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Research article

Downregulation of CDC14B in 5218 breast cancer patients: A novel prognosticator for triple-negative breast cancer

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Abstract: Breast cancer is the most common female malignancy worldwide and the prognosis of triple-negative breast cancer (TNBC) and advanced breast cancer patients is unsatisfying. The exploration of novel prognostic indicators and appropriate targets is crucial for improving the treatment outcomes of breast cancer patients. The cell division cycle protein 14B (CDC14B) is known for its roles in cell cycle control, but its expression status and molecular function in breast cancer is unknown. This study explores the expression patterns and clinical values of CDC14B in breast cancer tissues. For this research, the authors downloaded gene microarrays and RNA sequencing datasets to examine the expression levels of CDC14B in 5218 breast cancer tissues, comparing them to the expression levels in 1176 normal breast tissues. The relationships between CDC14B and clinicopathologic characteristics of breast cancer were also addressed. The mutation conditions of CDC14B were then clarified using cBioPortal. Finally, differentially expressed genes and co-expressed genes related to CDC14B were filtered using the Limma-Voom package. These genes were intersected to conduct functional annotations and to construct a protein-protein interaction network. It was observed that CDC14B was significantly downregulated in breast cancer tissues but not in normal breast tissues (standardized mean difference= −1.17 [−1.50–−0.85], area under the curve = 0.88). In addition, CDC14B downregulation was correlated with the poor prognosis of TNBC patients (hazard ratios < 1; $p < 0.05$). Amplification was detected to be the most frequent alteration of CDC14B. The presence of this alteration forecasted unfavourable overall

survival outcomes in breast cancer patients ($p < 0.05$). Dysregulated genes that co-expressed with CDC14B were pivotal in cell cycle (namely mitotic-nuclear division and DNA packaging complex) and cancer-related signaling pathways (namely the peroxisome proliferators activated receptor [PPAR] signalling pathway and the AMP-activated protein kinase [AMPK] signalling pathway). Moreover, the genes ADIPOQ and CCNE2 were identified as two promising prognostic factors in breast cancer. In summary, CDC14B was downregulated in breast cancer tissue and may be a promising hallmark in TNBC patients. The dysregulated genes co-expressed with CDC14B may play an important role in the development of breast cancer through PPAR and AMPK signalling pathways.

Keywords: CDC14B; breast cancer; microarray; RNA sequencing; clinical utility; genetic mutation; signaling pathway

Abbreviations: ER: estrogen receptor; PR: progesterone receptor; HER-2: human epidermal growth factor 2; TNBC: triple-negative breast cancer; CDC14B: cell division cycle protein 14B; SMD: standardized mean difference; AUC: areas under the curve; LR+: positive likelihood ratio; LR−: negative likelihood ratio; OS: overall survival; RFS: relapse-free survival; DMFS: distal-metastasis-free survival; HR: hazard ratio; DCIS: ductal carcinoma in situ; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; DO: Disease Ontology; SPSS: Statistical Product and Service Solutions; AMPK: AMP-activated protein kinase; PPAR: peroxisome proliferators-activated receptor

1. Introduction

Breast cancer, one of the most common female malignancies worldwide, has seen a rapid rise in its morbidity rate in recent years, and now also exhibits a lower-age tendency [1–3]. Invasive ductal carcinoma, or non-specific invasive carcinoma, accounts for most of the histological types of breast cancer [4]. Considering the clinical management and prognosis of breast cancer patients, the intrinsic molecular classifications of breast cancer have been universally used to guide the treatment and prognosis prediction in patients with breast cancer. These classifications are based on the differential expression statuses of the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER-2), and other novel hallmarks [5]. Although the pathogenesis of breast cancer has yet to be clarified, there has been a consensus that many complex factors are responsible for the formation of breast cancer, such as dietary habits, estrogen imbalance, environment, and pathogenic genetic alterations [6–8]. In addition, the natural course of breast cancer—onset, progression, and prognosis—is the complex result of intertwining intrinsic and external risk factors [9–11]. Recently, many studies have been illuminating the molecular pathological events occurring in breast cancer tissue, enriching the understanding of the mechanisms underlying breast cancer [12–14]. However, further studies are required to uncover the fundamental causes of breast cancer and to provide more information for the development of new therapeutic strategies.

