



Original Article



Identification of Key Pathways and Hub Genes in Breast Cancer Via a Systems Biology Approach

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Abstract

Background: Breast cancer (BC) remains the leading cause of cancer-related mortality among women globally. Despite significant advances in diagnosis and treatment, the molecular mechanisms driving breast tumorigenesis are not yet fully elucidated. This study aimed to identify key genes and signaling pathways associated with BC pathogenesis and prognosis through comprehensive bioinformatic analysis.

Methods: In this study, gene expression data from the GSE124646 dataset were retrieved from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were identified based on the criteria of $|\log_2 \text{fold change}| > 1.5$ and $P \text{ value} < 0.01$. Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, were conducted. In addition, protein-protein interaction (PPI) network was constructed using STRING database and visualized using Cytoscape. Hub genes were identified based on network topology ($\text{degree} \geq 7$; betweenness centrality between 0.005 and 1). Further validation was performed using the GEPIA web tool and Kaplan-Meier survival analysis.

Results: A total of 923 DEGs were identified, comprising 645 upregulated and 278 downregulated genes. Enrichment analysis revealed that these genes were predominantly involved in extracellular matrix (ECM) organization and localized within collagen-containing ECM components. Molecular function analysis indicated significant enrichment in glycosaminoglycan binding. KEGG pathway analysis highlighted the PI3K-Akt signaling pathway as a major pathway implicated in BC. 73 hub genes were identified and incorporated into the PPI network. Survival analysis demonstrated that elevated expression of several hub genes was significantly associated with poor prognosis. GEPIA analysis confirmed aberrant expression of these genes in BC tissues compared to normal controls.

Conclusion: These findings enhance our understanding of the molecular underpinnings of breast cancer and highlight potential diagnostic biomarkers and therapeutic targets. Furthermore, this study identifies a subset of previously under-characterized genes, which may contribute to refining the molecular taxonomy and treatment strategies of BC.

Introduction

In the United States, breast cancer remains one of the most deadly cancers affecting women, second only to other major cancer types. However, breast cancer mortality has declined in recent years.¹ It is also the most common cancer in women.² According to the available global data from 2022, almost 20 million people were diagnosed with cancer (9.7 million people died of cancer globally), which includes 36 cancer types in 185 countries. Breast cancer was the most diagnosed cancer in women globally, with about 2.3 million new cases and 665,684 deaths worldwide.³

BC can be classified into three major subtypes based on the presence or absence of molecular markers. Subtypes of breast cancer affect the optimal therapy for each patient

in addition to the anatomic cancer stage and patient preferences.⁴ BC is a multifactorial disease.⁵ Sex, age, and blood group are known risk factors for breast cancer.⁶⁻⁹ Reproductive factors such as the age of menarche, the age of menopause, pregnancy, and the ovulatory menstrual cycle significantly affect the development of breast cancer.^{7,9} Genetic factors are correlated with breast cancer.¹⁰ Obesity, alcohol consumption, and smoking are associated with an increased risk of breast cancer.¹¹⁻¹⁴

Early diagnosis and treatment of BC can lead to more successful treatments and a decrease in the mortality rate of patients with this disease.¹⁵ BC can be diagnosed through mammography, ultrasound, magnetic resonance imaging (MRI), and high-end molecular bioimaging. Unfortunately, these techniques detect BC at a

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later stage.^{16,17}

It is necessary to detect breast cancer at early stages to improve patient outcomes. Therefore, with the use of microarray technology to detect breast cancer, advancements may be achieved.¹⁸ Recent studies have shown that gene expression analysis may improve breast cancer prognosis and treatment.¹⁸⁻²¹ DNA and protein analysis can be performed with microarrays, which are powerful tools in biomedical research and are based on probe molecules attached to a planar surface in a miniaturized grid pattern.²²

For over two decades, the research community has analyzed gene expression in human cancers, resulting in a vast amount of data. The development of DNA microarray technology in the late 1990s revolutionized how gene expression was analyzed.^{23,24} The development of additional gene expression profiling technologies, such as RNA sequencing (RNA-seq), has shed light on the specific genes that are differentially expressed, enabling a deeper understanding of novel transcripts and providing a far more precise measurement of the levels of transcripts and their isoforms.^{25,26}

Systems biology is about increasing the awareness and understanding of biology and related fields by joining the rules of engineering, physics, and math to the complexity of living systems through a continuous process to illustrate the interrelated processes occurring within a cell. This integration elucidates how environmental inputs and network alterations resulting from genomic abnormalities in patient tumors influence cellular behavior and ultimately affect patient outcomes.²⁷ Systems biology represents an approach that emphasizes a global perspective by analyzing the entire network of interactions rather than focusing on individual proteins, genes, or enzymes. This field has demonstrated that cellular proteins do not function in isolation; instead, these genes and proteins are interconnected, forming a complex molecular network that collaborates to fulfill specific functions.²⁸

The objective of our study was to discover novel biomarkers or hub genes from DEGs via an integrated analysis of microarray data. We employed a bioinformatics approach to identify the hub genes and differentially expressed genes between breast tumors and normal tissues. The PPI network was created using the STRING database with the Cytoscape software for visualization.

Moreover, KEGG analysis and gene ontology (GO) utilising the Enricher web tool were performed on the DEGs to gain relevant insights. Additionally, survival analysis and heatmaps were generated. Through the analysis of gene expression levels and biological pathways, we studied the genetic sources of faulty pathways in cancerous cells to identify potential targets for cancer treatment. A novel discovery of our findings was a group of underrepresented genes that are not widely studied in the breast cancer literature, adding another layer of awareness and suggesting new molecular candidates beyond the standard biomarkers.

Materials and Methods

Data Collection

The gene expression data of 10 BC samples and 10 normal samples (GSE124646) from the GPL96 platform (Affymetrix Human Genome U133A Array) were obtained from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). Figure 1 shows a summary of the steps accomplished.

Data Preprocessing and Identification of Differentially Expressed Genes (DEGs)

The raw gene expression data were first processed using the MAS5 method, implemented through the Affy package in R. Boxplots and histograms were generated to visualize the identified DEGs. We then analyzed DEGs between BC and normal samples via significance analysis via the microarray method with the limma package. The filtering conditions were as follows: $|\log_2\text{-fold change}| > 1.5$ and $P < 0.01$. A heatmap was plotted for the samples and DEGs via the heatmap package in R software.

Construction of The Protein-Protein Interaction Network

For protein-level interactions, we used the STRING database (<http://string-db.org/>), which allows you to map protein-protein interactions. In the STRING analysis, we only used interaction scores ≥ 0.9 , which are considered reliable confidence scores and used to build a network.²⁹ To visualize the PPI network and identify core genes, Cytoscape software (version 3.6.0) was utilized. With the threshold of a degree ≥ 7 , $0.005 \leq \text{betweenness} \leq 1$ hub genes were found. The thresholds were chosen based on studies in the field of network biology, as well as studies that showed that highly connected and central nodes are biologically important nodes in protein-protein interaction (PPI) networks. Degree of network indicates how many interactions a node has directly, and the degree threshold was used to show several biologically relevant hub genes by utilizing strong connectivity.^{30,31} Betweenness centrality indicates how far a node lies on the shortest path between other nodes. Betweenness centrality indicates whether a node is bridging bottlenecks in the network.³²⁻³⁴ To confirm the choice of hub genes, functional enrichment, survival analysis, and expression profiling were performed. All of these indicate the resulting genes are relevant from a biological perspective and as prognostic factors.³⁵

The candidate modules were detected via Gephi. Gephi is software for graph visualization, network analysis, and module development.³⁶ By running the Fruchterman-Reingold Algorithm, we create a force-directed layout algorithm.³⁷ Several aspects of the hub gene network, including the eigenvector, network diameter, closeness, and betweenness, were examined in Gephi. Additionally, Gephi identified the hub gene clusters and candidate modules.

