Up-regulation of FN1, Activation of Maturation Promoting Factor and Associated Signaling Pathway Facilitates Epithelial-Mesenchymal Transition, Inhibits Apoptosis and Elevates Proliferation Rate of Breast Cancer Cells: In Silico Analysis of Microarray Datasets

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Research

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Abstract

Background: Knowing that the molecular mechanisms underlying breast cancer (BC) are not yet fully understood it was considered worth to launch investigation for the detection of key molecular pathways and associated genes and proteins.

Methods: In total two microarray based datasets (GSE10810 and GSE29431), consisting of 89 breast cancer samples and 31 controls, were retrieved from the Gene Expression Omnibus (GEO) database and processed to identify the differentially expressed genes (DEGs). The pathway and functional enrichment analyses of DEGs were performed using DAVID online tool. Protein-Protein interaction (PPI) network was constructed using an online tool STRING and visualized through Cytoscape software to identify the significant module and hub genes via MCODE and Cytohubba applications. The identified hub genes were then further analyzed to document their response in Kaplan-Meier (KM) survival curve analysis, investigate differential expression profile and its correlation with promoter's methylation status, and finally for the construction of the gene-drug interaction network.

Results: In total 449 DEGs were detected including 151 up-regulated and 298 down-regulated genes. The identified DEGs were enriched in various cancer related biological functions and pathways. Based on PPI network analysis of the DEGs, six hub genes, CDK1, FN1, AURKA, CCNB2, BIRC5, and TOP2A, were correlated with worse overall survival (OS) of the breast cancer patients.

Conclusion: The extensive *in silico* analysis has been helpful to evaluate evidence highlighting the prominent role played by the identified hub genes in stimulating breast tumor growth through the activation of Maturation Promoting Factor (MPF), PI3K/AKT signaling, and associated pathways that could be targeted for devising effective treatment strategies.

Background

Breast cancer (BC) is the most common type of non-cutaneous malignancy among female with a high recurrence rate. The global burden of breast cancer has been enhanced significantly in recent years and it is estimated that more than 1.7 million individuals are being diagnosed with breast cancer annually, worldwide, and approximately 521,000 individuals succumb to the disease [1]. Reproductive and dietary changes are contributing to the increased morbidity of breast cancer. However, the precise molecular mechanisms underlying breast carcinoma are still uncertain. The division of breast cancer into different subtypes is based on the difference of molecular characteristics, clinical outcomes and histopathological appearance. Based on the molecular characteristics breast cancer is categorized into six different subtypes: normal-like, luminal A and B, HER2-positive, basal-like, and claudin-low. The basal-like and claudin-low subtypes are recognized by the lack of estrogen receptor (ER) and progesterone receptor (PR), respectively. The lack of HER2 expression, belongs to triple-negative breast cancer (TNBC) which accounts for 10–20% of all breast cancer cases [2].

Nowadays 3 different biomarkers including the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor (EGF) receptor 2 (HER2) are being exploited for diagnosis, prognosis and the treatment of breast cancer [3]. However, no biomarker exists to date which can be employed for the early diagnosis and treatment of breast carcinoma [4]. Therefore, it is necessary to explore the molecular regulatory pathways to understand biology of breast carcinoma and identify molecular markers / targets that can be used in future as tools for early diagnosis and monitoring of the disease.

The role of microarray analysis in studying the genetic variability is well established in oncology research. In recent years, DNA microarray analysis technique has been emerged as a rapid, high-throughput detection technology due to its potential to simultaneously monitor the differential expression of various genes or miRNAs in diseased and normal samples [5, 6]. The microarray data of breast cancer patients and normal individuals have been stored in online electronic repositories such as GEO [7–9]. Therefore, in present work, we identified differentially expressed genes (DEGs) by analyzing two breast cancer mRNA microarray datasets (GSE10810 and GSE61304). We then aimed to perform gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of GEGs, construction of protein-protein interaction network (PPI) for significant module and hub genes identification. We hypothesized that the pathway enrichment analysis and further validation of Hub genes participation in tumor biology through *in-silico* analysis of available wet laboratories' data would be helpful for understanding the molecular mechanism underlying breast cancer. The information thus generated might also provide new insights for the identification of novel breast cancer biomarkers.

Material And Methods

Microarray data sets retrieval

The Gene Omnibus (GEO) (available at: <https://www.ncbi.nlm.nih.gov/gds>), a free international public repository of genomics data including microarray and next-generation sequencing (NGS) data, was accessed to retrieve the microarray datasets. In the present study, we retrieved two microarray datasets (GSE10810 and GSE61304) which contained microarray expression profile data using the Affymetrix U133 Plus 2.0 gene expression array from GEO. The data set GSE10810 consisted of total 58 samples including 27 normal and 31 breast cancer samples. While GSE61304 data set comprised of 62 sample including 4 normal and 58 breast cancer samples.

Identification of differentially expressed genes (DEGs)

GSE10810 and GSE61304 data sets were selected and analyzed to identify differentially expressed genes (DEGs) using software GEO2R (available at: <https://www.ncbi.nlm.nih.gov/geo/geo2r/>). In the present study, all the top 250 genes with fold change ≥ 1.5 and $p < 0.05$ were considered as DEGs.

Functional and pathway enrichment analysis

Gene Ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs were conducted using Database for Annotation, Visualization and Integration Discovery, DAVID version 6.8 [10]. A P value < 0.05 was chosen as the cut-off criterion. GO is a set of coherent terminology to describe the biological processes (BP). While KEGG analysis was performed to understand the signaling pathways involving DEGs.

PPI network construction, module and KEGG pathway analysis

The online tool STRING (available at: <http://string.embl.de/>) is a biological repository, designed to construct the PPI network of DEGs based on the known and predicted PPIs, and then analyze the functional interactions between proteins [11]. Using STRING, a PPI network of all the DEGs was constructed with a confidence score ≥ 0.7 . Subsequently, the PPI network was then visualized using Cytoscape software (version 3.5.1). Furthermore, the plug-in of Molecular Complex Detection (MCODE) in Cytoscape software (Bader and Hogue, 2003) was applied to explore the significant module containing hub genes in the constructed PPI network. The advanced options were set as degree cutoff = 2, K-Core = 2, and Node Score Cutoff = 0.2 for module identification and then top 6 DEGs with highest degree of centrality were also found using Cytohubba application through Cytoscape software. Subsequently, the KEGG pathway enrichment analysis of the identified hub genes was conducted using Database for Annotation, Visualization and Integration Discovery (DAVID) version 6.8 [10]. The P value < 0.05 was selected as the cut-off criterion.

