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The breast cancer biomarkers associated with the development of the disease; an in-silico-based study

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Abstract

Introduction: Breast cancer (BC) is among the top causes of mortality among women worldwide. Identifying genes by differential expression associated with the development of the disease helps us to better understanding the molecular mechanisms of BC.

Objectives: Our study used in-silico analysis to identify hub genes could trigger the development of BC.

Materials and Methods: In this cross-sectional in-silico study, we identified GSE38959 and GSE45827 for differentially expressed genes (DEGs) in the Gene Expression Omnibus (GEO) database, with an adjusted $P<0.05$. In both sets, logFC ≥ 2 and logFC ≤ -2 were observed in the DEGs that express themselves within cases and normal BC samples. A comparison was then performed, detecting two common datasets of DEGs using the GEO2R tool. Pathways were elucidated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology databases. Thereafter, protein-protein interactions (PPIs) were analyzed using Cytoscape and Gephi. Finally, a GEPIA analysis was conducted to validate the target genes.

Results: Using the GEO, 322 common DEGs were identified and 65 hub genes as PPIs. The DEGs were enriched in functions associated with cell division, chromosomes, centromeric regions, microtubule binding, and the cell cycle based on the gene ontology (GO) and KEGG pathways analysis. The expression of 6 genes, CDK1, CCNB1, TOP2A, CXCL12, IGF1, and KIT, represented statistically significant values when the normal and tumor samples were compared via GEPIA analysis.

Conclusion: This study introduced six genes (CDK1, CCNB1, TOP2A, CXCL12, IGF1, and KIT) with high expression significantly, which could act as a biomarker for BC development ($P<0.05$ for all genes). Further comprehensive experimental in vivo studies are needed to describe their role in BC.

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Introduction

Globally, the most frequent malignant neoplasm in women is breast cancer (BC). It is estimated that in 2018, approximately 2.089 million women were diagnosed with BC (1). The incidence of BC is rising worldwide, with the highest rates in industrialized nations, where nearly half of all cases can be found. This increase is attributed to lifestyle-related factors, such as poor diet, smoking, high stress, and lack of physical activity (2). Mammography is widely recognized as a key screening tool, particularly effective for women aged 50-69, with a sensitivity of 75%-95% and specificity of 80%-95% (3). Magnetic resonance mammography is used for screening for people with a genetic predisposition to BC. However, if mammography detects a suspicious lesion, an ultrasound and possibly a thick needle biopsy followed by a histopathological examination will be conducted (4).

Key point
- Identifying breast cancer (BC) biomarkers could trigger the disease development and helps better understand the molecular mechanisms of BC.
- We identified 6 genes (CDK1, CCNB1, TOP2A, CXCL12, IGF1, and KIT) by significant high expression, which could be introduced as potential biomarkers for BC.

Approximately 5%–10% of BC cases are related to genetic susceptibility. Interestingly, the most well-known genetic mutations linked to BC are found in the breast cancer gene 1 (BRCA1) and BRCA2 genes. As a

tumor suppressor, the BRCA1 gene on chromosome 17 could encode a nuclear protein essential for genome stability. This protein needs several factors to affect transcription, DNA repair, and recombination processes such as tumor suppressor genes and signal transduction genes (4,5). BRCA1 works alongside the product of BRCA2, which is another BC-related tumor suppressor gene on chromosome 13, uses homologous recombination for double-strand DNA break editing (6). Mutations in the BRCA1 and BRCA2 genes are present in only 3%–5% of BC patients. Hence, mutant carriers should be identified and put into preventive programs. Individuals with BRCA1/BRCA2 mutations have an estimated 10-fold increased risk of developing BC (7).

BRCA1 mutations are associated with triple-negative BC, while BRCA2 mutations are linked to estrogen receptor-positive BC (8,9). Other suppressor genes with high-penetrance mutations predisposing to BC include in Li-Fraumeni syndrome (TP53 mutants) and Cowden syndrome (by PTEN mutations). Mutations in those genes are rarer than BRCA1 and BRCA2. However, they also predispose women to a high risk of developing BC (10). Moreover, the NBN, ATM, BRIP1, CHEK2, RAD51C, and PALB2 mutations are moderately increase BC risk (11,12). It is believed that while less than 10% of BC are genetically determined, over 90% result from sporadic somatic mutations. Noteworthy, the BC risk doubles in women with a close relative who has a history of developing BC and increases three to six times if two close relatives have been affected by that malignancy. Moreover, this risk decreases with the relative's age at diagnosis (4).