Enrichment Analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses of the DEGs and hub genes

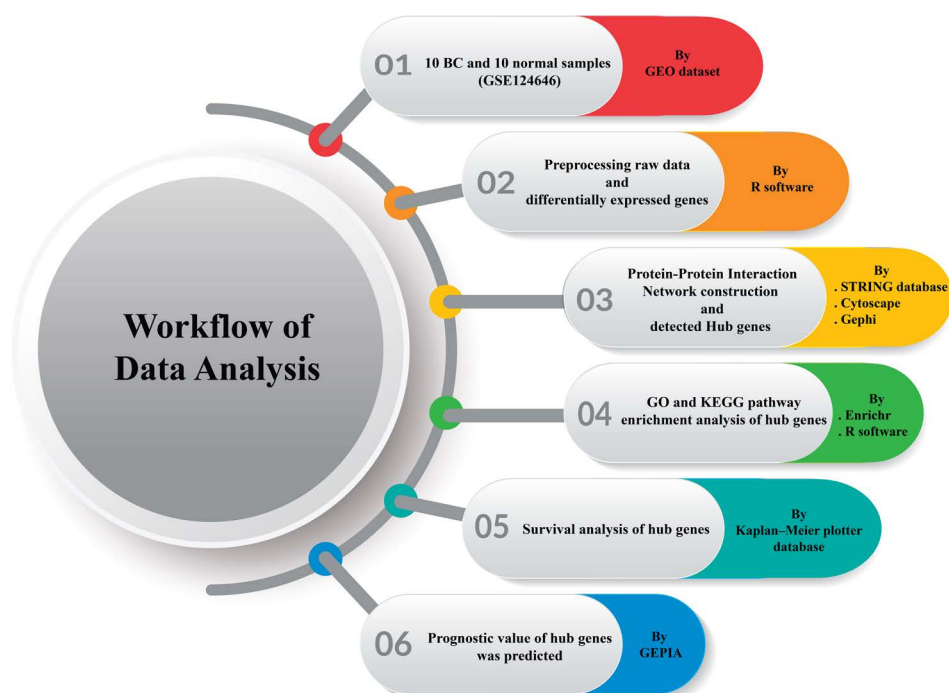


Figure 1. Flow diagram of the bioinformatics analysis in the present study

were performed via Enrichr (<https://maayanlab.cloud/Enrichr/>). Enrichr contains a wide range of gene sets and biological knowledge for further biological discoveries. Annotations of the cellular components, biological processes, and molecular functions of the DEGs were determined via Gene Ontology (GO) enrichment analysis. The R cluster profile package was used to explore the results of the GO enrichment analysis. Additionally, GO enrichment plots were drawn via a bioinformatics tool (<http://www.bioinformatics.com.cn/>). Gene Ontology introduced the concept of systematically linking a collection of genes to a functional biological term.³⁸ KEGG is also used to understand high-level and genomic functions. It consists of genomic, chemical, and network information.³⁹

Survival Analysis of The Hub Genes

The Kaplan–Meier plotter database (<http://kmplot.com/analysis/>) was used to perform the survival analysis. The Kaplan–Meier plotter is a website tool that can be utilized to assess the impact of numerous genes on survival based on the EGA, TCGA, and GEO databases. In order to evaluate how the identified hub genes could impact patient outcomes, we applied Kaplan–Meier analysis, a statistical method often used to evaluate survival probabilities over time ($P < 0.05$ was considered to indicate statistical significance).

Expression Analysis of The Hub Genes

Additionally, the GEPIA tool was used to validate the role of key genes in BC progression as well as their expression levels in normal breast and BC samples to predict the prognostic value of the hub genes. GEPIA is an online tool that can be employed for survival, correlation, gene expression, and dimensionality reduction analyses in

various cancers and normal tissues and contains 9,736 tumors (1085 breast cancer samples) and 8,587 normal samples from the TCGA and GTEx projects.⁴⁰

Results

Identification of Differentially Expressed Genes (Degs)

GSE124646 was selected. The data of 10 breast cancer samples and 10 normal breast samples, 100% cancer tissue, and 100% normal tissue were normalized with the MAS5 algorithm in the Affy package in R statistical software. A total of 22283 genes were identified. After filtering with criteria of $|\log_2FC|$ greater than 1.5 and P value < 0.01 , 923 differentially expressed genes were identified. Of these, 645 genes had increased expression levels and 278 genes were downregulated. (Figure 2) (Table 1).

PPI (Protein-Protein Interaction) Network and Module Analysis

The PPI network of DEGs was constructed with 307 nodes and 882 edges based on the STRING database, with an interaction score of 0.9 as the threshold. The network was analyzed via Cytoscape. Degree ≥ 7 and $0.005 \leq \text{betweenness} \leq 1$ were set as the cutoff criteria. A total of 73 genes were selected as hub genes. Among these hub genes, ALB, ALDH1A1, EGFR, BRCA1, EZH2, FN1, JUN, CXCL8, MMP9, FOS, CDK1, EGR1, STAT1, PTPRC, and IGF1 had relatively high betweenness centralities, and ALB, EGFR, FN1, JUN, MMP9, CXCL8, IGF1, STAT1, PTPRC, FOS, FGF2, CXCL12, PDGFRA, EZH2, and BRCA1 were among the top 15 genes with high degrees of connectivity. By using Gephi, the hub genes were divided into 4 significant modules, as presented in Figure 3.

Enrichment Analysis

Once again, our hub gene network was constructed in

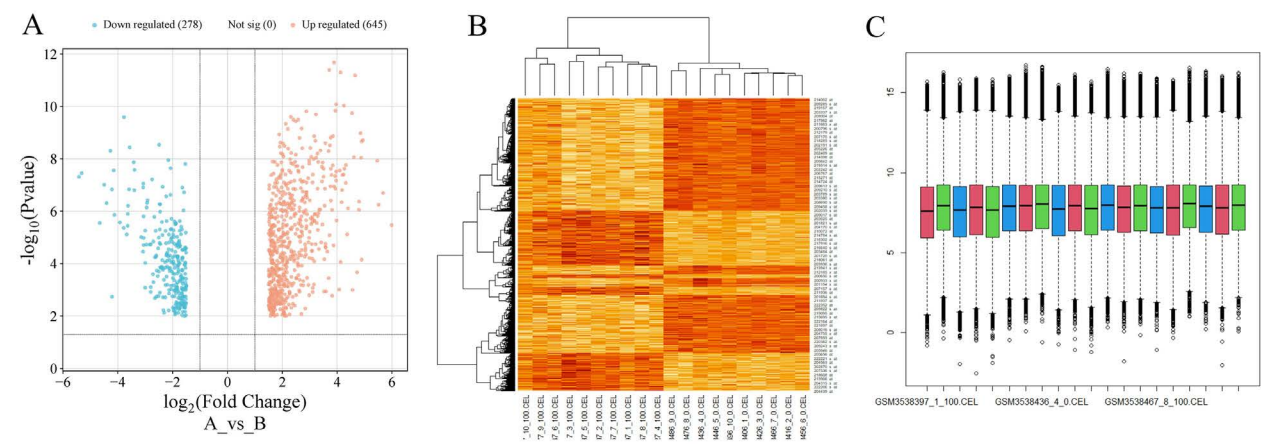


Figure 2. Identification of DEGs from the GSE124646 dataset in BC tissues compared with normal breast tissues. | Log2FC| $\geq$ 1.5 and P value $\leq$ 0.01 were used as selection criteria for DEGs. (A)Volcano plot of differential expression analysis. The red dots represent upregulated genes, and the blue dots represent downregulated genes. (B)Heatmap of 10 normal and 10 BC samples. The heatmap represents the differential expression profiles of DEGs ($|\log_2FC| \geq 1.5$, P value $\leq$ 0.01) in the GSE124646 microarray. (C)Box plot representations of the distribution of data after normalization via the MAS5 method. Abbreviations: DEGs, differentially expressed genes; BC: breast cancer