Furthermore, the appearance of precision medicine offers more opportunities for the treatment

of breast cancer [15]. The selection of clinical treatment protocols for advanced breast cancer patients is based on various individual indices, such as age, hormone receptor and HER-2 statuses, disease-free intervals, and clinical complications [16–18]. For example, it is often recommended that locally advanced breast cancer patients with positive HER-2 receive multidisciplinary treatment including systematic treatment, surgery, and radiotherapy [19]. In addition, anthracyclines- and taxus-based neoadjuvant treatments are preferred for patients diagnosed with locally advanced triple-negative breast cancer (TNBC) [20]. Despite the progress in treating advanced breast cancer patients, it is still difficult to achieve radical treatment, and the long-term prognosis of patients with advanced breast cancer remains unfavourable. Therefore, the exploration for more novel prognostic indicators and appropriate targets is crucial to improve clinical decisions and the treatments and prognoses of the breast cancer patients.

Cell division cycle protein 14B (CDC14B, or Cdc14B1, Cdc14B2, CDC14B3, and hCDC14B), located on human chromosome 9q22.32–q22.33, belongs to the dual-specificity tyrosine phosphatase, which possesses a highly conserved sequence [21]. Previous studies have mainly demonstrated the roles that CDC14B plays in controlling the cell cycle and participating in DNA damage repair. It was reported that CDC14B could counteract the activity of CDK through dephosphorylation, thus promoting the exit of cell division and mitosis. These processes are dependent on another protein phosphatase, protein phosphatase 2A regulatory subunit B55α [22,23]. CDC14B was illustrated to be involved in DNA-double-strand break repair by targeting one of its downstream coding genes, CDH1 (E-cadherin) [24]. In addition, it was found that CDC14B dephosphorylated and regulated the activity of the renowned tumor suppressing gene—p53 [25]. Studies of the relationship between CDC14B and tumors are scarce to date. Although in vitro and in vivo studies illuminated that CDC14B may be an oncogene and may participate in the Ras-MAP kinase pathway, CDC14B was reported to be downregulated in hepatocellular carcinoma, central nervous system tumors, and clear cell renal cell carcinoma, indicating the complicated characteristics of CDC14B in malignancies [26–28]. However, the associations between CDC14B expression and breast cancer have not been reported, and the gene's roles in breast cancer are elusive.

In this research, the authors compare the expression levels of CDC14B in 5218 breast cancer tissues to those of 1176 normal breast tissues. The study also aims to establish the relationships between CDC14B and clinicopathologic characteristics, especially the prognosis of CDC14B in breast cancer patients. In addition, another objective of this study is to unveil the pathogenic alterations of CDC14B in 5604 breast cancer patients with different histological features. This research focuses on the mechanisms of CDC14B to provide more useful insights into the initiation, development, treatment, and prognosis of breast cancer.

2. Materials and methods

2.1. Expression of CDC14B mRNA in breast cancer tissues based on in silico analysis

Microarrays and RNA sequencing data sets were initially screened and downloaded from Gene Expression Omnibus, The Cancer Genome Atlas (TCGA), ArrayExpress, Genotype-Tissue Expression (GTEx), and other scientific literature. The retrieval keywords were as follows, 'breast

cancer', 'breast carcinoma', 'breast tumor', 'mammary cancer', 'luminal A', 'luminal B', 'HER-2+', 'basal like', and 'triple-negative breast cancer'. The standards for inclusion were as follows: (1) the expression data were derived from *Homo sapiens* rather than cell lines or animals, such as *Mus musculus*; (2) the data sets contained the expression values of CDC14B; (3) the patients with breast cancer did not receive chemotherapy, radiotherapy or any other treatment; (4) the data were obtained from breast cancer tissues rather than stromal cells or interstitial tissues; and (5) the data were derived from primary breast cancers rather than metastatic cancers. The exclusion standards were as follows: (1) the data sets were duplicated; (2) the expression data were not available from the aforementioned databases.

