

Identification of CCNB2 expression in triple-negative breast cancer based on bioinformatics results

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Research

Keywords: Triple-negative breast cancer, Bioinformatics, Differentially expressed genes, Immunohistochemistry, CCNB2, Prognosis

Posted Date: May 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-506326/v1>

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Abstract

Background

The current epidemiology shows that the incidence of breast cancer is increasing year by year and tends to be younger. Triple-negative breast cancer is the most malignant of breast cancer subtypes. The application of bioinformatics in tumor research is becoming more and more extensive. This study provided research ideas and basis for exploring the potential targets of gene therapy for triple-negative breast cancer (TNBC).

Methods

We analyzed three gene expression profiles (GSE64790, GSE62931, GSE38959) selected from the Gene Expression Omnibus (GEO) database. The GEO2R online analysis tool was used to screen for differentially expressed genes (DEGs) between TNBC and normal tissues. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were applied to identify the pathways and functional annotation of DEGs. Protein–protein interaction network of these DEGs were visualized by the Metascape gene-list analysis tool so that we could find the protein complex containing the core genes. Subsequently, we investigated the transcriptional data of the core genes in patients with breast cancer from the OncoPrint database. Moreover, the online Kaplan–Meier plotter survival analysis tool was used to evaluate the prognostic value of core genes expression in TNBC patients. Finally, immunohistochemistry (IHC) was used to evaluate the expression level and subcellular localization of CCNB2 on TNBC tissues.

Results

A total of 66 DEGs were identified, including 33 up-regulated genes and 33 down-regulated genes. Among them, a potential protein complex containing five core genes was screened out. The high expression of these core genes was correlated to the poor prognosis of patients suffering breast cancer, especially the overexpression of CCNB2. CCNB2 protein positively expressed in the cytoplasm, and its expression in triple-negative breast cancer tissues was significantly higher than that in adjacent tissues.

Conclusions

CCNB2 may play a crucial role in the development of TNBC and has the potential as a prognostic biomarker of TNBC.

Background

Breast cancer is the most common malignant tumor in women [1]. According to reports, the incidence of breast cancer has increased year by year in the past few decades, and its prevalence has become the first among female malignancies. The treatment for breast cancer has been explored for many years, coupled with advances in diagnosis, though, around 400,000 patients die from breast cancer worldwide each year [2, 3]. Triple-negative breast cancer (TNBC), a special clinical subtype of breast cancer, which is characterized by negative expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2), accounts for 12–17% of all invasive breast cancers [4]. Since TNBC demonstrates highly malignant features as strong invasiveness, early metastasis, frequent recurrence, short survival and so on, it has attracted widespread attention [5].

Owing to the abundance of molecular information from several public databases, such as Gene Expression Omnibus (GEO: <https://www.ncbi.nlm.nih.gov/geo/>) database and ONCOMINE (<https://www.oncomine.org>) [6], the mechanism of cancer progression can be researched through unparalleled methods. Additionally, the differentially expressed genes (DEGs) between cancer and normal tissues were screened out based on bioinformatics analysis, and the identification of these oncogenes or tumor suppressor genes, leading to prediction of potential biomarkers for related cancers, may provide new therapeutic strategies. Among various bioinformatics methods, DEGs analysis is a widely used independent tool to study gene up-regulation and down-regulation [7].

In this study, we tried to explore novel therapeutic targets for improving overall prognosis of patients with TNBC. Firstly, we analyzed the gene expression profiling data downloaded from the GEO database to detect the DEGs between TNBC and normal breast tissues. Then, GO and KEGG pathway enrichment analysis were manipulated for the screened DEGs. Furthermore, we established a protein–protein

interaction (PPI) network to identify the potential protein complex containing core genes related to TNBC. we investigated the transcriptional and survival data of core genes using the Oncomine database and the Kaplan-Meier Plotter. Finally, we used the IHC method to detect the expression and localization of CCNB2 for verification.

Materials And Methods

Source of data

The GEO database stores microarray data, next-generation sequencing data, and other high-throughput sequencing data. In addition, it is currently the largest and most comprehensive public gene expression database storing various forms of data such as genomic DNA, gene expression profile data, protein molecular data, etc. In this study, the GEO database was used to download the original data including the expression profile of triple-negative breast cancer and non-triple-negative breast cancer. A total of 4,442 results for 'TNBC' in the GEO DataSets Database were retrieved. Through careful reading and comparison, we finally selected three TNBC-related gene expression profiles (GSE64790, GSE62931, GSE38959). All data resources were freely available on the Internet, and this study did not involve human and animal experiments.

Screening of DEGs

In each profile, the data was divided into TNBC samples and non-TNBC samples. The powerful online analysis tool GEO2R on the GEO website (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>) was used to perform analysis on these data. The adjusted P -value <0.05 and $|\log_{2}FC| \geq 1.5$ were defined as meaningful differentially expressed genes. We then performed statistical analysis on the three data sets, and used the venn graph network tool (<http://www.interactivenn.net/index.html>) to find out the intersecting part so as to enhance the reliability of DEGs' selection.