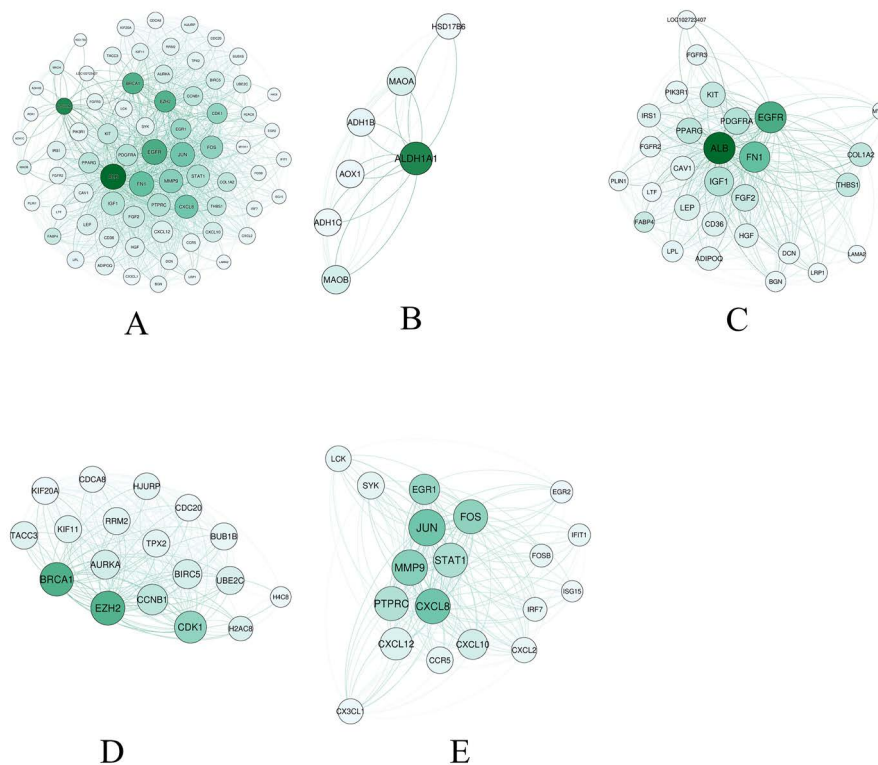


Figure 3. Protein-protein interaction network constructed with the hub genes and module screening constructed via the STRING online database and visualized via Cytoscape. Notes: The diameter of each node indicates the degree of connectivity, and the intensity of the color of each node indicates betweenness for each node in the network. A higher degree of the node represents a hub gene with more connections to other hub genes. The whole PPI network of proteins is encoded by the hub genes (A) and the network of four functional clusters (B-E). Abbreviations: PPI, protein-protein interaction

string, and by using Gephi, modules were constructed. Four out of five modules were significant, as shown in Figure 3 and Table 2. To better understand the biological functions of the DEGs and hub genes, GO and KEGG enrichment analyses were conducted. Highly expressed pathways and genes of each cluster were also identified and displayed. In the biological process analysis, the DEGs were involved mainly in in extracellular matrix

and structural organizations, and positive regulation of protein kinase B signaling. In the cellular component analysis, the DEGs were enriched mainly in the collagen-containing extracellular matrix, basement membrane, and endoplasmic reticulum lumen. According to the results of the molecular function analysis, the DEGs were enriched mainly in glycosaminoglycan binding, extracellular matrix structural constituent, and heparin binding (Figure 4). The

Table 1. Differentially expressed genes. 923 differentially expressed genes (DEGs), including 645 upregulated genes and 278 downregulated genes, were identified and confirmed from the Gene Expression Omnibus (GEO) database.