Survival analysis of hub genes

The Kaplan–Meier plotter (available at: <http://kmplot.com/analysis/>) could determine the impact of 54,675 genes on survival duration using 18,674 cancer samples [12]. The purpose of the tool is biomarker based meta-analysis to provide an advantage in clinical decisions, health care policies, and resource allocation [13]. In present study, we analyzed effect of each identified hub gene on the overall survival of patients through the Kaplan–Meier plotter. Patients were divided into two classes according to the median of each hub gene expression in Kaplan–Meier plotter for overall survival. This classification method could display the survival probability differences between high-expression class and low-expression class.

Validation of hub gene expression and their promoters' methylation analysis

The relative transcription and translation expression and methylation analysis of the six hub genes was performed using online platform, UALCAN (available at; <http://ualcan.path.uab.edu>), a user-friendly web

resource for analyzing cancer transcriptome data i.e. TCGA and MET500 transcriptome sequencing [14]. UALCAN allows users to perform *in silico* validation of potential genes of interest. One of the user-friendly features of this platform is that it allows user to perform the analysis of relative expression and promoter's methylation status of a query gene(s) across tumor and normal samples, as well as in subgroups based upon various clinicopathological features such as individual age, gender, tumor stages etc. The database uses quartile ranges from minimum to maximum values that indicate differential expression i.e., ranging from lower to higher, respectively. Expression of transcripts was measured as number of transcripts per million (TPM). UALCAN web portal integrates mass spectrometry-based proteomic data from The Clinical Proteomic Tumor Analysis Consortium (CPTAC) in various cancer types. To have an account of hub genes protein's expression we carried out CPTAC analysis. Z-value was used to measure the standard deviations from the median documented across samples of a particular group. UALCAN also links the gene expression with methylation status either hypo or hyper based on beta values i.e., 0 for fully unmethylated state and 1 for fully methylated state. The database uses cut-off values for hypo-methylation (0.3-0.2) and hyper-methylation (0.7-0.5). Therefore, we explored the relative expression and corresponding gene's promoter methylation status of six hub genes with the help of UALCAN based on various tumor molecular subtypes and clinicopathological features of breast cancer.

Hub gene-drug interaction network analysis

Comparative Toxicogenomics Database (CTD) was utilized to construct the hub gene-drug interaction for chemotherapeutic drugs that have the potential to reduce or enhance the mRNA or protein expression levels of the hub genes [15]. Briefly, six hub genes including CDK1, FN1, AURKA, CCNB2, BIRC5, and TOP2A were searched in CTD database, and the hub gene-drug interaction networks were constructed using Gliffy online tool (available at: <https://www.gliffy.com/examples/network-diagrams>).

Results

From both data sets (GSE10810 and GSE61304) a total 250 significantly differential expressed genes, either up- or down-regulated, were identified for each dataset. In data set GSE10810 out of 228 genes 37 genes were up-regulated and 191 were down-regulated genes. While in dataset GSE61304 out of total 221 DEGs 114 genes were up-regulated and 107 genes were down-regulated regulated including 33 common up-regulated / down-regulated genes. All the DEGs were clustered in the form of one table. Hence, a total of 418 genes were listed as DEGs that expressed a fold change of value ≥ 1.5 or ≤ -1.5 and $p < 0.05$.

GO term enrichment analysis of DEGs

For GO and KEGG pathway analysis, the identified significant DEGs were submitted to the online software DAVID. The results of the GO analysis showed that up-regulated DEGs were significantly enriched in biological processes like 'cell division', 'nuclear division', 'organelle fission', 'mitotic cell cycle process', and 'mitotic nuclear division' (Figure 1A; Table 1). The down-regulated DEGs were mainly enriched in 'Lipid

localization', 'muscle system processes, 'muscle organ development' and 'single-organ metabolic process (Figure 1B; Table 2).

Table 1
Gene ontology (GO) analysis of up-regulated DEGs associated with breast cancer

Gene Name	Gene term/Function	Gene Count	P-value
KIF4A, NEK2, AURKA, CEP55, PTTG1, KIF2C, SPC25, CCNA2, TOP2A, ASPM, KIF14, CDK1, NUF2, TPX2, NUSAP1, CENPF, BIRC5, UBE2C, CDK5, PPP1CA, CCNB2, KNL1, ZWINT, CKS2, KIF20A	GO:0051301~cell division	25	< 0.05
KIF14, CDK1, KIF4A, MKI67, NEK2, PTTG3P, TPX2, NUF2, NUSAP1, CENPF, BIRC5, AURKA, PTTG1, CEP55, UBE2C, SPC25, KIF2C, CCNB2, KNL1, ZWINT, CKS2, TOP2A, CCNA2, ASPM	GO:0000280~nuclear division	24	< 0.05
KIF14, CDK1, KIF4A, MKI67, NEK2, PTTG3P, TPX2, NUF2, NUSAP1, CENPF, BIRC5, AURKA, PTTG1, CEP55, UBE2C, SPC25, KIF2C, CCNB2, KNL1, ZWINT, CKS2, TOP2A, CCNA2, ASPM	GO:0048285~organelle fission	24	< 0.05
KIF4A, NEK2, AURKA, PTTG1, CEP55, GTSE1, KIF2C, SPC25, FANCI, TOP2A, CCNA2, ASPM, KIF14, CDK1, TPX2, NUF2, NUSAP1, CENPF, BIRC5, UBE2C, INHBA, CCNB2, KNL1, ZWINT, RRM2, CKS2, KIF20A	GO:1903047~mitotic cell cycle process	27	< 0.05
KIF14, CDK1, KIF4A, NEK2, NUF2, TPX2, NUSAP1, CENPF, BIRC5, AURKA, PTTG1, CEP55, UBE2C, KIF2C, SPC25, CCNB2, KNL1, ZWINT, CCNA2, ASPM	GO:0007067~mitotic nuclear division	20	< 0.05
KIF4A, NEK2, AURKA, PTTG1, CEP55, GTSE1, SPC25, KIF2C, FANCI, TOP2A, CCNA2, ASPM, KIF14, CDK1, MKI67, DTL, PTTG3P, TPX2, NUF2, CENPF, TBRG4, NUSAP1, BIRC5, UBE2C, CDK5, INHBA, CCNB2, KNL1, ZWINT, RRM2, CKS2, KIF20A	GO:0022402~cell cycle process	32	< 0.05
KIF14, KIF4A, NEK2, PTTG3P, NUF2, NUSAP1, CENPF, BIRC5, PTTG1, CEP55, UBE2C, KIF2C, SPC25, HJURP, KNL1, ZWINT, TOP2A	GO:0007059~chromosome segregation	17	< 0.05
KIF14, KIF4A, NEK2, NUF2, NUSAP1, CENPF, BIRC5, CEP55, UBE2C, SPC25, KIF2C, KNL1, ZWINT, TOP2A	GO:0000819~sister chromatid segregation	14	< 0.05
CTHRC1, HIST2H2AA4, U2AF2, AP2S1, AURKA, PTTG1, KIF2C, SAP30, WISP1, HPSE, TOP2A, CCNA2, COL11A1, ASPM, COL10A1, KIF14, CDK1, TPX2, NUSAP1, UBE2C, CDK5, TRPM2, ZNF335, MMP11, ADRM1, INHBA, PPP1CA, UHRF1, ZWINT, RRM2, CFL1, MFAP2, IBSP, KIF4A, SYNDIG1, NEK2, KIAA0101, CEP55, ATP6V0B, TPM3, TK1, SPC25, PFN1, SLC11A1, HJURP, COMP, FN1, MKI67, PTTG3P, NUF2, CENPF, BIRC5, STAT1, GJB2, POC1A, CCNB2, ARF1, KNL1, SULF1, CKS2, SH3BGRL3, HIST1H2AM, PARP1, KIF20A	GO:0016043~cellular component organization	64	< 0.05
KIF14, CDK1, KIF4A, HIST2H2AA4, MKI67, NEK2, PTTG3P, NUF2, CENPF, NUSAP1, BIRC5, AURKA,	GO:0051276~chromosome organization	26	< 0.05