Objectives

This research aims to identify and analyze hub genes associated with BC by analyzing common differentially expressed genes (DEGs) to understand better BC's molecular mechanisms, which could lead to novel therapeutic targets and improved patient outcomes.

Materials and Methods

Microarray data

In this cross-sectional in-silico study, we downloaded GSE38959 (Agilent-014850 Microarray 4x44K G4112F), which included 30 patients with BC and 13 healthy individuals, and GSE45827 (Affymetrix U133 Plus 2.0) into the Gene Expression Omnibus (GEO) platform (https://www.ncbi.nlm.nih.gov/geo/). GEO is a significant resource that allows users to download and use enormous microarray gene expression datasets for free. Both datasets in this investigation matched the following criteria: (a) inclusion of samples from Human BC, (b) existence of a case-control group, and (c) sample size of more than 40.

Common differentially expressed genes

Common DEGs were compared between patients and normal cases using GEO2R (www.ncbi.nlm.nih.gov/geo/geo2r/). Data with adjusted *P* values < 0.05, logFC (fold change) >2, and logFC (fold change) ≤-2 were introduced for network development as DEGs. For comparison of DEG genes, up- and downregulated genes via Venn diagrams were used (https://bioinformatics.psb.ugent.be/webtools/Venn/).

Enrichment analysis

For the enrichment analysis we used Gene Ontology (GO) (https://www.geneontology.org/). Moreover, the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) was used for assessing the pathways in which a certain gene is enriched. the DAVID database (http://www.david.ncifcrf.gov/) was used for DEGs functional analysis with a significance level set at $P < 0.05$.

PPI network and performance analysis

The STRING server (https://www.string-db.org/; version 11.5) is used for finding the hub genes through a network of protein-protein interaction (PPI) with the common DEGs and centrality parameters. The Cytoscape software (version 3.6.0) is used for the PPI network construction. The input file of STRING was fed with the data to analyze significant genes. The hub genes were identified in degree, betweenness, and closeness based on the centrality parameters. These hub genes have been further clustered by the Gephi package.

Verification and survival analysis

Differential expression of mRNA was analyzed in the search for biomarkers of BC using the GEPIA "Single Gene Analysis" module. GEPIA analyzes and visualizes the expression data derived from RNA sequencing.

Statistical analysis

The extracted DEGs from GEO datasets were subjected to analysis. Statistically significant findings were identified based on adjusted *P* values below 0.05 and integrated into the considerable dataset. For the GO and KEGG enrichment analysis, a significance threshold of *P* values < 0.05 was employed. The GEPIA Box Plots module used *P* values < 0.05, log2FC < 1, and matching TCGA normal to GTEx Data, facilitated examining gene expression levels associated with BC.

Results

Common differentially expressed genes

The GEO database was used to select GSE38959 and GSE45827. Venn diagram software was then used to discover shared DEGs across the two datasets (Figure 1). The research identified 322 common DEGs, including 117 upregulated and 205 downregulated DEGs. Table S1 (Supplementary file 1) includes a list of all 322 frequent DEGs.

GO and KEGG pathway enrichment

Using the DAVID and Enrichr, the top 10 enriched GO

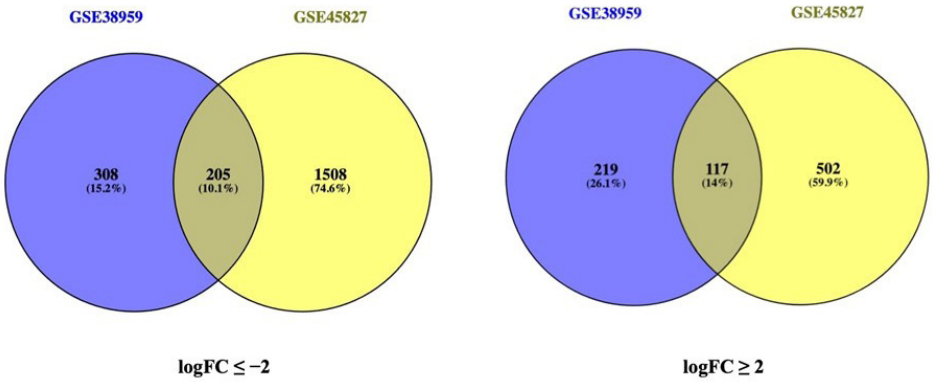

Figure 1. Venn diagram common DEGs (n=322) among GSE38959 and GSE45827 datasets. Different colors represent different datasets (logFC ≥ 2 and logFC ≤ −2).

terms and KEGG pathways were obtained (Table 1). Totally, 322 DEGs were significantly enriched in cell division, mitotic spindle assembly checkpoint, chromosomal segregation, and mitotic spindle organization.