Regulation	DEGs (gene symbol)
Upregulated	COL11A1, COL10A1, PITX1, NEK2, IFI44L, COL10A1, TOP2A, MMP1, INHBA, COMP, COL11A1, CXCL11, MMP11, NUSAP1, CST1, BIRC5, ASPM, ISG15, FOXM1, CXCL10, MMP9, STAT1, FGRF3, NDC80, CEP55, NA, RRM2, TOP2A, SAC3D1, NKG7, GINS2, TACC3, CXCL9, LMNB1, KIF20A, MMP11, EZH2, S100P, CDK1, CXCL11, IL21R, NA, PITX1, NA, RSAD2, NSD2, H2AC8, H2BC5, DLGAP5, SPP1, TNNT1, SNX10, MYBL1, NA, IL32, PLAUR, MKI67, CKS2, AURKA, SULF1, NA, RGS1, MELK, STAT1, NA, HJURP, CDCA3, IFI27, H2BC9, PCLAF, CDC20, NA, IFI6, ADAMDEC1, AURKA, NA, CD52, MNDA, NA, CENPM, CXCL8, HSD17B6, SLC2A6, FN1, NCAPG, NA, SLC15A3, SLAMF8, STAT1, GINS1, MILR1, LAMP3, TK1, H2BS1, NCAPG, STAT1, H1-2, NOD2, SERPINA6, SYK, OAS2, NPL, FANCI, NUP210, NA, PLXNC1, FN1, NSD2, KPNA2, LEF1, STAT1, MCM4, BIRC5, LILRB1, NUSAP1, TPX2, CDK1, BST2, FCMR, FN1, BGN, CD52, RRM2, E2F8, LYZ, NA, LILRB3, DPP3, CCN4, GTSE1, IDO1, RNF19B, SAMS1, SH2D1A, PAFAH1B3, GYPA, CDCA8, LILRB3, BGN, TFRC, RHOD, FN1, LILRB3, AQP9, CCNB2, NA, HSD17B6, NCLN, NA, KIFC1, CLIC3, YKT6, SERPINA1, CLEC4A, BUB1, AP1S1, NA, SQSTM1, IFIH1, MX1, NFKB2, SULF1, IFIT1, MAZ, CCR5, SULF1, NA, ATP13A2, TRAF3IP3, CDK1, H1-4, LTB, BGN, CENPN, PCYT1B, CENPE, MAD2L1, GK, TAP1, SERPINA1, NA, LST1, UBE2S, KIF11, PRC1, STK10, ATP2C1, KIF26B, H2AX, MYBL2, CTSD, CD72, KPNA2, SNX24, TGM2, METRN, PPIF, F12, GBP1, GZMB, RGS14, PBK, SLC6A9, THBS1, SQLE, LCK, TDO2, ADAM8, IRF7, UNC5B, HCP5, CANT1, TREM1, TYMP, APOC1, P2RY10, ELF4, LILRB1, GZMK, H4C8, MRPL35, BUB1, E2F5, CST5, HMGB3, HMMR, SHCBP1, NA, OAS1, PTPRC, HLA-C, SERPINH1, CCL19, HLA-DQB1, CSF2RA, TGM2, RGS1, ERCC6L, CNTNAP2, PMAIP1, COL1A2, NA, MSC, CFB, GK, CAPG, PTTG3P, EMC1, ACOT7, TRAT1, NKAIN1, BUB1B, NA, TFRC, SLC19A1, MSR1, CENPF, UBE2C, MFAP2, RAC2, FAM49B, NA, CENPF, JPT1, SPI1, CD86, TMEM127, ITGAL, CCNB1, H2BC5, TPI1, NA, HLA-DQB1, ITGAX, USP18, PSMC4, LRRC15, BRCA1
Downregulated	LTF, RBP4, ADH1B, PPP1R1A, NA, KRT15, NA, MYH11, NA, LPL, WIF1, SCGB1D2, DST, APOD, CSN3, DLK1, FOSB, LEP, NA, PLIN1, MYH11, NA, GPD1, DCX, HLF, FABP4, ELF5, COL17A1, ADH1B, CSN1S1, OGN, OXTR, LYVE1, DST, ACACB, MT1M, SVEP1, DZIP1, SOSTDC1, OLFM4, PAMR1, GPD1, PIP, ADIPOQ, CNN1, PDK4, MME, NA, ZBTB16, PTN, NA, KIT, SCGB2A1, HLF, CAPN6, KRT14, CXCL2, SLC26A3, TMPRSS2, FAXDC2, GULP1, NA, SFRP1, SCGB2A2, ABCA8, TIMP4, SRPX, SORBS1, LIPE, FHL1, FKBP5, FHL1, CLDN8, NTRK2, ITGA7, SCN3A, CFD, MYH11, LIFR, FHL1, GREM2, GPM6B, LPL, EZH1, SFRP1, CHRDL1, SLIT3, LMOD1, RELN, DUSP6, NA, GPC3, MAOB, RUNX1T1, NA, GABRP, NA, NA, GPM6B, HSPB2, NTRK2, SFRP1, NFIB, TPPP3, BBOX1, HLF, JCHAIN, GPM6B, PTN, MFAP4, HPSE2, SOX10, CXCL12, NA, SPRY2, CA4, ELF5, NA, FHL1, DPT, S100B, CD36, TF, EGFR, NPR1, DPT, FXR1, NA, NTRK2, HBB, PENK, NA, CES1, MME, PTN, NA, PEG3, MYH11, NA, NSG1, FHL1, LTBP4, NFIB, LAMA3, NA, CIDEA, CLDN5, NA, SYNM, NLGN4X, FOS, SPTBN1, HOXA5, ACACB, NA, TGFB3, CSN2, IGF1, MAOA, MIA, LDB2, CAB39L, EDN3, NA, DUSP1, LAMC3, EDNRB, TTYH1, LTBP4, KRT5, MATN2, NA, TGFB2, FGFR2, AREG, DMD, IGFBP6, PPP1R12B, PCOLCE2, CD36, LHFPL6, ACACB, PDE9A, NA, CDKN1C, NA, TAT, C1orf21, TNFRSF17, FAM107A, FAM13A, NA, GULP1, PLPP3, GPX3, ADH1C, OPRPN, CX3CL1, EIF1, CYBRD1, C7, NDRG2, GYG2, DCN, MYLK, ACKR1, ABCA6, NA, NAV3, MFAP5, IGF1, ITM2A, CX3CL1, EDNRB, ALB, PPP2R1B, ACSM5, RBM5, HBB, CDKN1C, CLC21, NA, NA, HBB, MYBPC1, HOXA7, HPGD, CAPN6, CRYAB, PPARG, NDRG2, GHR, MYOC, CRABP1, NA, GSN, FAM13A, ADH1B, ARL4A, LAMA2, IGF1, RUNX1T1, SOCS2, GSN, TCF7L2, EFHC1, RECK, NR4A2, MMRN1, CEL, PDGFD, F3, MEG3, PDGFRA, DCLK1, AASS, LAMA2, KLF4, MAOA, DCN, FMO2, AHNK, NA, GRAMD2B, EDNRB, PDZD2, AMIGO2, CITED1, CPE, CDO1, CIDEA, KCNJ8, NA, SNX1, NA, SCN3B, DPT, NA, ITGA7, ZNF334, TFPI, ATP1A2, ZNF711, TAC1, ACSM1, TCN1, MOCS1, MT1X, DPG6, TFPI, GIPC2, DCN, SGCE, NA, MEG3, NEDD4L, MEGF9, TP63, KLF4, CDKN1C, PLAGL1, SLC25A37, SERPINA5, TF, CORO2B, MEOX2, COL14A1, GPX3, PCK1, JUN, RGS2, RRAD, GPM6A, CPE, IGH, ARHGAP19, PELI2, CAV1, SLC6A14, PROS1, PPL, SLC22A3, FAM149A, NAALAD2, F10, PYGB, FBLN5, CHST3, MBD2, AGTR1, PDE2A, EGR1, BHMT2, MAOA, NOVA1, PLSCR4, OMD, SH3BP2, MAF, GPM6A, CAV1, MPPED2, ADAMTS5, PDGFRL, NA, AOC3, ITIH5, AK4, NA, NUDT2, ADAMTS1, NFIB, LAMA2, NFIB, NA, CBX7, PDLIM4, ID4, TNN, ALDH1A1, HEY2, EDH2, GYG2, FAM149A, CAV2, TFPI, CRY2, BMP4, ZNF230, SETD4, NRG2, PLPP3, KLHL29, DCN, TIE1, GPRASP1, LRRC17, KLF9, RND3, PLPP3, AK5, ANKZF1, GRIK2, ACTG2, LRP1, TCF7L2, IGF1, NCALD, CAVIN2, MFAP5, TFPI, HEY1, NFIB, SPATA6, PLA2R1, ZBTB20, SLPI, ITM2A, AGTR1, PLEKHS1, ACACB, NFIB, CSRN3, ADD3, LAMA4, XIST, NA, GRB10, CHL1, NFASC, SUN1, SORBS2, KLF9, COX7A1, PIK3R1, GPM6B, DUSP6, CFH, TCF7L2, PCDH9, DIXDC1, LOC389906, PTHLH, NR4A1, PPP1R15A, TRIM29, ARID5B, HSD17B2, GNAI, ARHGAP6, FOXI1, ID4, ALDH1A3, FBLN1, KLF3-AS1, NKTR, SNCAIP, MAFF, IL11RA, PER2, ENPEP, HNRNP1, P3H2, SORBS1, FAXDC2, SLIT3, NA, NA, METTL7A, NA, 1-Mar, ANGPTL4, EHF, NID1, CCN1, IGF1, NA, FOXO3, CAMSAP1, FZD7, MED6, SLC13A2, TSC22D3, PARD3, FMO1, ADD3, JUN, FHL5, VSTM4, KLK11, ALDH1L1, ADGRA2, SLC10A2, ANK2, TENM1, CGF2, NMNAT2, XIST, PRELP, TNMD, ASPH, DCX, SLC27A6, FBLN1, EYA2, AOX1, FGF1, EMP1, PLAGL1, ABLIM1, NA, SEMA3C, DKK3, TRIM33, RRP15, LIMCH1, CAV2, NA, RCAN1, ABCF2, ATP1A2, RRAGD, GPER1, LRRC36, JUN, ANPEP, TNS1, COL14A1, GULP1, OLFML2A, ADD3, PID1, STEAP4, ENPP2, NA, SLC44A1, CA3, KLK5, RAI2, TAGLN, SESN1, ATF3, CIRBP, COL4A3, ANKH, PART1, PHYHIP, EWSR1, TRPC1, PALMD, GAS7, PIK3R1, DST, DCLK1, TRMO, EBF2, GAS7, NA, PTPRB, KLK10, DPYSL2, CFAP69, TRIO, MT1E, PIK3R1, MAP1LC3C, NA, SCN7A, NA, TMEM47, DST, AOX1, TGFB2, DENND2A, EGR2, MPZL2, TCF7L2, KCNMA1, TXNIP, FER, EXOC7, RGCC, LRP2, KLK7, KCNAB1, WLS, MKRN2, FMOD, MEIS2, NES, PGF, EDN1, GOLGA8A, NA, ZBTB20, COL7A1, NRG2

biological process analysis indicated that these hub genes are likely to be vital components of specific functions such as the positive regulation of intracellular signal transduction. In the cellular component analysis, the hub genes were enriched mainly in spindle regulation. In the molecular function analysis, the hub genes were enriched mainly in integrin binding. The statistical significance of the GO term enrichment analysis is shown in [Supplementary file 1, Table S1](#) and [Figure 5](#). KEGG analysis revealed that the DEGs were enriched in the PI3K-Akt signaling pathway, focal adhesion, pathways in cancer, ECM-receptor interaction, and complement and coagulation cascades. The hub genes were enriched in pathways related to cancer, the PI3K-Akt signaling pathway, focal adhesion, proteoglycans in cancer, and pathways related to cancer.

