

Stillwater, B. J., Bull, A. C., Romagnolo, D. F., Neumayer, L. A., Donovan, M. G., & Selmin, O. I. (2020). Bisphenols and risk of breast cancer: A narrative review of the ımpact of diet and bioactive food components. *Frontiers in Nutrition*, *7*, 581388.

STRING, (2023). Protein-Protein Interaction Networks Functional Enrichment Analysis, https://string-db.org/, Last accessed on August 10, 2023.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of ıncidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209-249.

Tang, Z., Kang, B., Li, C., Chen, T., & Zhang, Z. (2019). GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Research*, *47*(W1), W556-W560.

TIMER2.0, (2023). Tumor IMmune Estimation Resource, http://cistrome.org/TIMER/, Last accessed on August 10, 2023.

Tillett, T. (2009). Bisphenol A, chapter 2: new data shed light on exposure, potential bioaccumulation. *Environmental Health Perspectives*, *117*(5), A210.

UALCAN, (2023). The University of ALabama at Birmingham Cancer Data Analysis Portal, https://ualcan.path.uab.edu/, Last accessed on August 10, 2023.

WHO, (2023). Official Website of World Health Organization, https://www.who.int/health-topics/cancer/, Last accessed on August 12, 2023.

Xue, M., Zhang, K., Mu, K., Xu, J., Yang, H., Liu, Y., ... & Zhuang, T. (2019). Regulation of estrogen signaling and breast cancer proliferation by an ubiquitin ligase TRIM56. *Oncogenesis*, *8*(5), 30.

Yadav, S. K., Bijalwan, V., Yadav, S., Sarkar, K., Das, S., & Singh, D. P. (2023). Susceptibility of male reproductive system to bisphenol A, an endocrine disruptor: Updates from epidemiological and experimental evidence. *Journal of Biochemical and Molecular Toxicology*, *37*(4), e23292.

Yesilkent, E. N., & Ceylan, H. (2022). Investigation of the multi-targeted protection potential of tannic acid against doxorubicin-induced kidney damage in rats. *Chemico-Biological Interactions*, *365*, 110111.

Zhang, X., Yang, L., Huang, B., Yin, J., & Wei, Y. (2023). Identification and validation of Cyclin A2 and Cyclin E2 as potential biomarkers in small cell lung cancer. *Oncol Res Treat*, *46*(6), 246-258.

Zhou, H., Lei, P. J., & Padera, T. P. (2021). Progression of metastasis through lymphatic system. *Cells*, *10*(3), 627.

Zuryn, A., Krajewski, A., Klimaszewska-Wisniewska, A., Grzanka, A., & Grzanka, D. (2019). Expression of cyclin B1, D1 and K in nonsmall cell lung cancer H1299 cells following treatment with sulforaphane. *Oncology Reports*, *41*(2), 1313-1323.

Cite as: Akkus, M., & Ceylan, H. (2023). Identification and verification of promising diagnostic genes in bisphenol A-associated breast cancer development via *in silico* analysis. *Front Life Sci RT*, *4*(2), 72-78.

Supplementary

Suppl. Table 1. GO and KEGG pathways enrihment analysis for **upregulated DEGs**.