GO/KEGG enrichment analysis

Functional analysis of large gene lists derived from emerging high-throughput genomics, proteomics, and bioinformatics scanning methods (such as expression microarrays, promoter microarrays, ChIP-on-CHIPs, etc.) is a challenging Task. DAVID (the Database for Annotation, Visualization and Integrated Discovery) biological information database online analysis tool is an efficient database with powerful analysis functions, from which we can simultaneously perform GO function annotation and KEGG pathway enrichment analysis. In GO analysis, $P < 0.01$ and count ≥ 10 were defined as statistically significant. KEGG pathway analysis took $P < 0.01$ as statistically significant.

PPI network construction and DisGeNET analysis

Metascape (<http://metascape.org/>) is a free, well-maintained and powerful gene annotation analysis tool that can visualize enrichment results as bar maps, heat maps or networks [8,9]. In this study, Metascape was applied to analyze the pathway and process enrichment of differential genes and their adjacent genes which were significantly related to the differential genes. The Gene Ontology (GO) terms for cellular component (CC), biological process (BP), and molecular function (MF) categories, were obtained from the Metascape online tool. Simultaneously, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were also gotten by this method. Only the conditions of P -value < 0.01 , enrichment factor > 1.5 , and minimum count of 3 were considered as meaningful. Select a subset of rich terms and draw a network plot to further determine the relationship of the terms. The following databases were used for protein-protein interaction enrichment analysis: BioGrid+, InWeb_IM+ and OmniPath+. Moreover, the Molecular Complex Detection (MCODE) algorithm was used to identify tightly connected network components. Metascape also provides the DisGeNET analysis, a versatile platform containing one of the largest publicly available collections of genes and variants associated to human diseases. This makes it easier to study and predict disease-related genes.

Oncomine database analysis

ONCOMINE (www.oncomine.org) is an online tumor data analysis platform that facilitates research by providing genome-wide expression analysis [10]. Using the Oncomine database, we identified the mRNA expression levels of the SKA1, CCNB2, CENPF, CENPA and BIRC5 genes in various cancers.

The Kaplan–Meier plotter analysis

Kaplan-Meier plotter (www.kmplot.com) is an online database containing survival information and microarray gene expression data. These data come from GEO, TCGA and the Cancer Biomedical Information Grid. It contains gene expression data and overall survival information of 1402 clinical breast cancer patients. In this study, Kaplan-Meier plotter was used to evaluate the prognostic value of mRNA expression of core gene members. By dividing the patient samples into two groups according to the median expression (high expression and low expression) and using the Kaplan-Meier survival plot for evaluation, the relapse-free survival (RFS) of TNBC patients was determined, and the risk ratio was 95% confidence interval and log Rank *p*-value.

Immunohistochemistry (IHC)

TNBC specimens were taken from the Pathology Department of the First Affiliated Hospital of Bengbu Medical College and confirmed by pathological diagnosis. The expression of CCNB2 protein was detected by immunohistochemical staining. The tissue sections were routinely deparaffinized and dehydrated, and the endogenous peroxidase activity was inactivated with 3% H₂O₂ methanol. The primary antibody, secondary antibody and tertiary antibody were added in turn. The tissue sections were stained with aminobenzidine (DAB) and counterstained with hematoxylin. The positive cells were judged by the obvious brown granules in the cell membrane or cytoplasm.

Results

Identification of DEGs

Logging in to the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>) to search for TNBC, a total of 4,442 results were displayed. Through our careful reading and comparison, we selected 3 TNBC-related gene expression profiles (GSE64790, GSE62931, GSE38959). GSE64790 contains 3 TNBC samples and 3 normal samples, GSE62931 has 47 TNBC samples and 53 normal samples, GSE38959 contains 30 TNBC samples and 13 normal samples (Table 1). All results were obtained by comparing the differential expression of each gene in normal tissue samples and TNBC samples with the help of GEO2R online data analysis tool. We identified the $P < 0.05$, $|\log_2FC| \geq 1.5$ as the standard. The results showed that there were 600 DEGs in GSE62931, containing 269 up-regulated genes, and 331 down-regulated genes; GSE38959 had 1550 DEGs, up-regulated 1010, down-regulated 540; In gene chip GSE64790, a total of 660 DEGs were detected, including 186 up-regulated genes and 374 down-regulated genes. Subsequently, the venn graph network drawing tool was used to obtain their intersection to ensure the reliability of results. Finally, there were 66 DEGs, 33 of which were both up-regulated and down-regulated genes.

Table 1 Statistics of the three microarray databases selected from the GEO database

Dataset ID	TNBC	Normal	Total Number
GSE64790	3	3	6
GSE62931	47	53	100
GSE38959	30	13	43

Abbreviations: GEO, Gene Expression Omnibus; TNBC, triple-negative breast cancer.

Functional enrichment analyses

Enter the differential gene list into the DAVID database for GO and KEGG pathway enrichment analysis. The enriched GO terms were divided into CC, BP, and MF. In GO analysis, the results revealed that the DEGs were mainly enriched in BP term including mitosis, cell proliferation and so on; in CC term, they were mainly enriched in nucleus and nucleoplasm; MF term showed that DEGs were mainly enriched in protein binding. In addition, KEGG pathway analysis results displayed that DEGs were mainly enriched in pathways related to Progesterone-mediated oocyte maturation, oocyte meiosis and cell cycle (Table 2).