In the KEGG enrichment figure, the $-\log_{10}(P \text{ value})$ scale represents statistical significance for each pathway, with higher numbers indicating greater enrichment ([Figure 6](#), [Supplementary file 2, Figure S1](#))

Hub Gene Validation: Survival Analysis and Gene Expression Profiling of The Hub Genes

Kaplan-Meier curves were plotted for the hub genes. The KM plot showed that the expression of most of the hub genes significantly differed between the high- and low-expression groups ([Supplementary file 3, Figure S2](#)). These results indicate that 78.08% of the hub genes have prognostic significance for breast cancer patients. Hazard ratios (HR) and log-rank P values for all hub genes assessed, which reflect the results of our survival analysis,

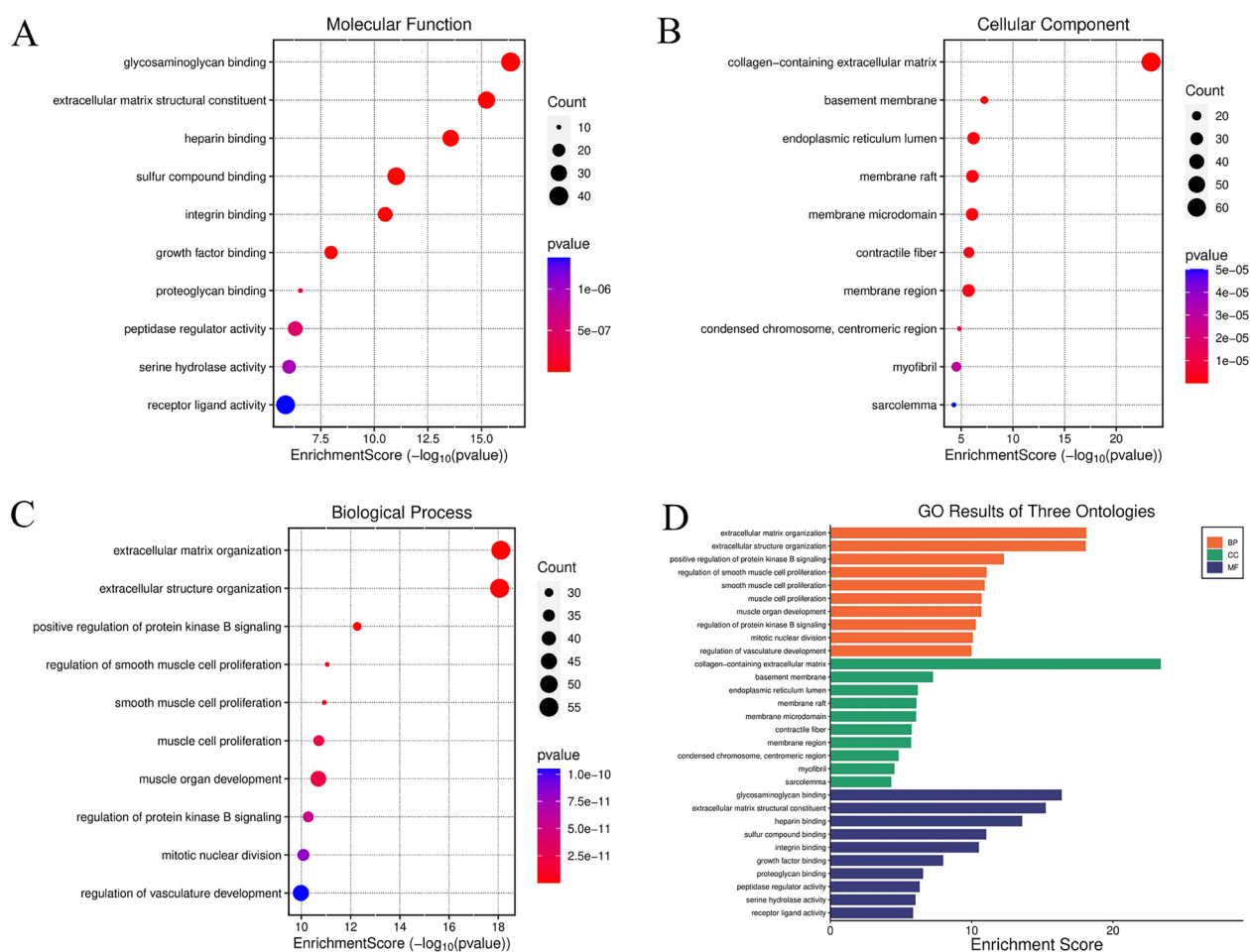


Figure 4. GO enrichment analysis of DEGs. (A-C) Dot plot of the results of the GO enrichment analysis of molecular function (A), cellular component (B), and biological process (C) terms. (D) Bar graph of the top 10 GO terms of the DEGs. Terms. Abbreviations: DEGs, differentially expressed genes; GO, gene ontology

are presented in [Supplementary file 4, Table S2](#). GEPIA was used to analyze the hub genes and compare their overall expression levels with those of normal tissues. The results indicated that the hub genes were abnormally expressed in breast cancer tissue compared with normal breast tissue ([Figure 7](#)).

Discussion

Breast cancer is a biologically diverse disease, characterized by considerable heterogeneity in both its molecular characteristics and clinical behavior, driven by DNA alterations resulting in the activation of oncogenes or the suppression of tumor suppressor genes. The development of array technology allows surveys of gene expression, and with the aim of bioinformatics tools, a better understanding of expression profiles has led to new approaches. Using these technologies enables researchers to define the functions of newly identified genes, delineate the pathway they're part of, study patterns of gene variation, and identify possible therapeutic targets.^{41,42} A better understanding of molecular mechanisms is necessary to identify target genes and novel therapeutic strategies.^{43,44}

In this study, 923 (278 downregulated and 645

upregulated) genes were differentially expressed. From a constructed protein-protein interaction network, 73 hub genes were selected. These 73 hub genes were differentially expressed between breast cancer and normal tissues. KEGG pathway enrichment analysis revealed that the differentially expressed genes and hub genes were particularly enriched in three pathways: pathways associated with cancer, the PI3K-Akt signaling pathway, and focal adhesion.

Hub genes primarily activate pathways involving PI3K-Akt signaling and focal adhesion, both of which are associated with the motility, growth, and survival of cancer cells. The phosphatidylinositol 3-kinase (PI3K) pathway coordinates intracellular responses such as survival, cell growth, differentiation, cellular metabolism, and cytoskeletal reorganization to extracellular stimulators. This pathway occurs in many human cancers.⁴⁵ The PI3K pathway is a novel pathway for therapeutic targeting. Many drugs that target various components of this pathway are now in clinical trials.⁴⁶ Focal adhesions (FAs) are multiprotein structures that connect the cytoskeleton of a cell to the extracellular matrix through integrins. Cellular adhesion is an essential process involved in motility.⁴⁷ Changes in the expression of these molecules can induce