The top four most abundant cellular components are the chromosome, centromeric region, spindle, kinetochore, and mitotic spindle. In GO molecular function analysis, the top four significantly enriched phrases were identified for binding to microtubule, protein, ATP, and integrin. Also they were for the cell cycle, signaling, progesterone-mediated oocyte maturation, and protein digestion and absorption.

Table 1. Common DEGs using functional and pathway enrichment analysis

Category	Term	Count	*P* value based on DAVID database analysis
GOTERM_BP_DIRECT	GO:0051301~cell division	44	4.20147490392258E-25
GOTERM_BP_DIRECT	GO:0007094~mitotic spindle assembly checkpoint	12	3.05304559382787E-13
GOTERM_BP_DIRECT	GO:0007059~chromosome segregation	17	9.86972071002192E-13
GOTERM_BP_DIRECT	GO:0007052~mitotic spindle organization	12	5.88694447831251E-10
GOTERM_BP_DIRECT	GO:0000070~mitotic sister chromatid segregation	10	7.07226199753302E-10
GOTERM_BP_DIRECT	GO:0051988~regulation of attachment of spindle microtubules to kinetochore	7	4.70717926996756E-09
GOTERM_BP_DIRECT	GO:0000278~mitotic cell cycle	15	1.95152825917979E-08
GOTERM_BP_DIRECT	GO:0008284~positive regulation of cell proliferation	24	2.46236492476566E-06
GOTERM_BP_DIRECT	GO:0000281~mitotic cytokinesis	9	4.99225006947273E-06
GOTERM_BP_DIRECT	GO:0007051~spindle organization	6	7.19826158078879E-06
GOTERM_CC_DIRECT	GO:0000775~chromosome, centromeric region	16	1.17243619208578E-14
GOTERM_CC_DIRECT	GO:0005819~spindle	19	6.64999689594979E-12
GOTERM_CC_DIRECT	GO:0000776~kinetochore	19	1.30028106213799E-11
GOTERM_CC_DIRECT	GO:0072686~mitotic spindle	18	3.01272178785005E-11
GOTERM_CC_DIRECT	GO:0030496~midbody	18	2.07255937150727E-09
GOTERM_CC_DIRECT	GO:0005813~centrosome	31	2.51051343070019E-08
GOTERM_CC_DIRECT	GO:0005634~nucleus	131	7.54829348395616E-08
GOTERM_CC_DIRECT	GO:0005737~cytoplasm	121	8.3304941575554E-07
GOTERM_CC_DIRECT	GO:0045171~intercellular bridge	11	2.08566786555995E-06
GOTERM_CC_DIRECT	GO:0005654~nucleoplasm	91	3.60504787145935E-06
GOTERM_MF_DIRECT	GO:0008017~microtubule binding	24	3.60757331908448E-11
GOTERM_MF_DIRECT	GO:0005515~protein binding	239	5.3007635075383E-09
GOTERM_MF_DIRECT	GO:0005524~ATP binding	50	7.12540018591269E-07
GOTERM_MF_DIRECT	GO:0005178~integrin binding	13	5.56709661105006E-06
GOTERM_MF_DIRECT	GO:0003777~microtubule motor activity	8	3.15917469610343E-05
GOTERM_MF_DIRECT	GO:0016887~ATPase activity	20	3.67525605040006E-05
GOTERM_MF_DIRECT	GO:0030020~extracellular matrix structural constituent conferring tensile strength	7	6.0177748109176E-05