Table 2 Significantly enriched GO terms and KEGG pathways

Category	Term	Description	Count	P-value
BP term	GO:0007067	Mitotic nuclear division	10	1.4E-7
BP term	GO:0008283	Cell proliferation	10	3.6E-6
CC term	GO:0005634	Nucleus	32	4.3E-4
CC term	GO:0005654	Nucleoplasm	20	1.4E-3
MF term	GO:0005515	Protein binding	42	4.8E-3
KEGG pathway	hsa04914	Progesterone-mediated oocyte maturation	4	3.7E-3
KEGG pathway	hsa04114	Oocyte meiosis	4	7.3E-3
KEGG pathway	hsa04110	Cell cycle	4	9.8E-3

Abbreviations: BP, biological process; CC, cellular component; MF, molecular function. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

PPI network construction and DisGeNET analysis

Metascape analysis results showed that the DEGs and their neighboring genes were mainly enriched in cell division, mitotic nuclear division and cell cycle phase transition (Figure 2: A and B). Meanwhile, the website used the MCODE algorithm to cluster the PPI network to identify the subnetwork, that was, the potential protein complexes containing five core genes (CCNB2, BIRC5, CENPA, CENPF, SKA1) (Figure 2: C and D). Herein, Metascape also provided a DisGeNET analysis. Through quality control and association analysis, these DEGs were significantly related to the occurrence of the invasive carcinoma of breast, carcinoma of male breast, malignant neoplasm of male breast and other diseases (Figure 2: E).

ONCOMINE Analysis

The graph reveals the numbers of datasets with statistically significant mRNA over-expression (red) or down-regulated expression (blue) of the target gene. The threshold was designed with following parameters: p-value of 1E-3 and fold change of 1.5. We compared the transcription levels of core genes in cancers with those in normal samples by using ONCOMINE databases (Figure 3 and Figure 4). ONCOMING analysis showed that the mRNA expression of BIRC5, CCNB2, CENPA, CENPF and SKA1 was upregulated in patients with breast cancer. In Curtis's dataset, BIRC5 was upregulated in medullary breast carcinoma compared with that in the normal samples, with a fold change of 6.014 and *p*-value of 9.13E-17. In Turashvili's dataset, CCNB2 was overexpressed in Invasive ductal breast carcinoma with a fold change of 4.653 and *p*-value of 6.05E-6. In Curtis's dataset, CENPA was overexpressed in Invasive ductal breast carcinoma compared with that in the normal samples, with a fold change of 2.183 and *p*-value of 1.27E-115. In the TCGA dataset, the transcription level of CENPF was significantly higher in patients with Invasive lobular breast carcinoma than that in the normal specimens, with a fold change of 6.980 and *p*-value of 1.31E-21. In Turashvili's dataset, the fold change of mRNA expression of SKA1 in Invasive ductal breast carcinoma was 7.501 and *p*-value of 2.48E-6.

The Kaplan–Meier Plotter Analysis

Five genes (CCNB2, CENPF, SKA1, CENPA and BIRC5) were found to be associated with relapse-free survival (RFS) in TNBC through the Kaplan–Meier Plotter analysis. Patients with a higher level of them had worse RFS compared to those with lower levels. Among them, the overexpression of CCNB2 was the most unfavorable prognostic factor of relapse-free survival in TNBC patients (HR=1.98; 95% CI: 1.28–3.06; P=0.0018; n=255) in accordance with the lowest logrank *p* value. To date, the TNBC cases in the database are still insufficient for overall survival analysis (Figure 5).

Immunohistochemistry staining

The results of immunohistochemical staining showed that the CCNB2 protein expression in TNBC tissues was significantly higher than that in adjacent tissues. And the protein was located in the cytoplasm and displayed mainly brown-yellow granular staining in TNBC tissues.

Discussion

Breast cancer is a malignant tumor that occurs in the epithelial tissue of the breast [11]. Breast cancer cells are easy to fall off due to loose connections and free cancer cells are easily spread in the blood or lymph fluid. Thereafter, these cancer cells disperse in the whole body, form metastasis, and endanger life. All of these make the breast cancer a serious threat to women's health. In particular, triple-negative breast cancer (TNBC) is a unique subtype of breast cancer. Its hormone receptors (ER: estrogen receptor, PR: progesterone receptor) and HER-2 receptors are not expressed, which makes clinical targeted therapy and endocrine therapy unsatisfactory [12]. Chemotherapy is currently the main method of adjuvant treatment for patients with TNBC. However, the efficacy is limited compared with comprehensive therapy, especially in patients who show resistance to chemotherapy drugs [13]. Therefore, it is urgent to dig out reliable biomarkers and effective targets of TNBC to improve the prognosis of patients.