CEP55, PTTG1, UBE2C, ZNF335, SPC25, KIF2C, SAP30, UHRF1, KNL1, HJURP, ZWINT, PARP1, HIST1H2AM, TOP2A			
CTHRC1, HIST2H2AA4, U2AF2, AP2S1, AURKA, PTTG1, KIF2C, SAP30, WISP1, HPSE, TOP2A, CCNA2, COL11A1, ASPM, COL10A1, KIF14, CDK1, TPX2, NUSAP1, UBE2C, CDK5, TRPM2, ZNF335, MMP11, ADRM1, INHBA, PPP1CA, UHRF1, ZWINT, RRM2, CFL1, MFAP2, IBSP, KIF4A, SYNDIG1, NEK2, KIAA0101, CEP55, ATP6V0B, TPM3, TK1, SPC25, PFN1, SLC11A1, HJURP, COMP, FN1, MKI67, PTTG3P, NUF2, CENPF, BIRC5, STAT1, GJB2, POC1A, CCNB2, ARF1, KNL1, SULF1, CKS2, SH3BGRL3, HIST1H2AM, PARP1, KIF20A	GO:0071840~ biogenesis	64	< 0.05
KIF14, CDK1, MKI67, DTL, NEK2, KIAA0101, TPX2, NUSAP1, TBRG4, CENPF, BIRC5, AURKA, UBE2C, CDK5, GTSE1, INHBA, CCNB2, FANCI, ZWINT, CKS2, TOP2A, CCNA2, ASPM	GO:0051726~regulation of cell cycle	23	< 0.05
KIF14, CDK1, MKI67, NEK2, TPX2, NUSAP1, CENPF, BIRC5, AURKA, UBE2C, GTSE1, FANCI, ZWINT, CKS2, CCNA2, TOP2A	GO:0007346~regulation of mitotic cell cycle	16	< 0.05
CDK1, DTL, FANCI, KNL1, ZWINT, CENPF, AURKA, BIRC5, CCNA2, TOP2A, GTSE1	GO:0000075~cell cycle checkpoint	11	< 0.05
KIF14, CDK1, KIF4A, NEK2, NUF2, KIAA0101, TPX2, CENPF, NUSAP1, BIRC5, AURKA, CEP55, UBE2C, CDK5, TRPM2, TPM3, SPC25, KIF2C, PFN1, ARF1, KNL1, ZWINT, CFL1, SH3BGRL3, TOP2A, ASPM, KIF20A	GO:1902589~single-organism organelle organization	27	< 0.05
KIF14, CDK1, NEK2, TPX2, CENPF, BIRC5, AURKA, UBE2C, GTSE1, INHBA, CCNB2, FANCI, RRM2, CKS2, CCNA2	GO:0044772~mitotic cell cycle phase transition	15	< 0.05
KIF14, KIF2C, KIF4A, NEK2, ZWINT, NUSAP1, CENPF, CEP55, UBE2C	GO:0000070~mitotic sister chromatid segregation	9	< 0.05
KIF4A, HIST2H2AA4, NEK2, U2AF2, KIAA0101, AURKA, CEP55, PTTG1, ATP6V0B, TPM3, SPC25, PFN1, KIF2C, SAP30, HJURP, TOP2A, CCNA2, ASPM, KIF14, CDK1, MKI67, PTTG3P, NUF2, TPX2, CENPF, NUSAP1, BIRC5, UBE2C, CDK5, ZNF335, TRPM2, UHRF1, CCNB2, ARF1, KNL1, ZWINT, CFL1, CKS2, SH3BGRL3, PARP1, HIST1H2AM, KIF20A	GO:0006996~organelle organization	42	< 0.05
KIF14, CDK1, KIF2C, KIF4A, NEK2, KIAA0101, TPX2, NUSAP1, AURKA, BIRC5, ASPM, KIF20A	GO:0000226~microtubule cytoskeleton organization	12	< 0.05
CDK1, TNFSF4, MKI67, DTL, NEK2, KIAA0101, S100A11, CENPF, PTTG1, TK1, PFN1, UHRF1, FANCI,	GO:0006259~DNA metabolic process	18	< 0.05

Table 2

Table 2: Gene ontology (GO) analysis of down-regulated DEGs associated with breast cancer