Table 1. Continued

Category	Term	Count	*P* value
GOTERM_MF_DIRECT	GO:0045236~CXCR chemokine receptor binding	5	6.42120564998702E-05
GOTERM_MF_DIRECT	GO:0005201~extracellular matrix structural constituent	10	8.47572290807155E-05
GOTERM_MF_DIRECT	GO:0008009~chemokine activity	7	1.23219289224074E-04
KEGG_PATHWAY	hsa04110: Cell cycle	20	6.20429214884524E-11
KEGG_PATHWAY	hsa04115: p53 signaling pathway	8	4.10049612661964E-04
KEGG_PATHWAY	hsa04914: Progesterone-mediated oocyte maturation	9	5.73155689520846E-04
KEGG_PATHWAY	hsa04974: Protein digestion and absorption	9	6.1183338836075E-04
KEGG_PATHWAY	hsa04151: PI3K-Akt signaling pathway	17	9.49302999257198E-04
KEGG_PATHWAY	hsa04512: ECM-receptor interaction	8	0.001246519637903790
KEGG_PATHWAY	hsa04933: AGE-RAGE signaling pathway in diabetic complications	8	0.002446354901595820
KEGG_PATHWAY	hsa05146: Amoebiasis	8	0.002737084461204500
KEGG_PATHWAY	hsa04114: Oocyte meiosis	9	0.002883176318966230
KEGG_PATHWAY	hsa04814: Motor proteins	11	0.003012716385507410

PPI network and hub genes

Via the STRING server, we drew the PPI network foundation and subsequently visualized them by the Cytoscape (Figure 2). Analysis of PPI networks allows for identifying influential molecular interactions contributing to disease progression. Two hundred seventy-five nodes were identified as DEGs (nodes: 275, coefficient: 0.527, centralization: 0.271). The hub genes were ranked based on centrality parameters (Table S2). Furthermore, by the STRING, we identified the top 65 common genes as key hubs within the network (nodes; 65, coefficient: 0.721, centralization: 0.307) (Table S3).

Clustering of hub genes

To reconstruct the PPI network, we utilized Gephi 0.9.2 (https://www.gephi.com/). Subsequent clustering of the hub genes resulted in the formation of distinct modules (Figure 3). Table 2 displays the Gephi top-ranked genes. Within the network, two modules were identified as clusters.

Verification of the hub genes

The analysis conducted using GEPIA revealed that certain genes exhibit significant prognostic value in BC. The higher gene expression in BC compared to normal

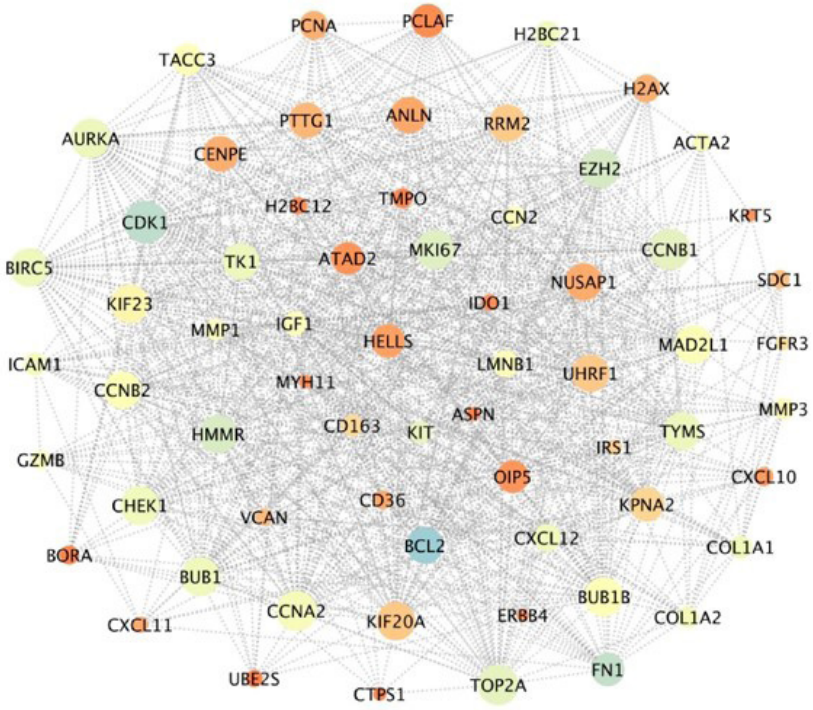

Figure 2. The PPI network analysis. The nodes size (degree) and color (betweenness) depict the DEGs from GSE38959 and GSE45827.