In our study, gene and protein expression analysis based on the publicly available bioinformatics databases was performed to screen out the potential key genes related to TNBC. DEGs between TNBC tissues and normal human breast tissues were identified by gene expression profiling data from the GEO database. Then, we identified 66 DEGs including 33 upregulated DEGs and 33 downregulated DEGs. These DEGs were correlated to the GO terms (Mitotic nuclear division, Cell proliferation, nucleus, nucleoplasm, Protein binding) and KEGG terms such as Progesterone-mediated oocyte maturation, Oocyte meiosis, Cell cycle. Hereafter, we constructed a PPI network to seek out the potential protein complex and obtain five core genes (BIRC5, CCNB2, CENPA, CENPF, SKA1). GEO analysis results showed that the five core genes were all up-regulated in TNBC, and the ONCOMINE database proved that they were significantly overexpressed in breast cancer compared to normal tissues. Finally, the Kaplan–Meier plotter was performed to estimate the relationship between the expression of core genes and prognosis of TNBC patients. Among the five core genes, we found that CCNB2 presented closest relevance to TNBC, indicating poor prognosis. And the cytoplasmic localization of the CCNB2 protein was detected by immunohistochemistry staining.

Cyclin family proteins which include cyclin B1 (CCNB1) and cyclin B2 (CCNB2), regulate the activities of cyclin-dependent kinases (CDKs) and different cyclins act in specific phases of the cell cycle [14–16]. CCNB2, as one of the cyclin family proteins, plays an important role in the regulation of the cell cycle. During the interphase and mitosis, CCNB2 is located in the Golgi apparatus and participates in its disassembly [17]. According to previous reports, CCNB2 usually triggers the process of G2/M transition by activating CDK1 kinase and the downregulation of CCNB2 helps to inhibit cell proliferation and promote cell cycle arrest in the G2/M phase [18–20]. Relevant studies have shown that metformin can down-regulate the expression of CCNB2 to enhance the rate of apoptosis and cell cycle arrest [21]. High level of CCNB2 protein is positively correlated with the degree of undifferentiation, tumor size, lymph node metastasis, distant metastasis and clinical stage status. In the past few years, the overexpression of CCNB2 in tumor tissues has been shown to be an unfavorable prognostic biomarker in many human cancers, for example, gastric cancer [22], breast cancer [23], pituitary adenoma [24], nasopharyngeal carcinoma [25], adrenocortical carcinoma [26] and so on. Our research found that compared with normal breast tissues, CCNB2 was upregulated in TNBC, and the overexpression of CCNB2 was associated with unfavorable relapse-free survival of TNBC patients. Herein, CCNB2 shows the potential as a therapeutic target and prognostic factor for TNBC.

In our study, BIRC5, SKA1, CENPA and CENPF also showed high expression in breast cancer compared to normal breast tissues, and CENPA, CENPF, SKA1 were significantly correlated with poor recurrence-free survival of TNBC (logrank $p < 0.05$). However, the role of these genes in TNBC is still unclear, and more experiments are needed for further research.

Conclusions

In summary, our bioinformatics analysis detected 66 DEGs from GEO database and clustered the functions and pathways of these genes. Transcriptional and prognostic analysis on the genes in the protein complex identified by the PPI network were then performed. We found that CCNB2 protein expression was significantly increased in TNBC tissues and associated with the malignant status and prognosis of TNBC patients. With no doubt, the clinical value of CCNB2 has yet to be confirmed by further research. Still, CCNB2 exhibits a promising prospect as a potential therapeutic target for TNBC.

Declarations

Competing interest

The authors declare that they have no competing interests in this work.

Authors' contributions

JC analyzed the data, drafted the initial manuscript and revised it for important content. ZF and NL led the conception and review of the paper. SS, RL, RM, XF, YH made contributions to the collection of relevant literature. All authors have read and approved the manuscript.

Funding

This work was supported by a grant from the Postgraduate Scientific Research Innovation Program of Bengbu Medical College (Byycx20003, Byycx1966), Anhui Provincial Natural Science Foundation (No.1908085MH275), the Key Foundation of Excellent Young Talents in Colleges and Universities in Anhui Province (no. gxyqZD2016161).