After acquiring the eligible microarrays and sequencing data, the researchers checked and normalized the data using the $\log_2(x+1)$ scale. Subsequently, the data sets that belonged to the same platforms were merged using the dplyr package in R v3.6.1. Since experimental error could be caused by the varying instruments and reagents among the different studies, Limma-Voom and sva packages were used to remove batch effects. In addition, principal component analysis (PCA) was conducted using FactoMineR and scatterplot3d packages. The standardized mean difference (SMD) of CDC14B mRNA expression levels in breast cancer tissue, in comparison with those in normal breast tissues, was calculated using STATA v12.0. Subgroup analysis was performed according to the molecular pathological classifications of breast cancer. Moreover, the SMD of CDC14B mRNA expression levels in TNBC tissues in comparison with those of non-TNBC tissues was computed. The expression patterns of CDC14B in breast cancer and in 32 other types of tumors were then compared using RNA sequencing data from TCGA and GTEx provided by using Gene Expression Profiling Interactive Analysis (http://gepia.cancer-pku.cn/). The Human Protein Atlas (THPA, https://www.proteinatlas.org/) was used to research the protein levels of CDC14B in these tumors.

2.2. Implications of CDC14B mRNA in the prognosis of breast cancer patients

The associations between CDC14B mRNA levels and clinicopathological features of breast cancer were determined through independent samples t-tests or variance analyses. For each data set, areas under the receiver operating characteristic curves (AUCs) were used to appraise the discriminatory abilities of CDC14B mRNA in breast cancer and normal breast tissues; AUC values between 0.5–0.7, 0.7–0.9, and 0.9–1.0 presented weak, moderate, and strong discriminatory abilities, respectively. In addition, the area under the summary receiver operating characteristic curve, sensitivity, specificity, positive likelihood ratio (LR+), and negative likelihood ratio (LR−), were used to determine the overall capability of CDC14B in differentiating between breast cancer tissues and normal breast tissues. Moreover, the prognostic value of CDC14B in breast cancer was comprehensively evaluated by performing overall survival (OS; n = 1402), relapse-free survival (RFS, n = 3951), and distal-metastasis-free survival (DMFS; n = 1746) analyses on the integrated cohorts of breast cancer from the Kaplan-Meier plotter database (https://kmplot.com/), which overcame the limitation of the small sample size of breast cancer cohorts. To verify the role of CDC14B in the prognosis of breast cancer patients, hazard ratios (HRs) of OS, DMFS, RFS, and disease-free survival (DFS) were calculated and collected from the Long-term Outcome and Gene Expression Profiling Database of pan-cancers (http://bioinfo.henu.edu.cn/Index.html) [29] and

PrognoScan (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html) [30]. Afterwards, HRs were pooled using STATA v12.0.

2.3. Genetic alterations of CDC14B in breast cancer and the gene's prognostic value

Chromothripsis Explorer (http://compbio.med.harvard.edu/chromothripsis) was used to learn the special mutations appearing in the chromosomes of breast cancer patients, such as chromothripsis, loss-of-heterozygosity, and structure mutations [31]. The results were visualized as circos plots. In addition, the chromothripsis regions of ductal carcinoma in situ (DCIS) and lobular breast carcinoma were compared. Based on cBioPortal (https://www.cbioportal.org), the genetic alteration patterns of CDC14B in different histological types of breast cancer were comprehensively analysed by integrating the five following studies: breast cancer (alpelisib plus AI, nature cancer 2020; n = 141), breast cancer (MSK, cancer cell 2018; n = 1918), breast cancer (METABRIC, Nature 2012 and nature communication 2016; n = 2509), breast cancer (SMC 2018; n = 187), breast invasive carcinoma (TCGA, firehose legacy; n = 1108). Moreover, the associations between CDC14B alterations and clinicopathological parameters of patients with breast cancer were explored using Chi-squared or Kruskal Wallis tests. OS and DFS analyses were applied to estimate the prognostic value of the genetic alterations of CDC14B in breast cancer patients.