Category	Term	PValue	Genes
BP	GO:0008284~positive regulation of cell proliferation	1,10E-03	*FGFBP1, TNC, LAMC2, SOX9, THBS1, PTHLH, CXCL5*
BP	GO:0008544~epidermis development	1,30E-03	*KRT14, LAMC2, CST6, PTHLH*
BP	GO:0000281~mitotic cytokinesis	2,50E-03	*KIF23, CEP55, ZNF365*
BP	GO:0030198~extracellular matrix organization	1,40E-02	*TNC, LAMC2, SOX9, THBS1*
BP	GO:0008608~attachment of spindle microtubules to kinetochore	2,30E-02	*KNL1, NDC80*
BP	GO:0007267~cell-cell signaling	2,70E-02	*FGFBP1, INHBA, PTHLH, CXCL5*
BP	GO:0000082~G1/S transition of mitotic cell cycle	2,80E-02	*RRM2, MCM10, INHBA*
BP	GO:0007062~sister chromatid cohesion	2,90E-02	*KNL1, NDC80, ZWINT*
BP	GO:0071397~cellular response to cholesterol	3,00E-02	*INHBA, LRP8*
BP	GO:0006271~DNA strand elongation involved in DNA replication	3,80E-02	*GINS1, GINS2*
BP	GO:0032270~positive regulation of cellular protein metabolic process	3,80E-02	*UHRF1, INHBA*
BP	GO:0042493~response to drug	4,30E-02	*INHBA, THBS1, LRP8, DUSP6*
CC	GO:0005615~extracellular space	4,30E-03	*FGFBP1, TCN1, LIPG, TNC, LAMC2, THBS1, CST6, F3, PTHLH, CXCL5*
CC	GO:0000811~GINS complex	7,20E-03	*GINS1, GINS2*
CC	GO:0005576~extracellular region	1,30E-02	*FGFBP1, TCN1, LIPG, TNC, LAMC2, INHBA, THBS1, LRP8, PTHLH, CXCL5*
CC	GO:0031298~replication fork protection complex	1,70E-02	*GINS2, MCM10*
CC	GO:0000777~condensed chromosome kinetochore	1,90E-02	*KNL1, NDC80, ZWINT*
CC	GO:0031012~extracellular matrix	3,40E-02	*TNC, THBS1, EDIL3, F3*
CC	GO:0009986~cell surface	4,10E-02	*FGFBP1, LIPG, THBS1, F3, PROM2*
MF	GO:0008201~heparin binding	7,30E-03	*FGFBP1, LIPG, LAMC2, THBS1*
MF	GO:0043138~3'-5' DNA helicase activity	1,70E-02	*GINS1, GINS2*
MF	GO:0005200~structural constituent of cytoskeleton	3,10E-02	*KRT14, NDC80, KRT6B*
KEGG_PATHWAY	hsa04512:ECM-receptor interaction	8,1E-03	*TNC, LAMC2, THBS1*
KEGG_PATHWAY	hsa04510:Focal adhesion	4,1E-03	*TNC, LAMC2, THBS1*

Suppl. Table 2. GO and KEGG pathways enrihment analysis for **downregulated DEGs**.

Category	Term	PValue	Genes
BP	GO:0006956~complement activation	1,70E-02	*C1S, C1R, CFB*
BP	GO:0043030~regulation of macrophage activation	2,00E-02	*RORA, SLC7A2*
BP	GO:0006809~nitric oxide biosynthetic process	2,90E-02	*RORA, SLC7A2*
BP	GO:0016601~Rac protein signal transduction	4,20E-02	*CDH13, RHOU*
BP	GO:0006810~transport	4,40E-02	*CLCA2, LAPTM5, ABCG1, SLC7A2*
BP	GO:0055114~oxidation-reduction process	4,40E-02	*SDR16C5, DHRS9, MAOA, HSD17B2, CYP4B1*
BP	GO:0010906~regulation of glucose metabolic process	4,90E-02	*PDK4, RORA*
CC	GO:0005886~plasma membrane	7,70E-03	*KLRC2, PLCL1, FHL1, CXCR4, SLC7A2, GHR, IL1RL1, CLEC2B, GPNMB, BASP1, CLCA2, CDH13, RHOU, IL6R, GPM6B, ABCG1, CFB*
CC	GO:0005887~integral component of plasma membrane	9,10E-03	*GHR, CLEC2B, GPNMB, KLRC2, CLCA2, LAPTM5, GPR137B, ABCG1, SLC7A2*
CC	GO:0005789~endoplasmic reticulum membrane	3,60E-02	*SDR16C5, RTN1, DHRS9, HSD17B2, CYP4B1, ABCG1*
CC	GO:0072562~blood microparticle	4,20E-02	*C1S, C1R, CFB*
MF	GO:0004896~cytokine receptor activity	2,70E-03	*GHR, IL1RL1, IL6R*
MF	GO:0030246~carbohydrate binding	8,30E-03	*CLEC2B, KLRC2, GALNT15, CHI3L1*
MF	GO:0047035~testosterone dehydrogenase (NAD+) activity	1,50E-02	*DHRS9, HSD17B2*
MF	GO:0004745~retinol dehydrogenase activity	3,80E-02	*SDR16C5, DHRS9*
KEGG_PATHWAY	hsa00830:Retinol metabolism	1,30E-02	*SDR16C5, DHRS9, ADH1B*
KEGG_PATHWAY	hsa04610:Complement and coagulation cascades	1,50E-02	*C1S, C1R, CFB*