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Figures

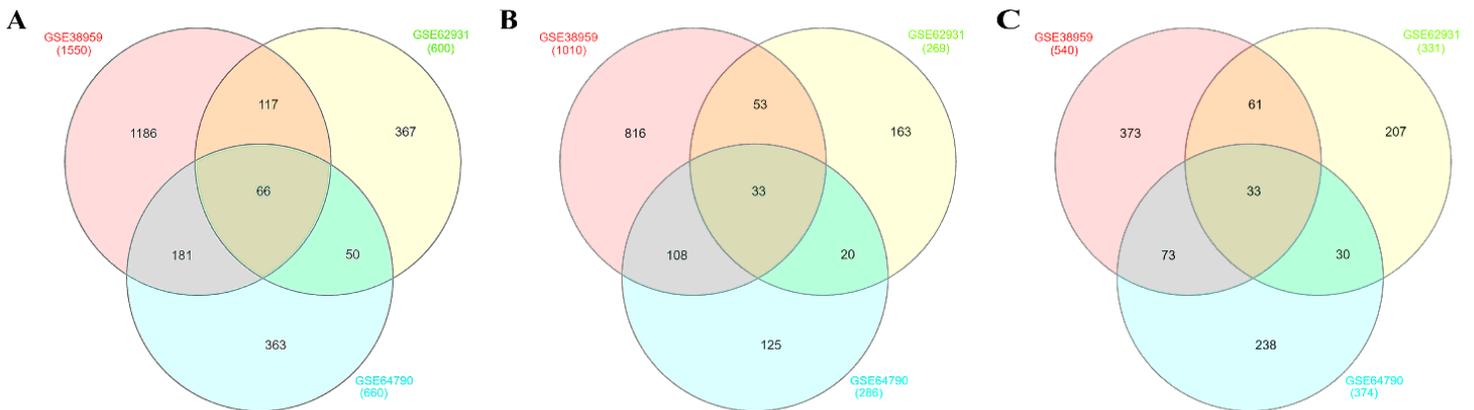


Figure 1

Venn diagram of DEGs common to the three GEO datasets. Notes: (A) DEGs. (B) Upregulated genes. (C) Downregulated genes. Abbreviations: DEG, differentially expressed gene; GEO, Gene Expression Omnibus.

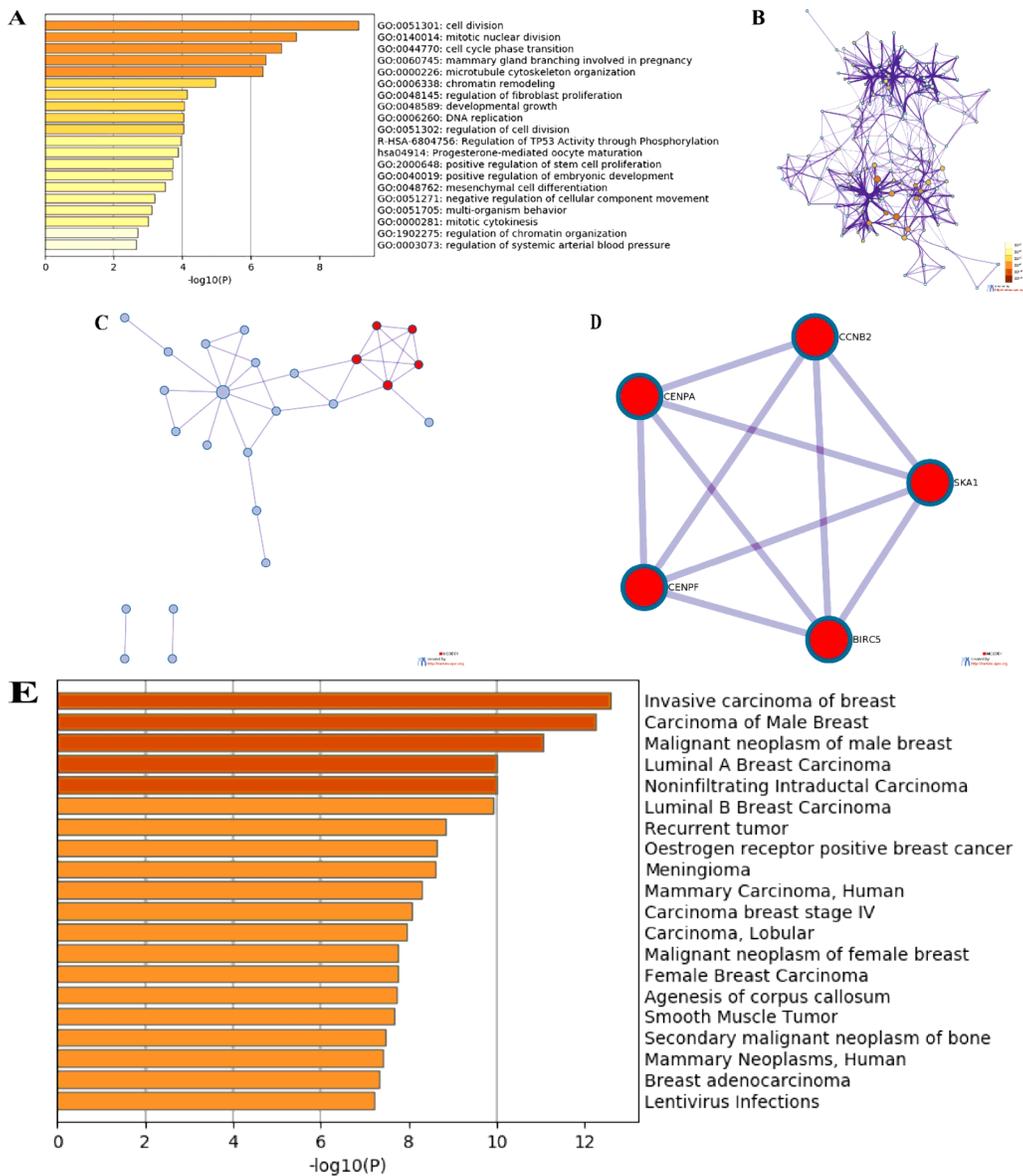


Figure 2

The enrichment analysis of DEGs and neighboring genes in TNBC (Metascape). (A) Heatmap of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enriched terms colored by p-values. (B) Network of GO and KEGG enriched terms colored by p-value, where terms containing more genes tend to have a more significant p-value. (C) Protein-protein interaction (PPI) network. (D) Five most significant MCODE components from the PPI network. (E) DisGeNET analysis of the DEGs.