2.4. Acquisition and annotations of differentially expressed genes and co-expressed genes of CDC14B in breast cancer

To clarify the potential molecular mechanism of CDC14B in breast cancer, the differentially expressed genes ($|\log_2 \text{FoldChange}| \geq 1$) in breast cancer and co-expressed genes of CDC14B in the gene regulatory network (|spearman's correlation| ≥ 0.5) were obtained by using the Limma-Voom package. For quality control, genes could only be preserved if they appeared in five or more different platform matrices at the same time. Dysregulated genes co-expressed with CDC14B were identified by intersecting the differentially expressed genes and co-expressed genes of CDC14B in breast cancer, the functions of which were subsequently analysed via Gene Ontology (GO), the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, Disease Ontology (DO), and Reactome analyses. Moreover, protein-to-protein interaction (PPI) network was constructed by using STRINGdb package in R v3.6.1 to observe the protein-protein interactions of differentially expressed genes that co-expressed with CDC14B in the gene regulatory network of breast cancer. Key genes in the regulatory network were determined by calculating connected degrees in Cytoscape v3.6.1. Finally, a prognostic model was established based on these key genes by performing a Cox regression analysis. The expression statuses and discriminatory ability of prognostic factors were then compared to CDC14B.

2.5. Statistical analysis

All the statistical calculations were completed using R v3.6.1, Statistical Product and Service Solutions (SPSS) v22.0, OrigrinPro 2020, and STATA v12.0. The expression values of CDC14B in

breast cancer tissues and normal breast tissues were calculated and recorded as mean and standard deviation. Unpaired independent-samples t-tests were used to measure the differential expression of CDC14B in breast cancer tissues and normal breast tissues. When calculating SMD and pooling HRs, a random-effect model was preferred on the condition that the heterogeneity appeared obvious ($I^2 > 50\%$, $p < 0.05$), whereas a fixed-effect model was chosen when the heterogeneity was insignificant ($I^2 < 50\%$, $p > 0.05$). If a large proportion of heterogeneity was observed, a sensitivity analysis was conducted to identify the source of heterogeneity. Data sets that might contribute to the high level of heterogeneity were then eliminated. The Begg's test was used to detect publication bias. If significant publication bias existed, a method of trim and fill would be considered to maintain the stability of results. Moreover, rates of true positive, true negative, false positive, and false negative were calculated to draw a summary receiver operating characteristic curve. A *p-value* (or *p*. adjusted) < 0.05 was recorded as statistically significant.

3. Results

3.1. Decreased expression levels of CDC14B in breast cancer patients

As presented in Table 1 and Figure 1, a total of 22 platform matrices, integrating 61 independent data sets with a total of 5218 breast cancer tissue samples and 1,176 normal breast tissue samples, were eligible for SMD calculation. The 3D scatter plots of the PCA showed that batch effects between the different data sets were removed (Figure 2A). When compared to normal breast tissues, mRNA expression levels of CDC14B were significantly decreased in breast cancer tissues in the following 17 platform matrices: GPL96, GPL570, GPL887, GPL1390, GPL3676, GPL5175, GPL6244, GPL6480, GPL6848, GPL8264, GPL8269, GPL8274, GPL13158, GPL13607, GPL13648, GPL17586, and TCGA-GTEx (Figure 2B). Since a high degree of heterogeneity existed, a random-effect model was selected when calculating SMD. The result of the sensitivity analysis showed that the significance of the SMD value did not change when omitting each included dataset (Figure S1A). There was no significant publication bias in these studies (Figure S1B). An SMD forest plot enables the authors to have an overview of the overall expression trends of CDC14B, which confirmed the decreased mRNA expression levels of CDC14B in breast cancer tissues in comparison with normal breast tissues (SMD = −1.17, 95% confidence interval [CI]: −1.50–−0.85; Figure 3A). To better comprehend the dysregulation patterns of CDC14B in breast cancer tissues, subgroup analysis was executed. Although the downregulation of CDC14B was universal in breast cancer with different PAM50 (Prediction Analysis of Microarray 50) characteristics (Figure S2A–B, S23 A–B), it was found that the decreased levels of CDC14B in the luminal B subtype of breast cancer were more significant in comparison with TNBC ($SMD_{\text{luminal B v.s. normal}} = -2.14$, 95% CI: −2.58–−1.70; $SMD_{\text{TNBC v.s. normal}} = -1.18$, 95% CI: −1.60–−0.76, *with no overlap of the 95% CIs*; Figure 4). Moreover, among the breast cancers, there was a decreased trend in CDC14B expression in non-TNBC tissues compared to that in TNBC tissues (Figure S4). An SMD value based on the expression level of CDC14B in TNBC compared to non-TNBC confirmed the differentially expressed status of CDC14B in breast cancer with different PAM50 subtypes (SMD = −0.48, 95% CI: −0.64–−0.33; Figure 3B). However, no consistent expression trend was observed among different types of cancers (Figure S5A), suggesting that CDC14B may have a distinct function