Gene name	Gene term/function	Gene count	P-value
ABCA8, RBP4, CAV1, ABCA9, PPARG, STAT5B, CIDEA, ABCB1, ACACB, FZD4, ABCA6, ACVR1C, ABCA5, LEP, SPX, PLA2R1, GPIHBP1, ANO6	GO:0010876~lipid localization	18	< 0.05
CFLAR, CAV1, SCN3B, CRYAB, PPP1R12B, UTRN, OXTR, KLF15, ATP1A2, KCNIP2, LEP, EDNRB, HSPB6, SORBS1, DMD, MYH11, SCN4B, CNN1, TMOD1	GO:0003012~muscle system process	19	< 0.05
HLF, RBP4, FGFR1, CAV2, CFLAR, CAV1, KCNAB1, CRYAB, FHL1, MRAS, UTRN, SMYD1, FOXP1, TAGLN, S100B, DMD, CFL2, ITGA7	GO:0007517~muscle organ development	18	< 0.05
HLF, RBP4, FGFR1, CAV2, CFLAR, CAV1, KCNAB1, CRYAB, FHL1, MRAS, UTRN, SMYD1, TCF7L2, FOXP1, EDNRB, S100B, TAGLN, DMD, CFL2, ITGA7, MYH11, TMOD1	GO:0061061~muscle structure development	22	< 0.05
ALDH1L1, ZAK, KCNAB1, PDGFA, STAT5B, PPARG, ADH1C, TMEM237, PDE3B, ADH1B, ACVR1C, SPRY2, EDNRB, ASPA, GPC3, PAK3, PPP1R1A, PAK5, LOXL4, PDGFD, FGF1, GHR, NMNAT2, AIFM2, FAXDC2, CRYAB, RDH5, GNAL, MARC1, CLIC5, CA4, HAS3, GPAM, UGP2, ADAMTS5, AOC3, CAV2, RBP4, FGFR1, CAV1, MRAP, ADCYAP1R1, AKAP12, MME, SNX1, OXTR, SLC19A3, CX3CL1, PLPP3, SORBS1, COL6A6, SH3GLB1, FMO2, DMD, PER1, GPD1, BHMT2, KLB, MAOA, NPR1, CIDEA, GYG2, ACACB, ATP1A2, FZD4, SOD3, PCK1, LEP, GLYAT, ATXN3, PDE2A, NTRK2, ALDH2, SPTBN1, HRASLS5, GRK3, ACSM5	GO:0044710~single-organism metabolic process	77	< 0.05
RHOJ, CFLAR, CAV1, SCN3B, PDGFA, MRAS, PDLIM3, ATP1A2, FOXP1, KANK1, SEMA5A, CORO2B, ATXN3, SORBS1, PAK3, DMD, CFL2, MYH11, SPTBN1, SCN4B, CNN1, PARVA, TMOD1	GO:0030029~actin filament-based process	23	< 0.05
ABCA8, RBP4, CAV1, ABCA9, PPARG, ABCB1, ACACB, FZD4, ABCA6, ABCA5, LEP, SPX, PLA2R1, GPIHBP1, ANO6	GO:0006869~lipid transport	15	< 0.05
RHOJ, FGFR1, RBP4, CAV1, PDGFA, KCNAB1, PPARG, PDLIM3, PDE3B, OXTR, CX3CL1, TCF7L2, PLPP3, SEMA5A, GPC3, TMEM100, PDGFD, FGF1, NPR1, SMYD1, ACACB, MCAM, FZD4, FOXP1, LEP, NTRK2, MYH11, PARVA	GO:0072359~circulatory system development	28	< 0.05
RHOJ, FGFR1, RBP4, CAV1, PDGFA, KCNAB1, PPARG, PDLIM3, PDE3B, OXTR, CX3CL1, TCF7L2, PLPP3, SEMA5A, GPC3, TMEM100, PDGFD, FGF1, NPR1, SMYD1, ACACB, MCAM, FZD4, FOXP1, LEP, NTRK2, MYH11, PARVA	cardiovascular system development	28	< 0.05

CACHD1, SCN3B, PDGFA, KCNAB1, TLN2, STAT5B, UTRN, PPARG, ITSN1, KCNIP2, KANK1, ACVR1C, SPRY2, EDNRB, SRPX, GPC3, SPX, CFD, GPIHBP1, ANO6, GHR, CLCA4, NUP88, STXBP1, CLIC5, CD300LG, CA4, RELN, GPAM, PROS1, ABCA8, TF, RBP4, FGFR1, CAV1, ABCA9, FHL1, ADCYAP1R1, SNX1, OXTR, SLC19A3, TCF7L2, ABCA6, ABCA5, SEMA5A, SORBS1, SH3GLB1, DMD, PER1, PLA2R1, NPR1, CIDEA, ABCB1, ATP1A2, ACACB, KLF15, FZD4, FOXP1, LEP, SYNE3, PDE2A, SLC16A7, FBLN5, ANXA11, NTRK2, SCN4B, SPTBN1, GRK3, SCARA5	GO:1902578~single-organism localization	69	< 0.05
PDGFA, STAT5B, PPARG, PDE3B, KCNIP2, KANK1, ACVR1C, SPRY2, EDNRB, GPC3, SPX, SAA2, PAK3, SEMA3G, ZNF106, TMEM100, PDGFD, FGF1, SIK2, GPIHBP1, ANO6, PPP1R14A, GHR, CRYAB, LIFR, SLIT3, MARC1, HSPB7, TFPI, HSPB2, RELN, GPAM, CHL1, AOC3, PARVA, ABCA8, TF, CAV2, RBP4, FGFR1, CAV1, GNAI1, ADCYAP1R1, SOX5, MME, OXTR, TIMP4, CX3CL1, TCF7L2, SEMA5A, SORBS1, FMO2, PER1, PLA2R1, GPD1, KLB, CIDEA, ABCB1, ACACB, KLF15, ATP1A2, FZD4, SOD3, FOXP1, PCK1, LEP, CDKN1C, GLYAT, ATXN3, PDE2A, S100B, FBLN5, ANXA11, NTRK2, SPTBN1, OR10D3	GO:0042221~response to chemical	76	< 0.05
ZAK, KCNAB1, PDGFA, SCN3B, PPARG, UTRN, EIF5, TCEAL7, KCNIP2, ITSN1, ACVR1C, EDNRB, SPRY2, GPC3, ARHGAP20, CDKN2C, PAK3, PPP1R1A, ITIH5, PDGFD, FGF1, GPIHBP1, PPP1R14A, GHR, CRYAB, LDB2, PCOLCE2, GNAL, TFPI, HSPB2, RELN, PROS1, CCDC8, FGFR1, CAV2, CAV1, FHL1, ADCYAP1R1, PPP1R12B, TIMP4, DENND2A, CX3CL1, TCF7L2, FAM13A, PLPP3, SH3GLB1, DMD, PLA2R1, CFLAR, KLB, ACACB, ATP1A2, FZD4, LEP, CDKN1C, NTRK2, SPTBN1, SCN4B	GO:0065009~regulation of molecular function	58	< 0.05
RBP4, FGFR1, CFLAR, CRYAB, FHL1, IGFBP6, PPARG, STAT5B, NPR1, ACACB, DCAF1, FOXP1, SLIT3, SEMA5A, LEP, CDKN1C, SPRY2, FAM107A, GPC3, CDKN2C, SEMA3G, FBLN5, PAK5, FGF1, GPAM, EMP1, GHR	GO:0040007~growth	27	< 0.05
FGFR1, RBP4, CAV1, SCN3B, KCNAB1, PPP1R12B, UTRN, PPARG, SOBP, MME, OXTR, KCNIP2, EDNRB, SPRY2, SPX, SORBS1, PDE1C, DMD, PAK5, CNN1, TMEM100, CFLAR, CRYAB, NPR1, KLF15, ATP1A2, MCAM, FZD4, RDH5, CCDC50, LEP, GNAL, PDE2A, S100B, HSPB6, CLIC5, HSPB7, NTRK2, MYH11, CELF2, SCN4B, OR10D3, RELN, CHL1, TMOD1	GO:0003008~system process	45	< 0.05
RHOJ, ZAK, PDGFA, EZH1, TMEM237, SOBP, PDE3B, KANK1, SPRY2, GPC3, SEMA3G, PAK3, TMEM100, FGF1, GHR, TBX15, CRYAB, STXBP1, SLIT3, CLIC5, CFL2, RELN, ADAMTS5, CHL1, PARVA, FGFR1, RBP4, CAV1, FHL1, SNX1, PALMD, CX3CL1, TCF7L2, PLPP3, SEMA5A, DMD, PALM2, CFLAR, NPR1, MCAM, FZD4, FOXP1, SYNE3, LEP, CDKN1C, LYVE1, S100B, FREM1, NTRK2, ITGA7, MYH11, SPTBN1, SH3D19, TMOD1	GO:0009653~anatomical structure morphogenesis	54	< 0.05