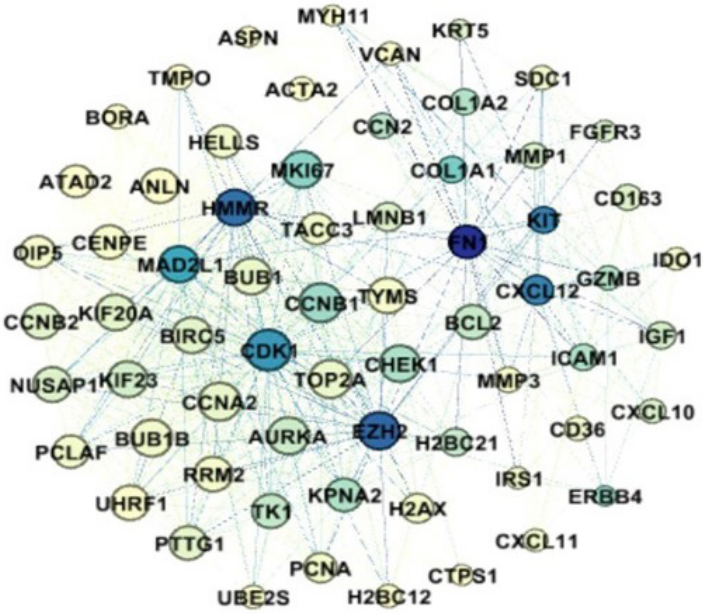

Figure 3. Gephi PPI network visualization and analysis. The size represents degree and the color represents betweenness.

samples indicates their potential utility as biomarkers (Figure 4). Notably, this study focused on six genes that demonstrated significant differential expression between normal and tumor samples. Moreover, the genes with a high degree in module 1 included cyclin-dependent kinase 1 (CDK1), cyclin B1 (CCNB1), DNA topoisomerase II alpha (TOP2A), and genes in module 2 included C-X-C motif chemokine ligand 12 (CXCL12), insulin-like growth factor 1 (IGF1) and KIT proto-oncogene, receptor tyrosine kinase (KIT) were down-regulated in BC samples, Therefore, these genes might have the potential to be used as biomarkers for BC ($P<0.05$ for all genes) (Figure 4).

Table 2. The Gephi list of top-ranked genes

Module 1		Module 1	
Gene symbol	**Degree**	**Gene symbol**	**Degree**
CDK1	42	BCL2	31
CCNB1	39	FN1	28
TOP2A	38	CXCL12	22
CCNA2	38	IGF1	18
EZH2	36	KIT	17
BUB1B	36	COL1A1	17
AURKA	36	ICAM1	17
CHEK1	35	COL1A2	16
BUB1	35	CD163	16
MKI67	35	CCN2	15
KIF23	34	MMP3	15
KIF20A	34	MMP1	15
HMMR	34	GZMB	13
MAD2L1	34	CXCL10	13
BIRC5	34	SDC1	13
TYMS	33	CD36	13
UHRF1	32	ACTA2	12
RRM2	32	VCAN	11
NUSAP1	32	IDO1	10
CCNB2	32	CXCL11	10
ANLN	31	IRS1	9
TK1	31	FGFR3	8
PTTG1	30	ASPN	7
CENPE	30	MYH11	7
KPNA2	29	KRT5	6
PCLAF	28	ERBB4	6
HELLS	28		
ATAD2	27		
OIP5	27		
TACC3	26		
PCNA	23		
H2AX	22		
LMNB1	21		
H2BC21	19		
TMPO	14		
BORA	13		
UBE2S	11		
H2BC12	11		
CTPS1	7		

Discussion

Despite recent advances in early detection and medication, BC remains the top cause of women's cancer-related deaths, globally. Developing nations have the greatest death rates due to restricted screening, diagnostic, and therapeutic options (4). The current work sought to find hub genes using GSE38959 and GSE45827 GEO databases. We discovered 322 common DEGs, 117 upregulated and 205 downregulated, which were subsequently evaluated using GO and KEGG pathway enrichment approaches. The major findings from these studies, as well as the subsequent building and analysis of the PPI network, revealed information regarding the molecular processes causing BC and possible targets for therapeutic intervention design and development.