in specific tumor types. Similar distinguishable expression levels of CDC14B protein were noted in pan-cancers. For breast cancer, two among ten (20%) cases showed moderately positive staining (Figure S5B) and the positive ratio of CDC14B seemed to be lower than that in other malignancies including melanoma, skin cancer and pancreatic cancer, which may further confirm the downregulation of CDC14B in breast cancer tissues.

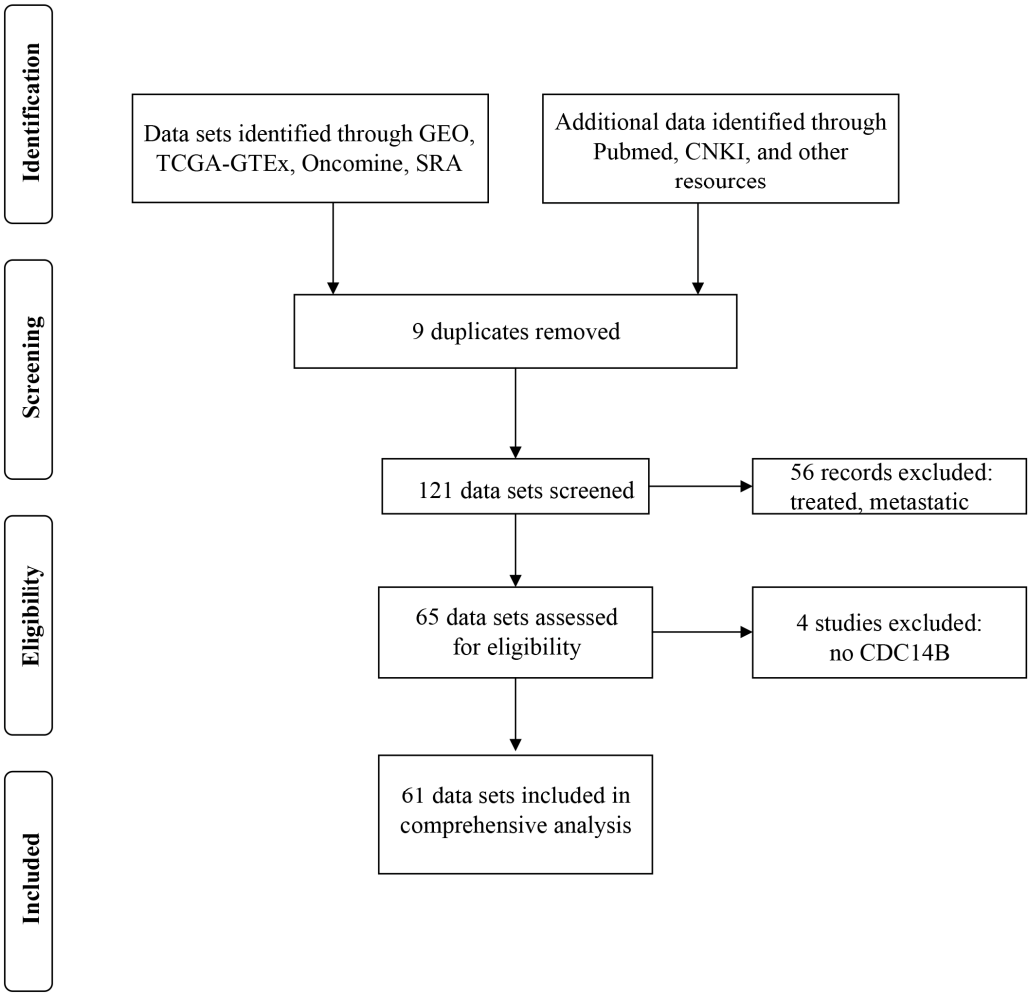
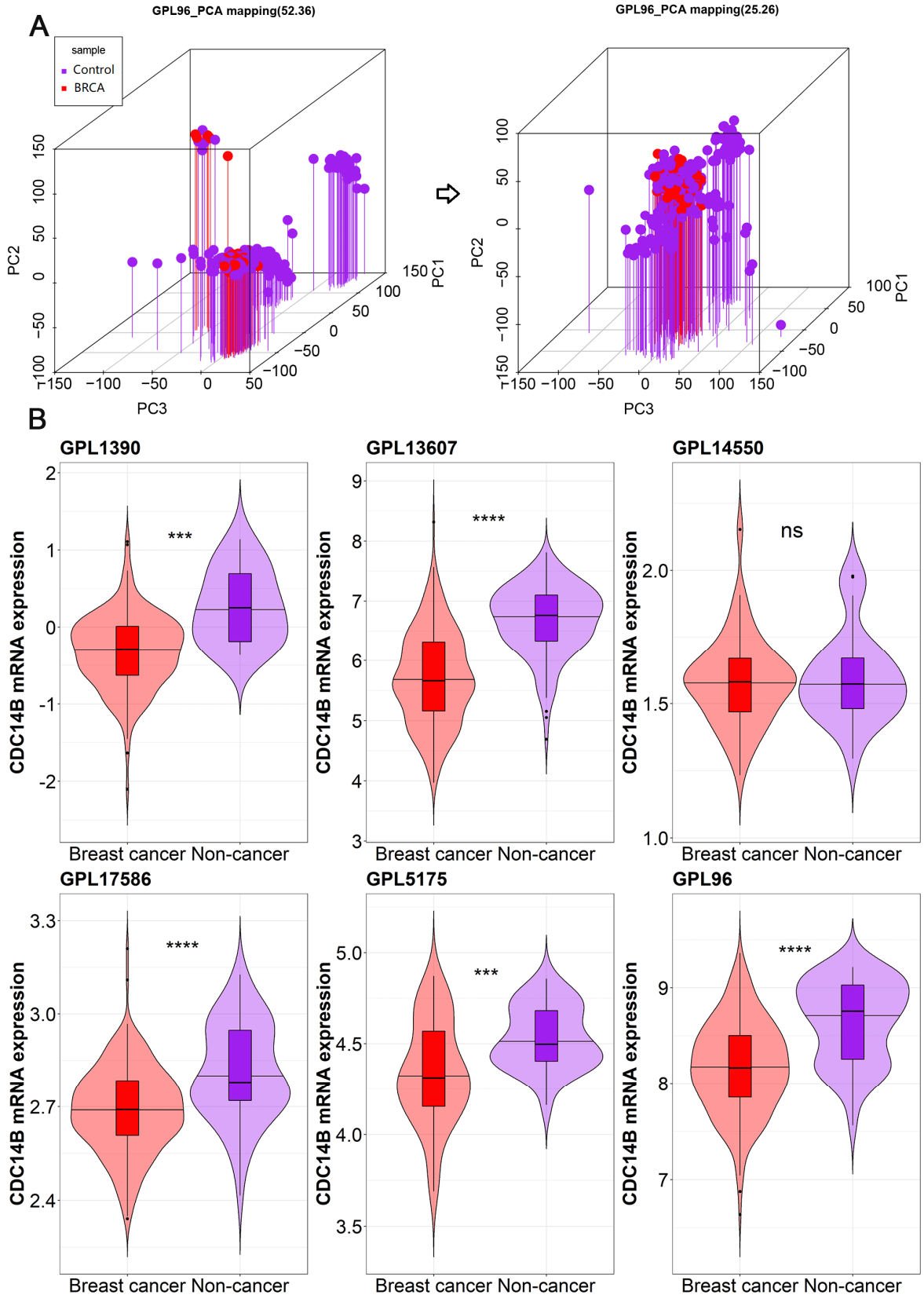


Figure 1. Flow diagram of data sets inclusion.