LEP, CAV1, STAT5B, PPARG, CIDEA, ACACB, ACVR1C	GO:0019915~lipid storage	7	< 0.05
CAV2, CAV1, GNAI1, PPARG, STAT5B, PDE3B, OXTR, TIMP4, KLF15, KANK1, PCK1, ACVR1C, LEP, SORBS1, ZNF106, SIK2, GHR	GO:1901652~response to peptide	17	< 0.05
RHOJ, ZAK, PDGFA, TLN2, MRAS, PDLIM3, KANK1, SEMA5A, CORO2B, SPRY2, SORBS1, PAK3, DMD, STARD9, PAK5, CNN1, CFLAR, CRYAB, FOXP1, SYNE3, ATXN3, CFL2, CEP68, MYH11, SPTBN1, SH3D19, MAP7D3, TMOD1, CCDC8, PARVA	GO:0007010~cytoskeleton organization	30	< 0.05
CAV2, CAV1, GNAI1, PPARG, STAT5B, PDE3B, OXTR, TIMP4, KANK1, PCK1, ACVR1C, LEP, SORBS1, ZNF106, SIK2, GHR	GO:0043434~response to peptide hormone	16	< 0.05
RBP4, FGFR1, CRYAB, FHL1, IGFBP6, PPARG, STAT5B, NPR1, ACACB, DCAF1, FOXP1, SLIT3, SEMA5A, LEP, GPC3, FAM107A, CDKN2C, SEMA3G, FBLN5, GPAM, GHR	GO:0040008~regulation of growth	21	< 0.05

KEGG pathway analysis of DEGs

KEGG pathway analysis of up-regulated DEGs revealed their involvement in various pathways including 'oocyte meiosis', 'p53 signaling pathway', 'focal adhesion' and 'ECM-receptor interaction' (Figure 2A; Table 3). While the KEGG pathway analysis of the down-regulated DEGs revealed their enrichment in 'focal adhesion', 'Rennin secretion', 'regulation of active cytoskeleton', 'ABC transporters' and 'axon guidance' (Figure 2B; Table 4)

Table 3

Kyoto encyclopedia of genes and genomes pathway analysis of up-regulated DEGs related with breast cancer

Pathway ID	Pathway Name	Gene count	P-value	Gene name
04114	Oocyte meiosis	5	< 0.05	CDK1, PPP1CA, CCNB2, AURKA, PTTG1
04115	p53 signaling pathway	4	< 0.05	CDK1, CCNB2, RRM2, GTSE1
04510	Focal adhesion	6	< 0.05	IBSP, PPP1CA, COMP, COL11A1, FN1, PIK3R2
04512	ECM-receptor interaction	4	< 0.05	IBSP, COMP, COL11A1, FN1
04914	Progesterone-mediated oocyte maturation	4	< 0.05	CDK1, CCNB2, CCNA2, PIK3R2
04110	Cell cycle	4	< 0.05	CDK1, CCNB2, PTTG1, CCNA
05161	Hepatitis B	4	< 0.05	BIRC5, STAT1, CCNA2, PIK3R2

Table 4

Kyoto encyclopedia of genes and genomes pathway analysis of down-regulated DEGs related with breast cancer

Pathway ID	Pathway Name	Gene count	P-value	Gene name
04510	Focal adhesion	12	< 0.05	CAV2, CAV1, COL6A6, PAK3, PDGFA, TLN2, PPP1R12B, PAK5, ITGA7, RELN, PDGFD, PARVA
04924	Renin secretion	6	< 0.05	CLCA4, GNAI1, PDE1C, ADCYAP1R1, PDE3B, NPR1
04810	Regulation of actin cytoskeleton	10	< 0.05	FGFR1, PAK3, PDGFA, MRAS, CFL2, PPP1R12B, PAK5, ITGA7, PDGFD, FGF1
02010	ABC transporters	5	< 0.05	ABCA8, ABCA9, ABCB1, ABCA6, ABCA5
04360	Axon guidance	7	< 0.05	SEMA5A, PAK3, SEMA3G, GNAI1, CFL2, PAK5, SLIT3
00350	Tyrosine metabolism	4	< 0.05	MAOA, ADH1C, ADH1B, AOC3
00360	Phenylalanine metabolism	3	< 0.05	GLYAT, MAOA, AOC3
05205	Proteoglycans in cancer	8	< 0.05	CAV2, FGFR1, CAV1, GPC3, MRAS, PPP1R12B, HSPB2, FZD4
04022	cGMP-PKG signaling pathway	7	< 0.05	EDNRB, PDE2A, GNAI1, PDE3B, NPR1, CREB5, ATP1A2
05215	Prostate cancer	5	< 0.05	FGFR1, PDGFA, CREB5, PDGFD, TCF7L2
00340	Histidine metabolism	3	< 0.05	ASPA, MAOA, ALDH2
04964	Proximal tubule bicarbonate reclamation	3	< 0.05	CA4, ATP1A2, PCK1
05032	Morphine addiction	5	< 0.01	PDE2A, GNAI1, PDE1C, PDE3B, GRK3
04922	Glucagon signaling pathway	5	< 0.05	PDE3B, CREB5, ACACB, SIK2, PCK1

04151	PI3K-Akt signaling pathway	10	< 0.05	FGFR1, COL6A6, PDGFA, ITGA7, RELN, CREB5, PDGFD, FGF1, GHR, PCK1
04024	cAMP signaling pathway	7	< 0.05	GNAI1, ADCYAP1R1, PDE3B, NPR1, OXTR, CREB5, ATP1A2
00010	Glycolysis / Gluconeogenesis	4	< 0.05	ADH1C, ALDH2, ADH1B, PCK1
00982	Drug metabolism - cytochrome P450	4	< 0.05	FMO2, MAOA, ADH1C, ADH1B
05218	Melanoma	4	< 0.05	FGFR1, PDGFA, PDGFD, FGF1
04015	Rap1 signaling pathway	7	< 0.05	FGFR1, GNAI1, PDGFA, TLN2, MRAS, PDGFD, FGF1

PPI (Protein-Protein Interaction) network construction, module identification and KEGG pathway analysis

PPI network analysis of the identified DEGs revealed that there were 334 nodes and 1127 edges interactions (Figure 3). These proteins were selected on the basis of combined score ≥ 0.4 in STRING analysis. In addition, one significant module with a score ≥ 5 was screened out via MCODE. The CDK1, FN1, AURKA, CCNB2, BIRC5, and TOP2A genes were identified as hub genes with highest degree of centrality in the identified module (Table 5). KEGG pathways analysis of the hub genes has been displayed in Table 6. The most significant pathways were enriched in the Oocyte meiosis, p53 signaling pathway, Progesterone-mediated oocyte maturation, and cell Cycle pathways (Table 6).