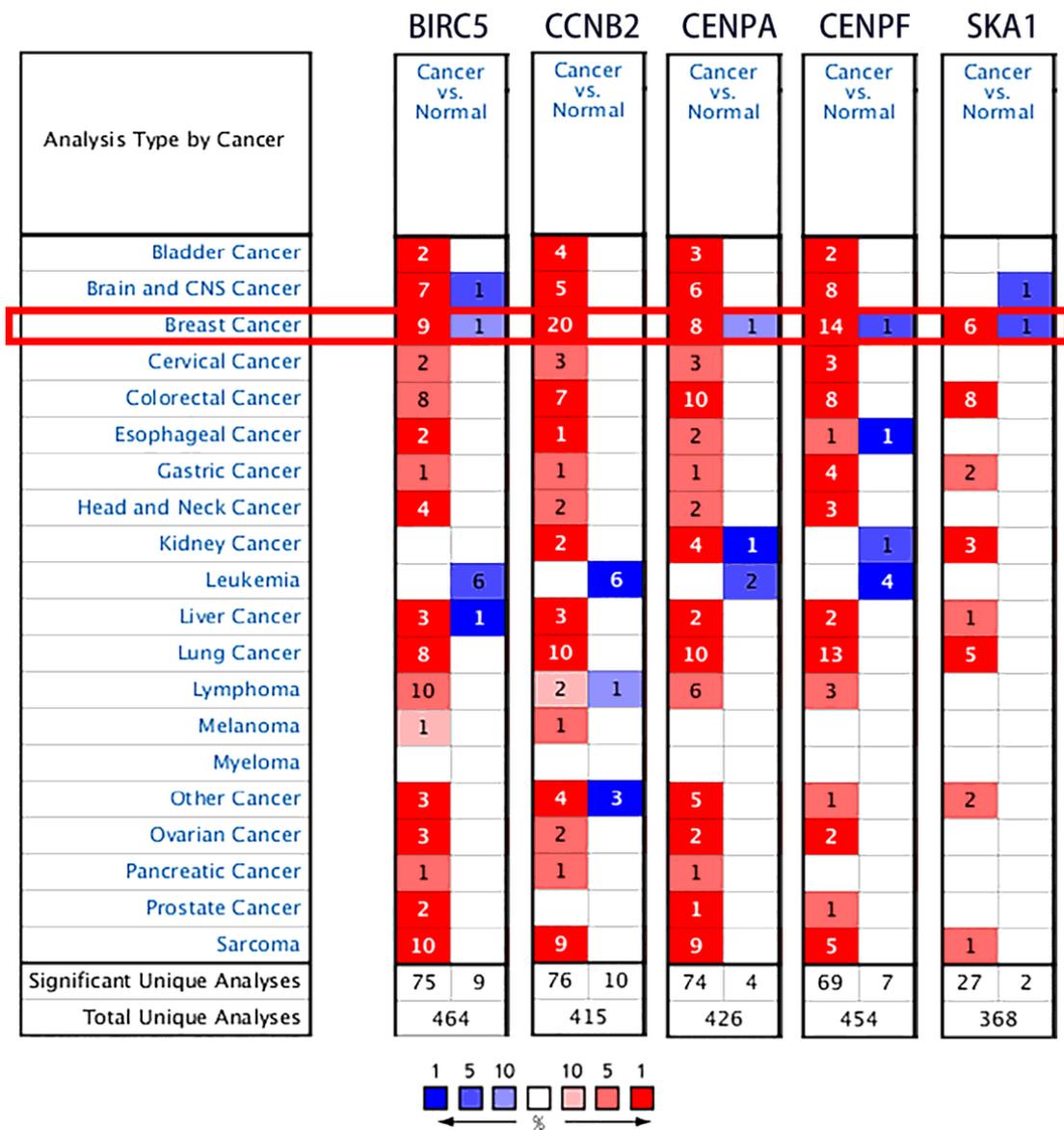


Figure 3

The transcription levels of core genes in different types of cancers (ONCOMINE).