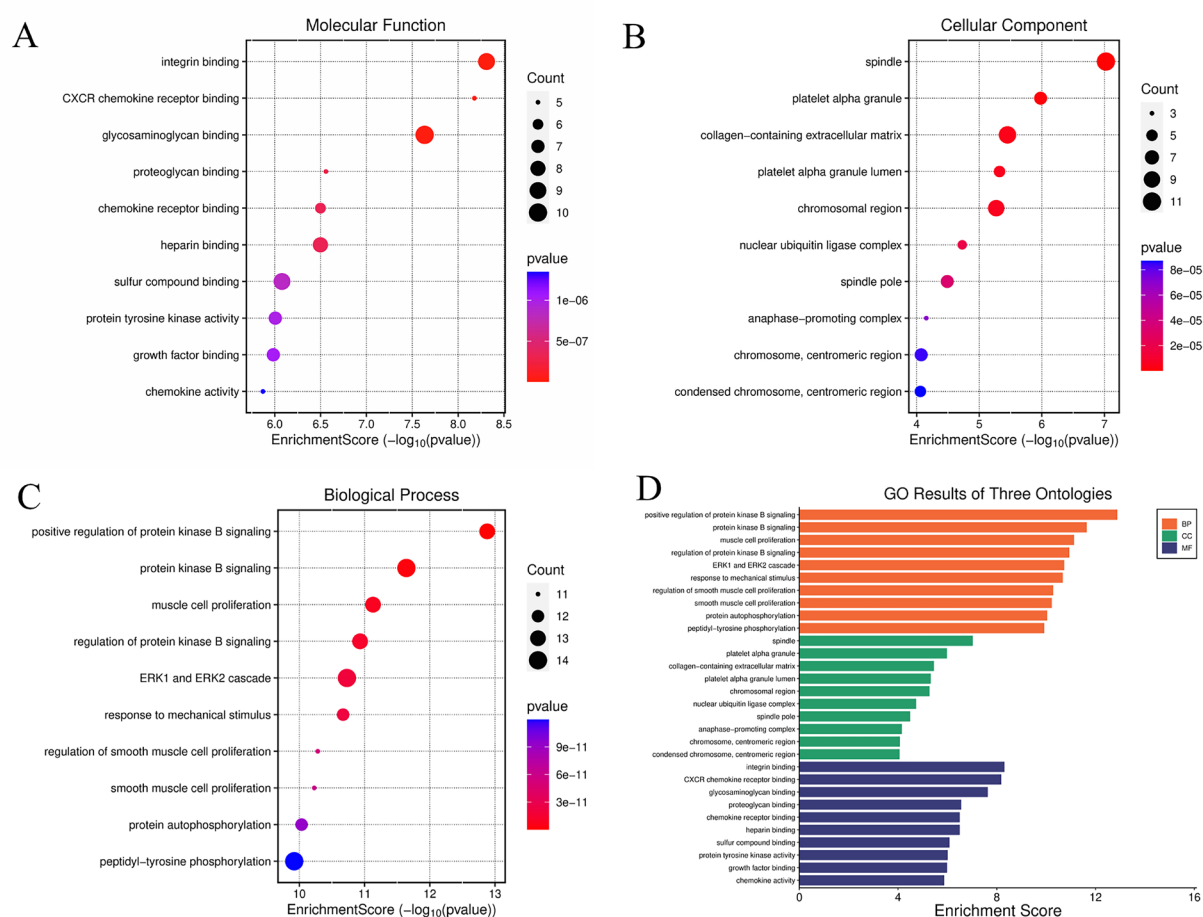


Figure 5. GO enrichment analysis of the hub genes. (A-C) Dot plot of the results of the GO enrichment analysis of molecular function (A), cellular component (B), and biological process (C) terms. (D) Bar graph of the top 10 GO terms of the hub genes. Abbreviations: GO, gene ontology

Table 2. Gene sets of each module and the top KEGG pathway terms

module	gene	Pathways in KEGG analysis
0	ADH1B, ALDH1A1, MAOB, MAOA, AOX1, ADH1C, HSD17B6	PI3K-Akt signaling pathway Focal adhesion Pathways in cancer ECM-receptor interaction Complement and coagulation cascades Malaria PPAR signaling pathway
1	FABP4, ADIPOQ, HGF, LRP1, FGF2, PPARG, ALB, COL1A2, PLIN1, IRS1, LEP, CAV1, FN1, IGF1, LPL, CD36, DCN, LTF, PDGFRA, THBS1, EGFR, KIT, BGN, LOC102723407, LAMA2, FGFR3, FGFR2, PIK3R1, MYH11	PI3K-Akt signaling pathway Focal adhesion Proteoglycans in cancer Rap1 signaling pathway Pathways in cancer Ras signaling pathway AMPK signaling pathway
2	CXCL10, CXCL8, STAT1, JUN, MMP9, CXCL12, PTPRC, CX3CL1, EGR1, CCR5, LCK, SYK, FOS, IRF7, CXCL2, FOSB, IFIT1, ISG15, EGR2	IL-17 signaling pathway Kaposi sarcoma-associated herpesvirus infection Hepatitis B Chemokine signaling pathway Viral protein interaction with cytokine and cytokine receptor Toll-like receptor signaling pathway TNF signaling pathway
3	CCNB1, KIF11, CDK1, BRCA1, AURKA, EZH2, HJURP, RRM2, BUB1B, KIF20A, CDCA8, UBE2C, BIRC5, CDC20, TACC3, TPX2, H2AC8, H4C8	Cell cycle Oocyte meiosis p53 signaling pathway Progesterone-mediated oocyte maturation Ubiquitin mediated proteolysis Viral carcinogenesis Systemic lupus erythematosus

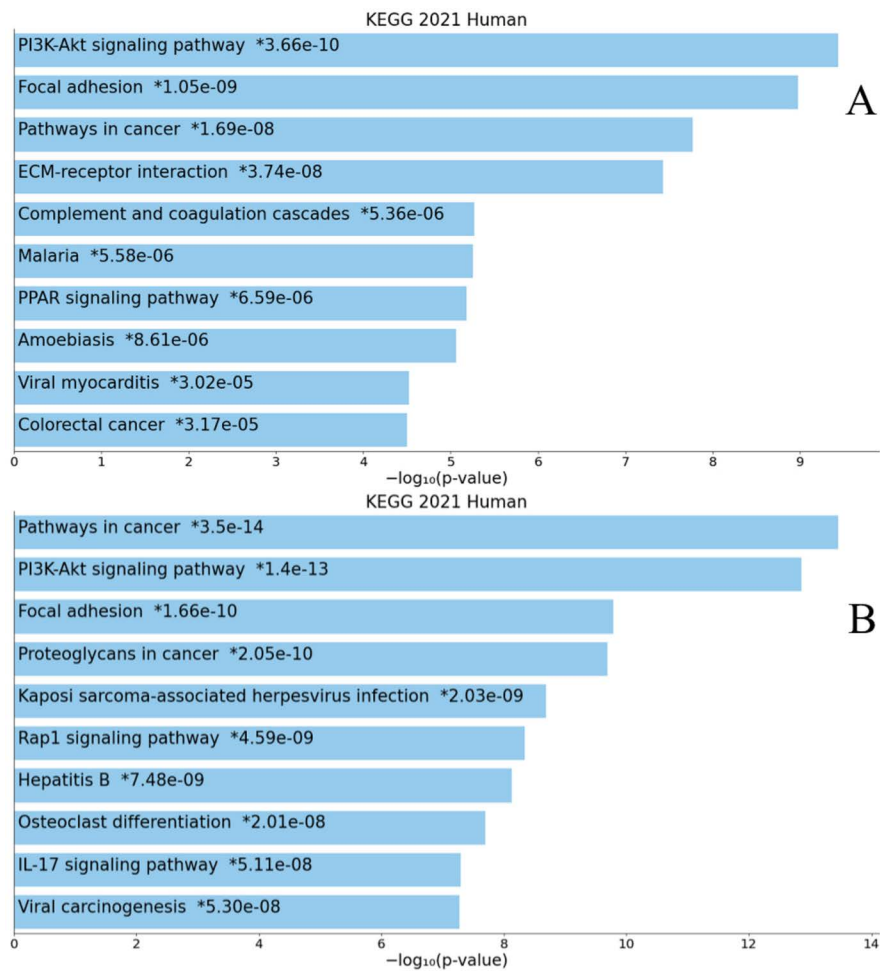


Figure 6. KEGG enrichment analysis of DEGs (A) and hub genes (B). Abbreviations: DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes

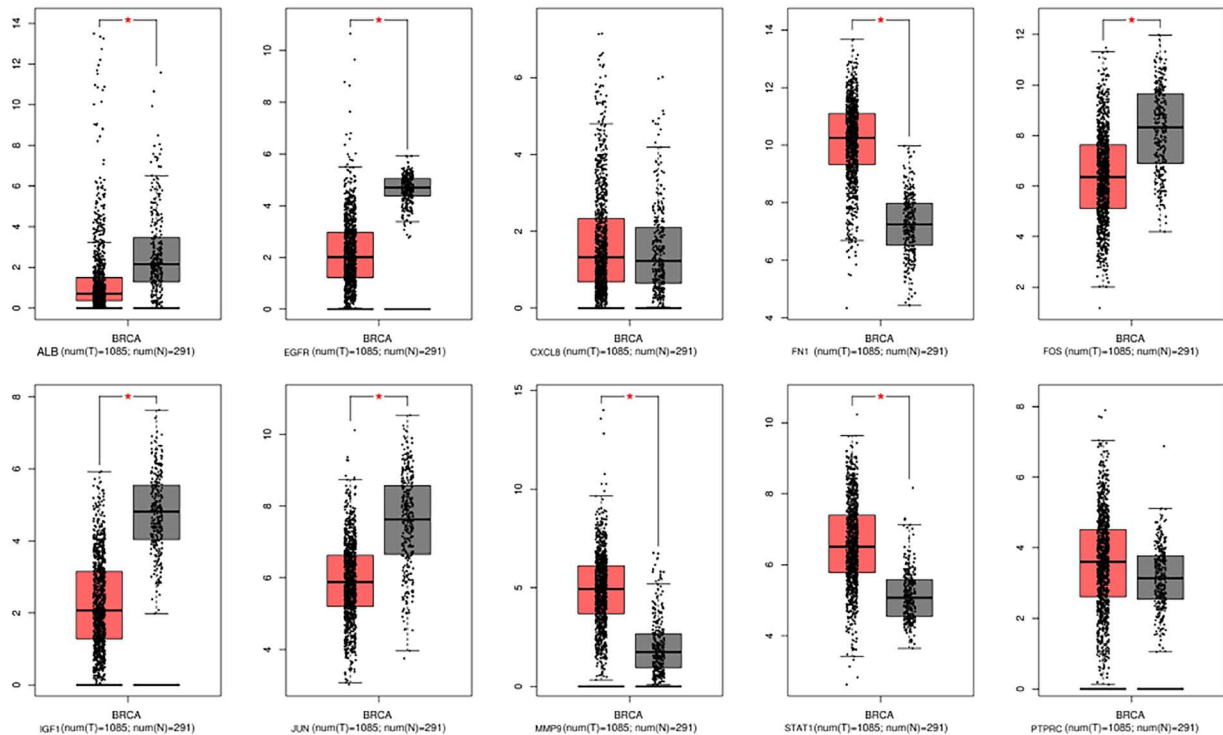


Figure 7. Expression profiles of the hub genes in BC patients. The top 10 hub genes with the highest degree were analyzed via the GEPIA online database to further verify the expression level of the hub genes between BC tissues and normal tissues. The pink box represents BC samples, and the gray box represents normal samples

cell death or change the size of individual cell-matrix interactions.⁴⁸ The targeting of FA proteins can lead to the sensitization of cancer cells to treatment.⁴⁹ Enrichment analysis also showed that module 3 genes work in a role involving cell cycle regulation, a therapeutic target that is established in breast cancer. For instance, CDK1, a hub gene from module 3, coordinates cell division in mammalian cells by regulating the G1/S transition by coupling Cyclin D1 with extracellular signals.⁵⁰ In several cancers, cell cycle progression is correlated with the dysregulation of CDKs (cyclin dependent kinases), and this mechanism contributes to abnormal cell proliferation.⁵¹ Selective CDK inhibitors against CDK4 and CDK6 have been approved by the FDA for patients with metastatic hormone receptor-positive breast cancer, while earlier-generation pan-CDK inhibitors, those that inhibit multiple cyclin-dependent kinases, have not been able to gain approval for clinical use. First-generation pan-CDKIs, such as Flavopiridol and Rocovitin, and second-generation CDKIs, such as Dinaciclib and Roniciclib, are some inhibitors that are under development for targeting cancer.⁵² Our analysis revealed that CCNB1, CDC20, BUB1B are module 3 genes that affects the cell cycle pathway in breast cancer tissues. Recent studies have also shown that high expression of CCNB1,⁵³ CDC20,⁵⁴ BUB1B⁵⁵ is associated with poor prognosis in patients with breast cancer. Module two genes promote mainly the IL-17 signaling pathway. The pro-inflammatory cytokine interleukin-17 is important in promoting tumor proliferation and metastasis and is significantly correlated with poor prognosis in breast cancer patients.⁵⁶ The IL-17 superfamily of T-cell-derived cytokines consists of six ligands (IL-17A/IL-17, IL-17B, IL-17C, IL-17D, IL-17E/IL-25, and IL-17F) and five receptors (IL-17RA, IL-17RB/IL-25R, IL-17RC, IL-17RD/SEF and IL-17RE).⁵⁷ In breast cancer, tumor-infiltrating lymphocytes (TILs) produce mainly IL-17A.⁵⁸ Recent studies in mice have found therapeutic value in IL-17A, IL-17B, and IL-17RB inhibitors as targeted therapies for breast cancer. Antitumor activities are enhanced with the use of IL-17E with cisplatin or paclitaxel.⁵⁹

By constructing a PPI network, ALB, EGFR, EZH2, FN1, JUN, CXCL8, MMP9, FOS, STAT1, and PTPRC were found to have elevated degrees of connectivity and betweenness centralities across the network. EGFR is also a tyrosine kinase receptor that normally functions to promote cell proliferation. However, its overexpression leads to tumorigenesis and aggressive growth, and several anti-EGFR therapies are in development.^{60,61} EZH2 is a transcriptional repressor that has been documented as a biomarker in advanced breast cancer, and we know that there are several inhibitors in development.⁶² High levels of EZH2 transcript and protein, when expressed in immortalized human mammary epithelial cell lines, enable those cells to grow independently and are linked with invasive carcinoma as well as breast cancer metastases.^{63,64} A recent study indicated that when EZH2 was inhibited in inflammatory BC cells, it inhibited tumor growth.⁶⁵ FN1 (fibronectin 1) gene targets a glycoprotein

that has poor prognosis for many cancers; inhibition of FN1 has inhibited proliferation and metastasis in models of breast cancer.⁶⁶⁻⁷⁰ The JUN proto-oncogene enhances angiogenesis and proliferation, c-JUN protein also regulates glutaminase and its sensitivity to therapy.⁷¹⁻⁷³ C-X-C motif chemokine ligand 8 (CXCL8, interleukin 8) is known to enhance cell proliferation and inhibit apoptosis in different cancers, including breast cancer.^{74,75} When CXCL8 is overexpressed, it can promote tumorigenesis and metastasis and should be considered a potential biomarker of metastasis for a variety of cancers, including BC.⁷⁶⁻⁷⁹ Matrix metalloproteinase 9 (MMP-9) has roles in the remodeling and invasion of the extracellular matrix, which is elevated in BC. In breast cancer, there are various biosensors currently improving the detection of MMP-9.^{80,81} Additionally, FOS family protein dysregulation, along with the JUN family proteins in AP-1 (transcriptional) complexes, is associated with cellular proliferation and breast cancer development.^{82,83}