Table 5
List of identified hub genes

Name of the gene	Degree of centrality	No. of nodes	Closeness of centrality
CDK1	46	46	0.38288288
FN1	43	43	0.42079208
AURKA	41	41	0.4028436
CCNB2	40	40	0.38694992
BIRC5	40	40	0.38694992
TOP2A	40	40	0.35027473
Closeness of centrality indicates how close a node is to all other nodes in the network.			

Table 6

Kyoto encyclopedia of genes and genomes pathway analysis of hub genes related with breast cancer

Pathway ID	Pathway Name	Gene count	P-value	Gene name
04114	Oocyte meiosis	3	< 0.05	CDK1, CCNB2, AURKA
04115	p53 signaling pathway	2	< 0.05	CDK1, CCNB2
04914	Progesterone-mediated Oocyte maturation	2	< 0.05	CDK1, CCNB2

Kaplan Meier Survival analysis of identified hub genes

The prognostic value of the identified six hub genes was documented with the help of Kaplan–Meier plotter website. A high expression of CDK1 (HR 1.55 [1.25–1.92], $P = 6.1\text{e-}05$), FN1 (HR 0.92 [0.74–1.14], $P = 0.44$), AURKA (HR 1.83 [1.47–2.28], $P = 3.6\text{e-}08$), CCNB2 (HR 2.06 [1.65–2.56], $P = 5.2\text{e-}11$), BIRC5 (HR 1.52 [1.23–1.89], $P = 0.00011$), and TOP2A (HR 1.84 [1.48–2.29], $P = 3.1\text{e-}08$), was related to a worse OS in breast cancer patients (Figure 2). However, results of the present study also shown that high expression of FN1 (HR 0.92 [0.74–1.14], $P = 0.44$) was related with good OS in breast cancer patients but the correlation was found insignificant ($p > 0.05$) (Figure 4).

Validation of hub genes participation in breast cancer: through analysis of expression profile in relation to various clinicopathological features and promoter's methylation status

Then, the online tool UALCAN was used to document alterations, if any, in the expression levels of six hub genes in breast normal and cancerous tissues. The transcription and translation expression levels of all the hub genes were found significantly ($p < 0.05$) higher in cancerous tissues as compared to the normal tissues (Figure 5 and Supplementary data Figure 6). Further analysis of patients subgroups based on multiple clinicopathological features of breast cancer samples in the TCGA database showed consistently significant ($p < 0.05$) overexpression in the levels of the six hub genes. The expression levels of the detected hub genes were significantly ($p < 0.05$) elevated in breast cancer samples than the normal samples in patients of different cancer stages and subclasses of tumors (Supplementary data Figure 1-

2), age, ethnicity, and menopause status of patients (Supplementary data Figures 3–5). In addition, we also explored that the expression levels of hub genes well correlated with the methylation status of the corresponding promoters. Unexpectedly, based upon up regulated expression levels, the promoter's methylation status of all the six hub genes was found to be hypermethylated (Figure 6).

Hub Gene-Drug interaction network analysis

To explore the interaction between hub genes and available therapeutic drugs of cancer, the gene-drug interaction network was constructed using CTD. As shown in Figure 9, a variety of drugs could potentially affect the expression of all the six identified hub genes including CDK1, FN1, AURKA, CCNB2, BIRC5, and TOP2A. For example, doxorubicin and bisphenol A could reduce CDK1 expression level while surmestine could elevate CDK1 expression level (Figure 7).

Discussion

Breast cancer is a group of heterogeneous diseases with multiple clinical presentations, treatment responses and subtype related to therapeutic measures. Molecular classification method of breast cancer is well recognized around the world. However, scientist gradually noticed that different molecular subtypes of breast cancer were correlated with distinct treatment responses and metastasis traits, which are useful to predict the duration of the survival and guide the proper administration of the drugs for the better treatment [16]. Till to date there is no diagnostic method available for the early detection of the breast cancer [17]. A number of therapies are available for the treatment of different breast cancer subtypes like hormonal therapy for HER-2 positive is well established. However, the response of HER2 based therapies is expected to be failure in basal-like breast cancer (BLBC) because this subtype usually expresses low or neither of these oncogenes. The BLBC expresses some normal myoepithelial cell makers such as cytokeratins (CK5/6, CK14 and CK17), HER1 and c-KIT but these markers are still unable to represent the complete characteristics of the BLBC [18, 19].

By looking at the complete scenario, it is worth to utilize bioinformatics methods to identify the novel diagnostic and prognostic biomarkers by analyzing the online available microarray based datasets. In the present research, we retrieved the RNA microarray datasets from GEO database and identified the DEGs in breast cancer patients utilizing bioinformatics tools. We further validated the role of the identified hub genes in breast cancer development and progression by evaluating breast invasive carcinoma datasets (BRICS) of TCGA database integrated with UALCAN platform. We also aimed to construct the drug-gene interaction network and performed correlation analysis between the expression level of hub genes and the methylation status of their promoters. In total 449 DEGs were identified (151 up-regulated and 298 down-regulated genes) including 33 common up-regulated / down-regulated genes.

KEGG pathway and GO functional annotation analysis of up-regulated DEGs revealed their involvement in various pathways including 'oocyte meiosis', 'P53 signaling pathway', 'focal adhesion', 'ECM-receptor interaction', 'Rennin secretion', 'regulation of active cytoskeleton', 'ABC transporters' and 'axon guidance'.

The role of major pathways identified through KEGG analysis like 'oocyte meiosis', 'p53 signaling pathway', 'focal adhesion' and 'ECM-receptor interaction' pathways in the development of various cancers including breast has already been reported by *Sun et al. and Bao et al.* [20, 21]. Similarly, the role of 'Rennin secretion', 'regulation of active cytoskeleton', 'ABC transporters' and 'axon guidance' pathways in breast cancer has also been suggested by *Vinson et al. and Baumann et al.* [22–25]. However, these individual studies were the small scale studies containing less number of samples. While the present study was performed on datasets consisting of a large sample size and higher number of target genes. Hence, it revealed a better picture of the molecular pathways contributing to the pathogenesis of breast cancer. The information obtained from this study is persistent with the existing state of knowledge, and provide further confirmation regarding the role of oocyte meiosis, 'p53 signaling pathway', 'focal adhesion', 'ECM-receptor interaction', 'Rennin secretion', 'regulation of active cytoskeleton', 'ABC transporters' and 'axon guidance' pathways in the breast cancer.