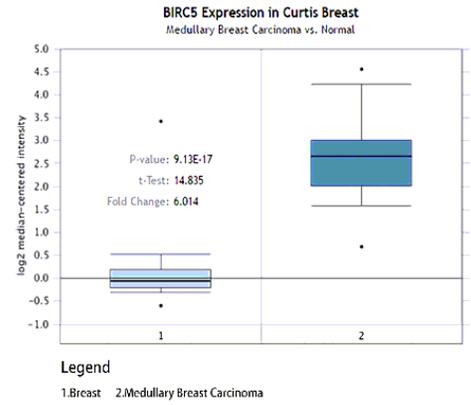
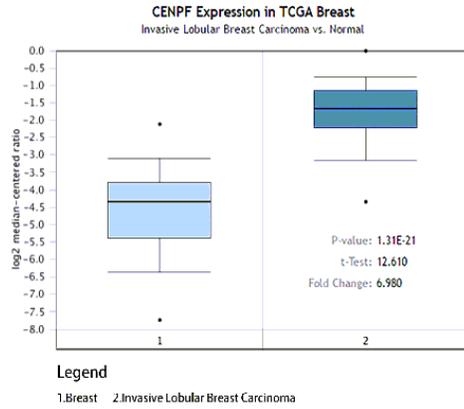
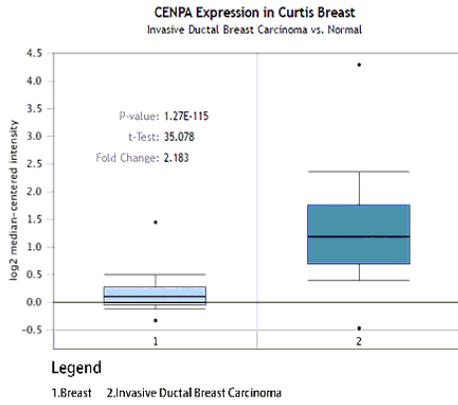
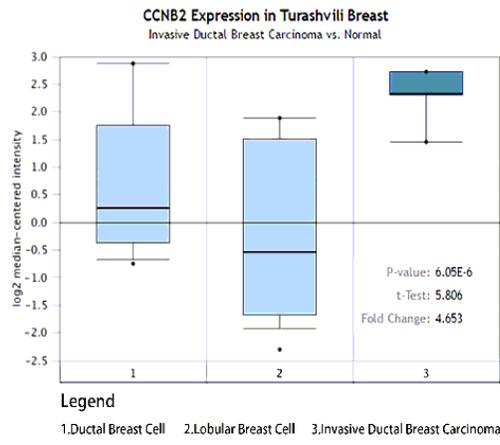
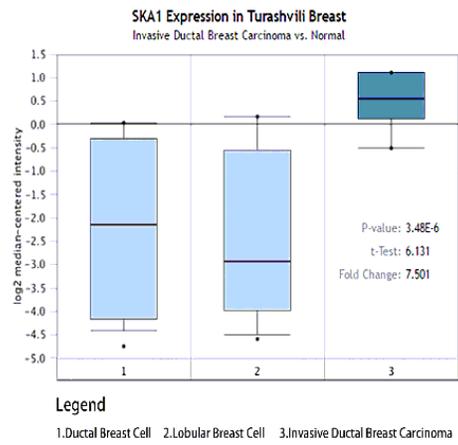