Our findings align with those of Xing et al, who identified the overexpression of cyclin-associated gene clusters (CDK1, CCNA2, and CCNB1) in BC tissues. These genes were correlated with advanced tumor stages and poorer survival outcomes (13), supporting our identification of CDK1 and CCNB1 as key hub genes in BC. Therefore, their potential as prognostic biomarkers and therapeutic targets can be considered. Qian et al also showed the significance of CDK1 and demonstrated that the RNA-binding protein KIAA1429 regulates CDK1 expression in an m6A-independent manner, which

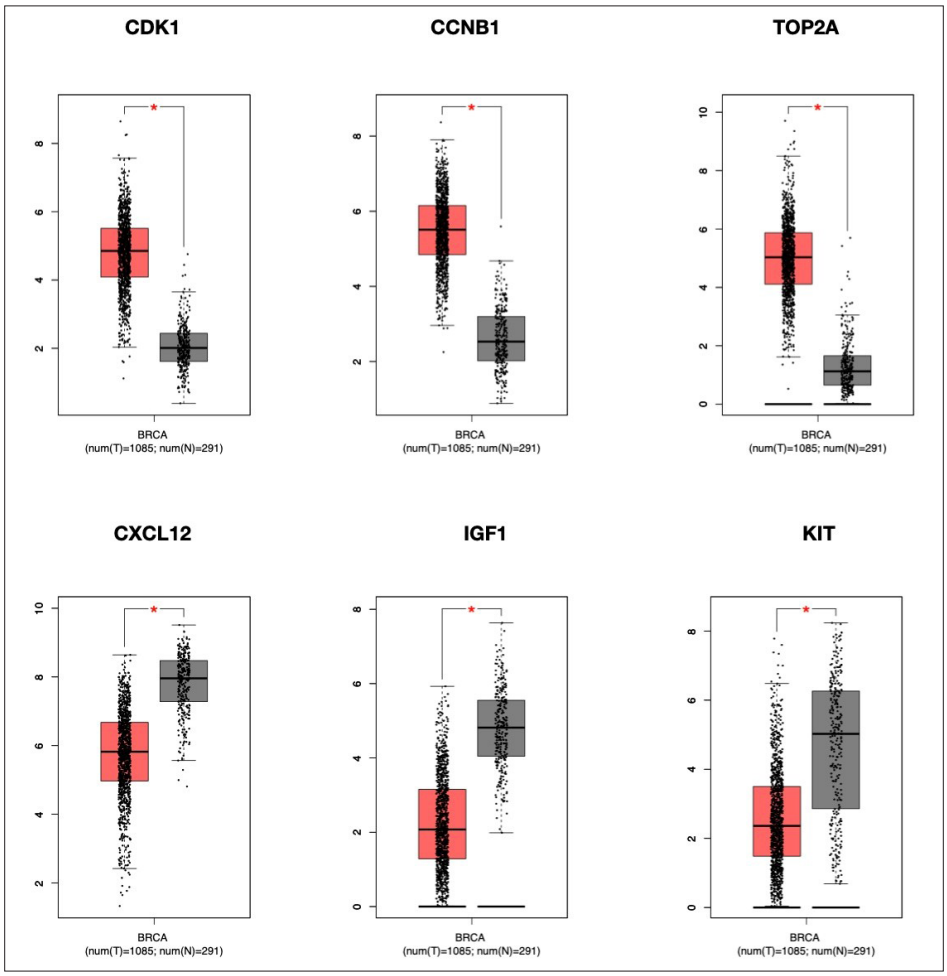

Figure 4. The core genes' plots included CDK1, CCNB1, TOP2A, CXCL12, IGF1, and KIT of normal and tumor samples by significant differences (*$P < 0.05$).