The PPI network of all the DEGs was then constructed to identify the module and hub genes. A total of 36 key genes were found in the identified module, out of which six up-regulated genes including CDK1, FN1, AURKA, CCNB2, BIRC5 and TOP2A showed the highest degree of centrality and were considered for the further analysis. KEGG pathway analysis of the identified hub genes showed their significant enrichment in terms of Oocyte meiosis, p53 signaling pathway, Progesterone-mediated oocyte maturation and Cell cycle pathways (Fig. 8).

The cell cycle dysregulation is the major event that occurs in cancer cells [26] and Cyclin-dependent kinases (CDKs) are the well-known proteins involved in the regulation of cell cycle [27]. The down-regulation of CDK1 has been associated with breast cancer by *Lie Ding et al.* [28]. Moreover, *Lie Ding et al.* [28] also reported that breast cancer patients with overexpression of CDK1 and CDK2 had significantly poorer OS compared to those with low CDK1 and CDK2 expression. Similar role of CDKs has also been reported in renal cell carcinoma [29]. *Izadi et al.* [30] revealed that down-regulation of CDK1 leads to the increased cell proliferation in breast cancer. They further added that the selective blockage of CDK1 alone or in combination with other therapeutics leads to potent anti-cancer outcomes. Contrary to the earlier reports we investigated the overexpression of CDK1 in breast cancer which suggests that elevated expression of CDK1 may serve as novel potential biomarkers for predicting the outcome of breast cancer.

FN1 is an extracellular matrix glycoprotein which is involved in cell proliferation, embryogenesis, wound healing, host defense, epithelial-mesenchymal transition (EMT), metastasis, and oncogenic transformation [31]. Previous reports revealed its involvement in the pathogenesis of various tumors and up-regulation in multiple cancer types, such as esophageal squamous cell carcinoma, colorectal cancer (CLC), and thyroid cancer [32–34]. Elevated expression and accumulation of FN1 has been reported in vincristine-resistant myeloma cells, while in other ECM components, including type II collagen $\alpha 1$, its level was found to be down-regulated [35]. However, the role of FN1 is not extensively studied in breast cancer. Until now, only a single study [36] has identified the role of FN1 overexpression in breast cancer and suggested it as a potential biomarker in breast cancer. Our findings are similar to this report and further validate the conclusions of *Wang et al.* [36].

AURKA is an important molecule for cell cycle progression. It has been frequently found overexpressed or mutated in various types of human tumor [37, 38]. However, its role in breast cancer is still uncertain due to the conflicting results of the various studies. For example, *Tanaka et al.* [39] investigated 33 cases of breast cancer and found overexpression of AURKA in 94% of the cases. *Miyoshi et al.* observed AURKA overexpression in 64% of the breast cancer cases using reverse transcription polymerase chain reaction (RT-PCR) in a group of 47 patients [40]. However, comparatively a larger study including 112 breast cancer patients did not find any association between AURKA expression and patient's survival [41]. However, *Nadler et al.* observed elevated expression of AURKA in breast tumors and related it with OS [42]. In our study we also observed the overexpression of AURKA and thus suggest it as a potential biomarker of breast cancer.

Cyclin B2 (CCNB2) is a member of cyclin family. The major function of CCNB2 is to regulate the cell cycle by promoting the activation of CDC2 kinase in eukaryotes, and inhibition of CCNB2 induced cell cycle arrest. Various studies had reported its overexpression in multiple types of cancers including bladder cancer, uterine corpus endometrial carcinoma, prostate cancer, and gastric cancer [43, 44]. In breast cancer, CCNB2 protein levels have been reported as an independent prognostic factor which significantly correlated with a poor disease specific survival [45]. Our results indicated up-regulation of CCNB2 in breast cancer samples as compared to the normal controls and highlighted it as potential biomarker in breast cancer. However, the underlying mechanisms of CCNB2 on tumor progression need to be further investigated.

BIRC5 (Survivin) is a multifunctional protein that controls rate of mitosis and participates in the regulation of apoptosis, and the cellular stress response [46]. Overexpression of BIRC5 in breast cancer is associated with increased resistance to chemotherapy, radiotherapy, increased grade and tumour size, as well as with the decreased survival rate [47]. According to *Marsicano et al.* breast cancer patients with higher expression of BIRC5 have significantly greater chance of developing metastasis than patients with lower expression [48]. Similar results were observed during present study which suggested that BIRC5 overexpression could be a potential prognostic and diagnostic biomarker in breast cancer.

DNA topoisomerase 2-alpha (TOP2A) gene encodes a protein that controls and alters topologic states of DNA during the process of transcription. TOP2A is a significant prognostic factor in predicting the breast cancer patients' OS, and low expression of TOP2A has been associated with a better clinical outcome [49]. In addition TOP2A amplification or deletion is also known to enhance responsiveness to anthracycline-containing chemotherapy regimens relative to non-anthracycline regimens [50]. Various other studies have evaluated the predictive value of TOP2A using different methodologies and inconsistent results related with TOP2A differential expression analysis have been reported. Indeed, the correlation among techniques employed for the assessment of TOP2A status has not been well evaluated [51]. However, in the light of our results we conclude that the elevated expression of TOP2A could be a prognostic factor for breast cancer and indicates a role of anthracycline-containing chemotherapy regimens for effective treatment of the disease.

The survival data obtained from Kaplan-Meier method showed that the OS of breast cancer patients harboring high expression of CDK1, AURKA, CCNB2, BIRC5 and TOP2A genes was significantly worsened than that of low-expression patients. However, the elevated expression of FN1 was found insignificantly ($p < 0.05$) correlated with good OS of breast cancer patients which need to be further validated through voluminous testing.

The elevated expression levels of identified hub genes were further validated on all the BRIC datasets present of TCGA database through UALCAN platform. Results revealed the persistent significant ($p < 0.05$) up-regulation of transcription and translation level of the all the hub genes in breast cancer samples including various subgroups of patients categorized on the basis of various clinicopathological features (i.e., tumor stage, breast cancer major subclasses, age, race and menopause status) as compared to the normal controls.

A correlation between expression levels of all the hub genes and the methylation status of their promoters was also accessed and it was found that the expression levels of hub genes have a significant ($p < 0.05$) positive correlation with the methylation extent of their corresponding promoters. Here, the methylation scenario challenges the classical view of the methylation where the up-regulation was always negatively correlated with the expression level. However, further studies need to validate these findings.

We further studied the response of available therapeutics drugs in regulating the expression levels of identified six hub genes. The information thus generated can serve as a guide during the treatment of patients exhibiting up-regulated expression of these hub genes and selection of appropriate drugs. Whether breast cancer patients with over-expression of these hub genes will get any benefit from the suppression of hub genes, or whether these hub genes are promising therapeutic targets still need additional experimental support through voluminous pre-clinical and clinical trials.