Table 1. Detailed information of 22 integrated expression matrices.

Accession	Breast cancer			normal			*p*	TP	FP	FN	TN	Precision	Recall	AUC
	N1	M1	SD1	N2	M2	SD2								
GPL1390	171	−0.3148	0.4806	13	0.2714	0.4778	0.0000	151	5	20	8	0.9679	0.8830	0.7990
GPL13607	250	5.2601	1.7232	151	6.3706	1.5188	0.0000	189	29	61	122	0.8670	0.7560	0.8380
GPL14550	48	1.5829	0.1659	24	1.5976	0.1708	0.7263	10	2	38	22	0.8333	0.2083	0.4900
GPL17586	214	2.6897	0.1371	57	2.8122	0.1566	0.0000	126	13	88	44	0.9065	0.5888	0.7210
GPL5175	45	4.3415	0.2827	28	4.5325	0.1722	0.0006	27	2	18	26	0.9310	0.6000	0.7250
GPL96	232	8.1733	0.4821	62	8.6477	0.4254	0.0000	186	23	46	39	0.8900	0.8017	0.7680
GPL13158	65	4.0799	0.1546	10	4.2269	0.1142	0.0052	51	2	14	8	0.9623	0.7846	0.8110
GPL571	28	3.8601	0.6377	5	4.3937	0.4612	0.0851	18	0	10	5	1.0000	0.6429	0.7710
GPL16025	6	9.8607	0.7544	3	9.7236	0.0279	0.6751	3	0	3	3	1.0000	0.5000	0.5000
GPL19612	3	4.9426	0.4759	3	4.7173	0.8103	0.6993	3	2	0	1	0.6000	1.0000	0.4440
GPL3676	150	−0.1384	0.6600	11	0.9060	0.7196	0.0000	116	1	34	10	0.9915	0.7733	0.8800
GPL13648	26	6.7894	0.1025	5	7.0805	0.0519	0.0000	26	0	0	5	1.0000	1.0000	1.0000
GPL570	1611	4.9365	0.4880	361	5.5123	0.4796	0.0000	1140	80	471	281	0.9344	0.7076	0.8000
GPL6244	294	6.4304	0.3918	91	6.9532	0.3764	0.0000	202	12	92	79	0.9439	0.6871	0.8380
GPL6480	225	2.7683	0.5123	42	3.2520	0.3791	0.0000	137	5	88	37	0.9648	0.6089	0.8080
GPL6848	109	−0.0665	0.3131	6	0.2677	0.3485	0.0127	92	2	17	4	0.9787	0.8440	0.7720
GPL8264	12	−0.6038	0.6200	9	0.1190	0.3827	0.0062	8	0	4	9	1.0000	0.6667	0.8430
GPL8269	226	0.7053	0.2390	41	1.0761	0.2155	0.0000	172	6	54	35	0.9663	0.7611	0.8720
GPL8274	26	−0.6476	0.5183	20	0.5437	0.3815	0.0000	22	0	4	20	1.0000	0.8462	0.9790
GPL887	304	−0.5590	0.4190	34	-0.1163	0.4431	0.0000	167	4	137	30	0.9766	0.5493	0.7650
TCGA-GTEx	1104	1.1145	0.4254	193	2.3084	0.4484	0.0000	1018	14	86	179	0.9864	0.9221	0.9740
GPL19956	69	6.5007	0.5131	7	6.8775	0.6823	0.0765	51	2	18	5	0.9623	0.7391	0.7040

Footnote: TP, true positive rate; FP, false positive rate; FN, false negative rate; TN, true negative rate.