Besides the core hub genes EGFR, EZH2, FN1, CXCL8, MMP9, JUN, and FOS, STAT1, PTPRC, and especially ALB have received less description within breast cancer literature. ALB encodes the most abundant protein in extracellular fluids.⁸⁴ Albumin is responsible for maintaining colloid osmotic pressure and acts as a carrier for many endogenous and exogenous compounds. Human serum albumin (HAS) is a distinguished biomarker for many diseases, including cancer, and albumin is clinically used for the treatment of various diseases.⁸⁵ Many studies have shown that hypoalbuminemia is associated with many cancers because of malnutrition and systemic inflammatory responses.⁸⁶⁻⁸⁸ In a previous study, ALB was also a hub gene with the highest degree in Wilms tumor,⁸⁹ whereas upregulation of ALB could be associated with colorectal cancer liver metastasis and hepatocellular carcinoma. Many studies have shown that lower levels of serum ALB have been associated with poor survival in many cancers for decades. However, few studies have investigated the prognostic value of ALB in patients with breast cancer, and few studies have specifically focused on the ALB expression level in patients with BC.⁹⁰⁻⁹³

Our analysis revealed that the signal transducer and activator of transcription 1 (STAT1), an essential component of interferon (IFN) signaling, is underexpressed. Stat1 can act as an oncogene or an antioncogene. The decision as to whether STAT1 is oncogenic or antioncogenic depends on the specific genetic background and type of cancer.⁹⁴ Previous studies reported conflicting findings about the role of STAT1 in the primary tumor development and growth of breast cancer. Stat1 has been proposed to serve a suppressive role in tumor development and may even help in suppressing cancer development.⁹⁵⁻⁹⁸ Although one study found that increased STAT1 activation is associated with more favorable outcomes for breast cancer patients,⁹⁹ two other studies found that increased STAT1 mRNA is associated with worse survival. This difference underscores the context-dependent and contradictory role of STAT1 in breast cancer progression.^{100,101} However,

another study revealed that the overexpression of Stat1 can play an oncogenic role in breast tumor growth.¹⁰² In addition, anthracyclines, such as doxorubicin, are anticancer treatments in the clinic that can increase the activation of STAT1 in breast cancer cells.^{103,104}

Protein tyrosine phosphatases (PTPs) have a specific subtype known as receptor-type PTPs (PTPRs).¹⁰⁵ The protein tyrosine phosphatase receptor type C (PTPRC) gene encodes PTPRC, also known as the CD45 antigen or leukocyte common antigen (LCA), which is a transmembrane glycoprotein and a vital regulatory factor that is involved in the modulation of antigen receptor signaling pathways in both T lymphocytes and B lymphocytes.^{106,107} PTPRC is amplified in 11.2% (108 of 962) of breast cancer samples, with the highest proportion of copy number amplifications among the PTPR genes reported in the TCGA database for all cancers.¹⁰⁵ A study showed that lower expression of PTPRC caused increased resistance to paclitaxel in triple-negative breast cancer cell lines.¹⁰⁸ Little research has been conducted on PTPRC expression in BC; however, our analysis revealed that this gene was under-expressed. These results demonstrated that the core genes might be key players in the progression of BC.

Finally, survival data analyses demonstrated a robust relationship between the expression levels of several hub genes and clinical outcome in breast cancer patients. Kinesin family member 11 (KIF11), a motor protein critical for spindle dynamics, showed poor OS (HR = 1.54, log-rank $P < 1E-16$),¹⁰⁹ is associated with poor prognosis. Similarly, BRCA1 alterations in its expression were related to poor prognosis in BC patients (HR = 1.41 (1.28–1.56), log-rank $P = 2.5E-11$).¹¹⁰

Overexpression of AURKA, a driver of tumorigenesis, was associated with poor OS (HR = 1.89, log-rank $P < 1E-16$), supporting its candidacy for targeted therapies.^{111–113} CCNB1, a cell cycle regulator, also had prognostic value (HR = 1.53, log-rank $P = 3.8E-08$).^{114–117}

Further analysis revealed that RRM2 (HR = 1.83, log-rank $P < 1E-16$),^{118,119} cell division cycle 20 (CDC20) (HR = 1.9 (1.71–2.11), log-rank $P < 1E-16$),^{120–123} CDC2 (CDK1) (HR = 1.68 (1.52–1.68), log-rank $P < 1E-16$),^{124,125} were also associated with poor OS in BC patients. Lastly, SSK (also known as BUB1B) gene expression is crucial for the production of BubR1, a key protein that mediates spindle checkpoint activation.¹²⁶ Increased expression of BUB1B was associated with worse OS in BC patients.^{127,128}

By studying 73 hub genes associated with breast cancer, this study not only validated known biomarkers, but also augmented methodological integrity by validating previous experimental and review based results. Interestingly, a gene subset including AOX1, LOC102723407, HSD17B6, ADH1C, ADH1B, EGR2, LTF, LAMA2, H2AC8, and H4C8 was not well researched to date in breast cancer. While these were discovered through integrative enrichment and survival analyses, which demonstrate an added element of novelty, the ability to present such genes can have implications for future avenues of research as

noteworthy candidates. These genes may provide possible therapeutic significance and unexplored mechanistic potential. In general, these results further the biology of breast cancer and provide new molecular targets.

AOX1 is under expression in some cancers, and is proposed to influence chemoresistance and redox homeostasis.^{129–132} The LOC102723407 gene is expressed at significant levels in some cancers, though research is limited.¹³³ HSD17B6 regulates steroid hormone metabolism, and has been associated with lung, prostate, and hepatocellular cancer.^{134,135} Levels of ADH1B and ADH1C, which are the main enzymes involved in alcohol and retinol metabolism, were associated with tumor aggression and metabolic adaptation, particularly in triple-negative breast cancer.^{136,137} Recent studies showed that EGR2 emerged as a central transcription factor that was induced in tumor-infiltrating CD8⁺ T cells, showing a role in T-cell exhaustion and tumor immune evasion, particularly in HER2-enriched tumor microenvironments.¹³⁸ LTF encodes lactotransferrin, one of the tumour-suppressing proteins, and may serve as a prognostic marker in several cancers, mediating immune effects.^{139,140} LAMA2 encodes an important structural component of the basement membrane and is a tumor suppressor in several cancers. Its reduced expression through promoter hypermethylation is associated with malignant traits such as invasion and metastasis through MAPK and PI3K/AKT pathway.^{141,142} The histone cluster variants H2AC8 and H4C8 exhibit atypical expression in high-grade tumors, suggesting they are involved in chromatin instability and epigenetic dysregulation.^{143–145}

There were several limitations in this study, including the following. First, only one dataset was observed in this study. Compared with studies with multiple microarray datasets, these results may be less reliable. Second, when the DEGs were analyzed, several factors, such as age, race, tumor stage, and patient classification, were not considered. Third, subtype-specific survival analysis was not undertaken which diminishes the clinical relevance of the findings of this research due to the heterogeneity of breast cancer. Future studies should incorporate subtype-stratified analyses to better capture prognostic differences across molecular classifications. This would enhance the translational value of the research and support more personalized treatment strategies.

Conclusion

In summary, bioinformatics analysis revealed 73 hub genes that were significantly enriched in important signaling pathways, such as PI3K-Akt and focal adhesion, among others. The study presented survival analysis and demonstrated that more than 78.08% of hub genes exhibited expression patterns that correlated with poor prognosis in BC patients. Together, these results elucidated the molecular basis for breast cancer progression, and these genes may serve as targets for biomarker identification and targeted therapy. Although the results shown above require verification via in vivo and in vitro analyses, our

study provides a new direction for further studies on breast cancer.

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Availability of Data and Materials

The GSE124646 dataset supporting the conclusions of this article is available on the GEO website (<https://www.ncbi.nlm.nih.gov/gds>).

Competing Interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethical Approval

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Supplementary File

Supplementary file 1, Table S1: The statistical significance of the GO term enrichment analysis

Supplementary file 2, Figure S1: Cnet plot of GO enrichment analysis of molecular function (A), cellular component (B), and biological process (C) molecular function (D), cellular component (E), and biological process (F) terms.

Supplementary file 3, Figure S2: Survival analysis (Kaplan-Meier plot) for hub genes.

Supplementary file 4, Table S2: Hazard ratios and log-rank p-values for hub genes

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