Currently, few relevant studies based on the same datasets that we used in the present study have been published. In one such study, *Jin et al.* [52] analyzed the microarray dataset GSE10810 in a consortium with few other datasets and identified 15 different hub genes by constructing PPI network. They also analyzed the effect of hub genes expression on the survival duration of patients. Similarly the dataset GSE29431 was also analyzed by *Lin et al.* [53] along with two different other datasets and they identified 10 hub genes in that study. Compared to their results, the hub genes we identified in the present study are not exactly compatible with already identified hub genes. The unique combinations of these datasets and different analysis methods have been used in the current study, which might partly account for the reasons for these differences. Some advantages of our study mainly lie in the following points: First of all we validated the expression of all the identified hub genes on BRIC datasets present on TCGA database and further investigated the status of hub genes expression in different sub groups of patients based upon various clinicopathological features such as breast cancer stages, major subclasses, age, race, and menopause status. Secondly, we performed the correlational analysis between the expression of hub genes and their respective promoters' methylation statuses. Finally, exploring the impact of many

commonly used chemotherapeutic drugs on regulation of the hub genes expression may potentially contribute to identify new biomarkers and targets for better management of breast cancer.

Conclusion

The present study provided a comprehensive overview of DEGs and associated biological processes which are dysregulated and may be involved in breast cancer progression. We documented up-regulation of the six hub genes including CDK1, FN1, AURKA, CCNB2, BIRC5, and TOP2A and propose that the detected hub genes are the most likely players which can be linked with breast cancer development and progression. The validation of hub genes overexpression in TCGA BRIC patients having diverse clinicopathological parameters further reinforced the role of these hub genes in pathogenesis of breast cancer. Results also revealed the higher expression levels of hub genes as a poor clinical outcome in breast cancer patients. The present study has significantly enhanced our understanding regarding breast cancer pathogenesis and progression. Moreover, the findings of the present study have provided new insights into the diagnosis, prognosis and treatment of breast cancer. However, the specific functions of the identified genes in breast cancer should be confirmed by further molecular biological investigations.

List Of Abbreviations

BC = Breast cancer

ER = Estrogen receptor

PR = Progesterone receptor

EGF = Epidermal growth factor

HER2 = Human epidermal growth factor receptor 2

GEO = Gene Omnibus

CTD = Comparative Toxicogenomics Database

GO= Gene ontology

KEGG = Kyoto Encyclopedia of Genes and Genomes

GEGs = differentially expressed genes

PPI = Protein-Protein Interaction

KM = Kaplan-Meier

CDKS = Cyclin-dependent kinases

CCNB2 = Cyclin B2

Declarations

Ethics approval and consent to participate

NA

Consent for publication

NA

Availability of data and materials

The two datasets (GSE10810 and GSE61304) on which the conclusion of present study based are available in GEO database (at: <https://www.ncbi.nlm.nih.gov/geo/>)

Competing interests

None

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Yasir Hameed], and [Samina Ejaz]. The first draft of the manuscript was written by [Yasir Hameed] which was finalized by [Samina Ejaz], all the authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Enriched GO functions analysis results of DEGs. (A) Up-regulated genes GO analysis. (B) Down-regulated genes GO analysis. GO = gene ontology; DEGs = differentially expressed genes.

Figure 2

KEGG pathway analysis results of differentially expressed genes. (A) Up-regulated genes KEGG pathway analysis results. (B) Down-regulated genes KEGG pathway analysis results. KEGG = Kyoto Encyclopedia of Genes and Genomes.

Figure 3

protein-protein interaction network of all the DEGs Yellow color indicates the main identified module containing CDK1, FN1, AURKA, CCNB2, BIRC5, and TOP2A hub genes. p-value ($p < 0.05$) was used to represent the significance of the correlation between the observed parameters.

Figure 4

Kaplan–Meier survival curves of six hub genes in breast cancer patients. Overall survival (OS) by low and high level of (A) CDK1, (B) FN1, (C) AURKA, (D) CCNB2, (E) BIRC5, and (F) TOP2A expression. p-value ($p < 0.05$) was used to represent the significance of the correlation between the observed parameters.

Figure 5

Relative expression of six hub genes in normal tissues and breast cancer tissues. (A) CDK1, (B) FN1, (C) AURKA, (D) CCNB2, (E) BIRC5, and (F) TOP2A. p-value ($p<0.05$) was used to represent the significance of the correlation between the observed parameters.

Figure 6

Relative methylation status of six hub genes in normal individual and breast cancer patients. (A) CDK1, (B) FN1, (C) AURKA, (D) CCNB2, (E) BIRC5, and (F) TOP2A. p-value ($p<0.05$) was used to represent the significance of the correlation between the observed parameters.

Figure 7

Gene-drug interaction network constructed with six hub genes and chemotherapeutic drugs. Panels A–F indicates available chemotherapeutic drugs that decrease or increase the expression levels of hub genes at mRNA or protein level. (A) CDK1, (B) FN1, (C) AURKA, (D) CCNB2, (E) BIRC5, and (F) TOP2A. Red arrows: chemotherapeutic drugs increase the expression of hub genes; green arrows: chemotherapeutic drugs decrease the expression of hub genes. The numbers of arrows between chemotherapeutic drugs and hub genes in the network represent the supported numbers of literatures by previous studies.

Figure 8

Crosstalk between the hub genes in the identified pathways Cyclin B2 (CCNB2) is a mitotic cyclin that is activated in mitosis and binds to CDK1 to form a complex and thus helps in its activation through phosphorylation by WEE1 kinases. Once CDK1 is activated it further activates BIRC5, AURKA, TOP2A, and SP1 through phosphorylation. After completion of its job, CDK1 is inhibited by TP53. The complex between CCNB2 and CDK1 also known as maturation promoting factor or mitosis promoting factor (MPF) because of its major role in the meiosis process [54]. In the present study, the up-regulation of CDK1 is supposed to elevate the activity of its major target proteins including BIRC5, AURKA, TOP2A, and SP1. The BIRC5 (Survivin) is an anti-apoptosis factor and is normally expressed at a very low level in normal cells [55]. Its high level observed in the present study is supposed to be a major obstacle in the apoptosis of abnormal cells. AURKA and TOP2A both are cell cycle and centrosome cycle regulators which are essential for mitotic onset and cell cycle transition from G2 to M phase [56]. On the basis of our results, it is proposed that overexpressed AURKA and TOP2A increase the G2-M transition and lead to the high proliferation of the cells. SP1 is a zinc finger transcription factor [57] and its elevated expression observed in the current study is expected to up-regulate the expression level of one of its key target gene FN1 which further helps to activate PI3k/Akt signaling pathway and also contributes to the high proliferation of the cells with a different mechanism. In addition, the up-regulation of BIRC5 inhibits the

apoptosis of such highly proliferative cells and ultimately results in the formation of a tumor. The brown upward arrow show the up-regulation.

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