Figure 4

Box plot of the core genes in breast cancer and normal cell

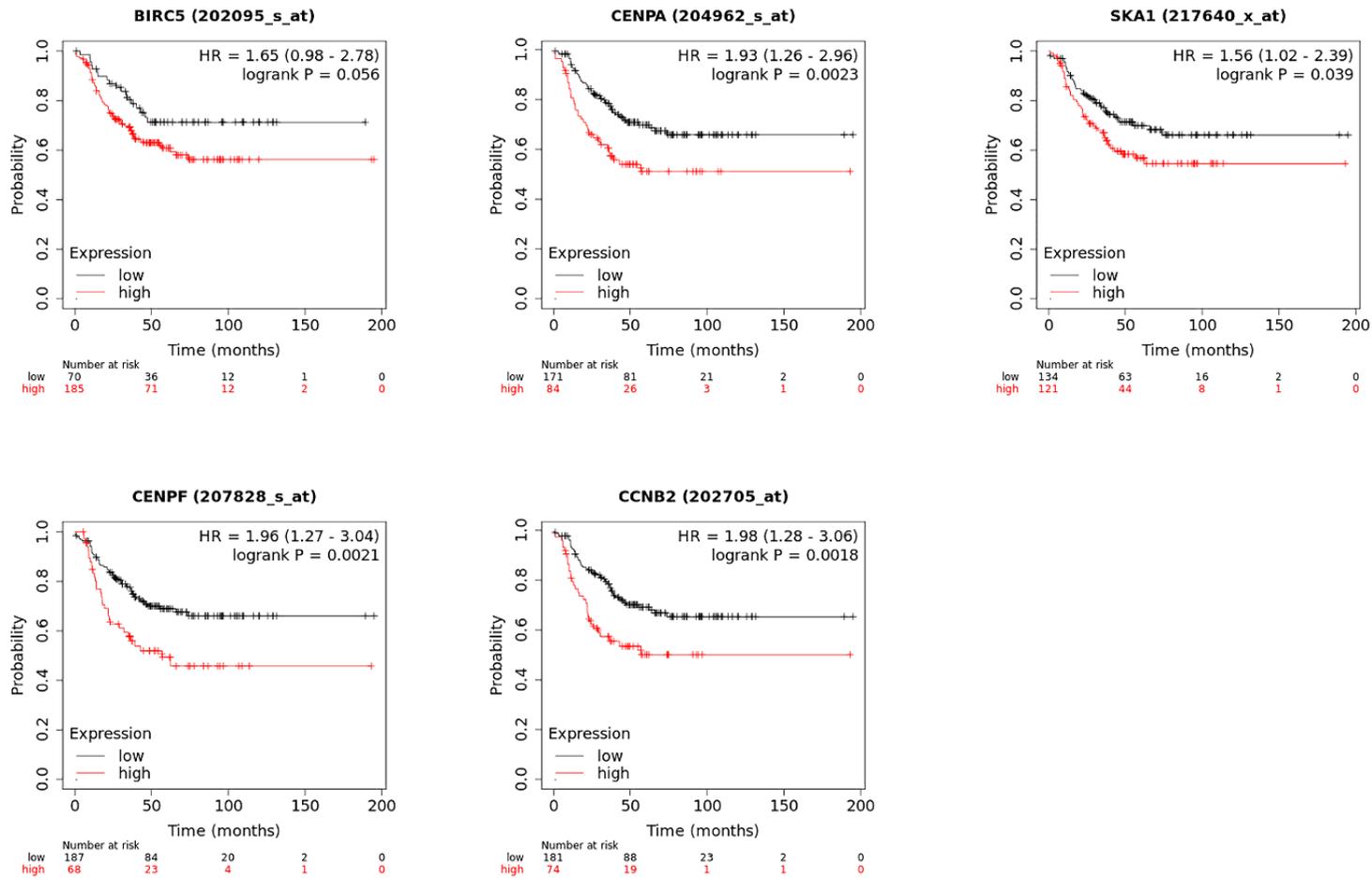


Figure 5

The prognostic value of mRNA level of core genes in TNBC patients (Kaplan–Meier plotter).

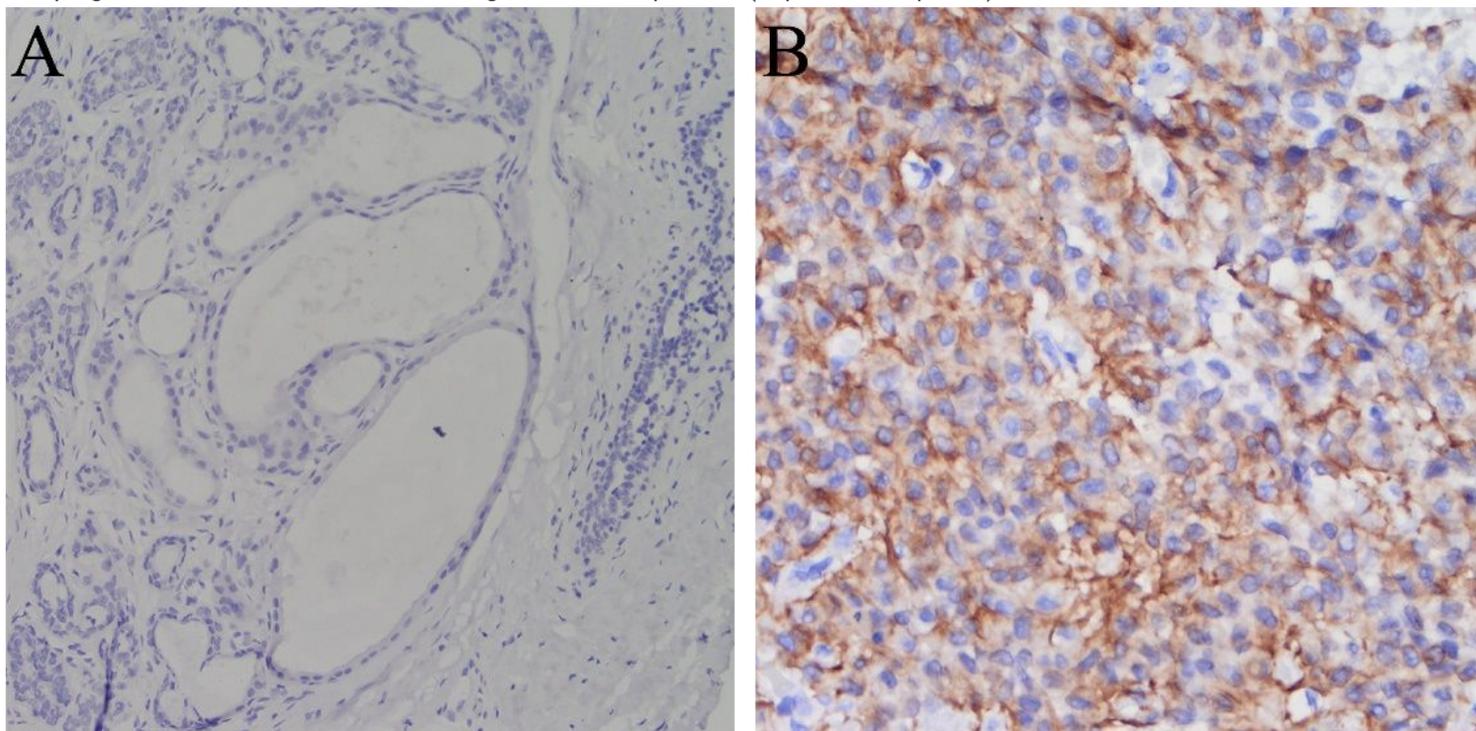


Figure 6

Immunohistochemical detection of CCNB2 expression. (A) CCNB2 protein was lowly expressed in adjacent tissues. (B) CCNB2 protein was highly expressed in TNBC tissues.