promotes BC proliferation and metastasis (14). Moreover, Li et al focused on the genetic variants of CCNB1 and CDK1 in the Chinese Han population to find if there are any associations with BC susceptibility, progression, and survival. Their identification of specific SNPs linked to BC risk and progression supports the importance of these genes, reinforcing our results that position CCNB1 and CDK1 as important players in BC pathogenesis (15). Mehraj et al conducted a bioinformatic analysis that demonstrated the deregulation of CDKs, including CDK1, correlates with poor overall and relapse-free survival in BC. This study and our findings showed targeting CDKs is a promising approach for BC treatment, particularly about the high CDK1 expression in metastatic tumors (16). Xi et al identified RBM7 as a regulator of CDK1, stabilizing its mRNA and promoting BC cell proliferation, which provided further evidence of CDK1's critical function (17). Fang and colleagues' analysis of DEGs in BC also highlighted CDK1 and CCNB1 as potential therapeutic targets, emphasizing their overexpression across all BC stages. This comprehensive identification of hub genes aligns with our study's results, further validating the role of CDK1 and CCNB1 in BC (18). Fu et al (19) and Aljohani et al (20) both showed the prognostic value of CCNB1 in BC. Fu et al highlighted the association between this gene and survival time and immune cell infiltration (19), while Aljohani et al found high CCNB1 expression linked to aggressive tumor behavior and poor clinical outcomes (20). These findings corroborate our results, suggesting that CCNB1 is a crucial biomarker for BC prognosis.

Furthermore, our findings are consistent with earlier research highlighting the important involvement of TOP2A and HER2 in BC. Engstrøm et al observed a significant link between TOP2A alterations and HER2 status. They found that HER2 amplification predicts a poor outcome during the first five years after diagnosis, independent of TOP2A status. This implies that whereas TOP2A alterations are widespread in BC, their predictive significance may be restricted compared to HER2 (21). Chen et al reported that although TOP2A amplification is less frequent, it is significantly associated with HER2 amplification and poorer overall survival, reinforcing that HER2 status is a more robust prognostic marker than TOP2A (22). Nielsen et al demonstrated that simultaneous amplification of TOP2A and HER2 occurs in a subset of BC, though different mechanisms drive these amplifications. This study also highlighted that TOP2A and HER2 amplifications often do not co-occur,

suggesting complex genetic interactions and chromosomal rearrangements in these tumors (23). Research conducted by Qiao et al showed that TOP2A expression correlates significantly with ER, KI-67, and HER2 status, but its prognostic significance is limited (24).

Our study identified CXCL12 as one of the hub genes with altered expression in BC, in line with the reports from de Oliveira et al and Sun et al, who reported that lower CXCL12 expression, associated with a specific SNP (rs1801157), correlates with poorer clinical outcomes in estrogen receptor-positive BC patients (25,26). Sun et al demonstrated that the CXCL12-CXCR4 axis is crucial in promoting BC metastasis, with high CXCR4 expression linked to poor prognosis. These studies represent targeting potential of the CXCL12-CXCR4 axis into the therapeutic strategy for BC (25).

Our research also identified IGF1 as a key hub gene. This is comparable to the findings of Rigiracciolo et al, who discovered that high levels of IGF1 and its receptor IGF1R are related to triple-negative breast cancer (TNBC) by poor clinical outcomes. The IGF1/IGF1R-FAK-YAP signaling pathway has been demonstrated to increase TNBC cell proliferation and aggressiveness, indicating that it might be a promising target for developing novel therapies for this aggressive BC subtype (27). Rodríguez-Valentín et al, studied how genetic variations in energy homeostasis genes affect blood levels of IGF1 and IGFBP-3. They discovered that some SNPs can change the link between these serum concentrations and BC risk (28).

Conclusion

In conclusion, this study identified significant DEGs and hub genes associated with BC through comprehensive bioinformatics analyses. Identifying and verifying hub genes offer promising insights for developing new biomarkers and targeted therapies for BC and, therefore, can contribute to improved diagnosis, prognosis, and treatment of this prevalent malignancy. We recommend that future studies focus on the functional validation of these hub genes and their roles in BC to elucidate their potential clinical applications further.

Limitations of the study

In the present study, experimental assessments of identified biomarkers were neglected due to limited sources of funding.

Authors' contribution

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Resources: Mohammadreza Babaei, Azra Asghari Marzidare.
Software: Niloufar Sadat Kalaki, Fahimeh Safarnezhad Tameshkel.
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Validation: Mohammad Hadi Karbalaie Niya, Soheila Sayad.
Visualization: Fahimeh Safarnezhad Tameshkel, Azra Asghari Marzidare.
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Writing–review & editing: Mohammad Hadi Karbalaie Niya, Mohammad Hossein Razizadeh.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The authors have fully complied with ethical issues, such as plagiarism, data fabrication, and double publication.

Funding/Support

There was no funding agency to cover the study expenses.

Supplementary files

Supplementary file 1 contains Tables S1-S3.

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