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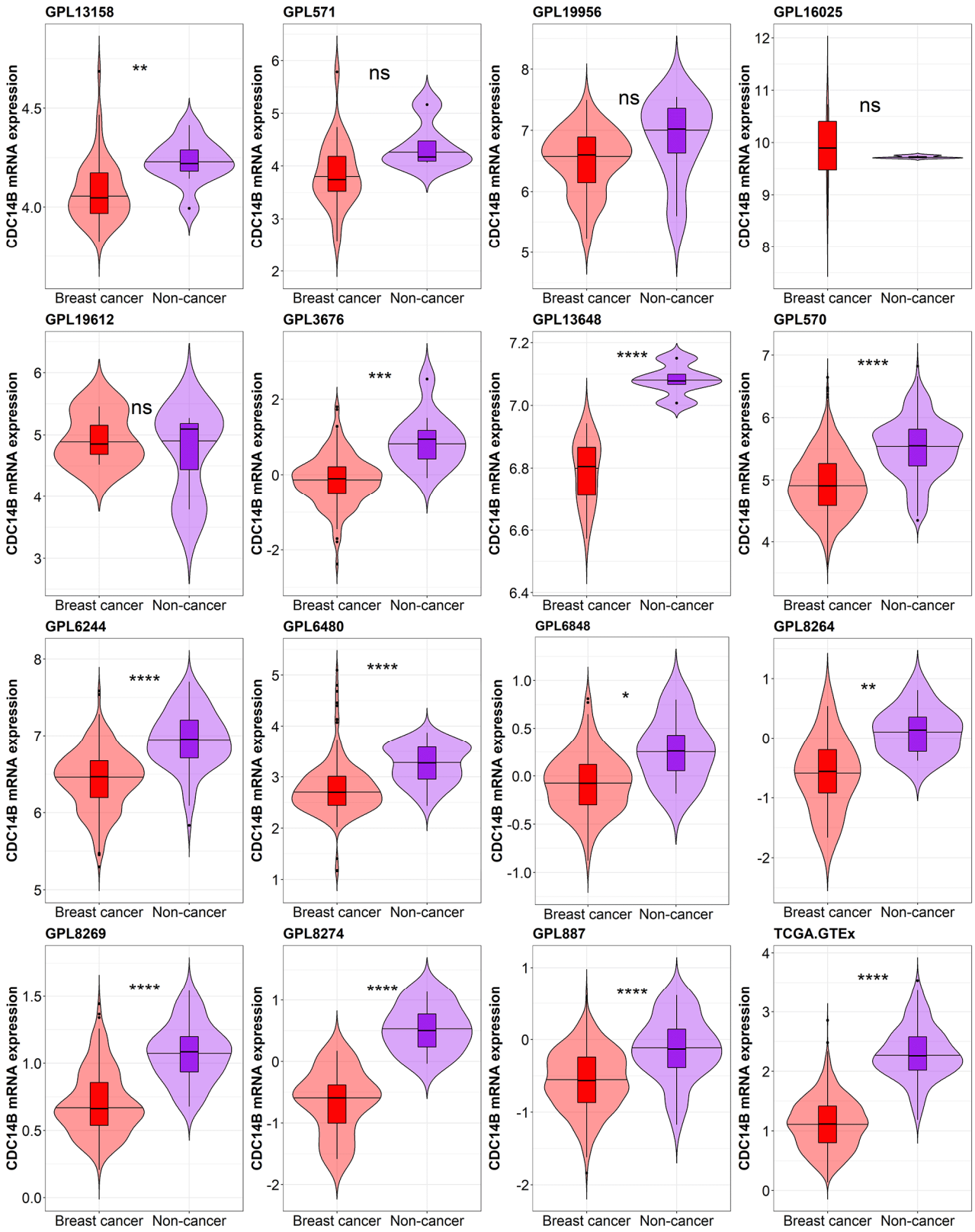

Figure 2. Expression levels of CDC14B in breast cancer. A: 3D principal component analysis presents that batch effects between different data sets are removed. B: CDC14B expression levels are significantly decreased in breast cancer tissues when compared to normal breast tissues.

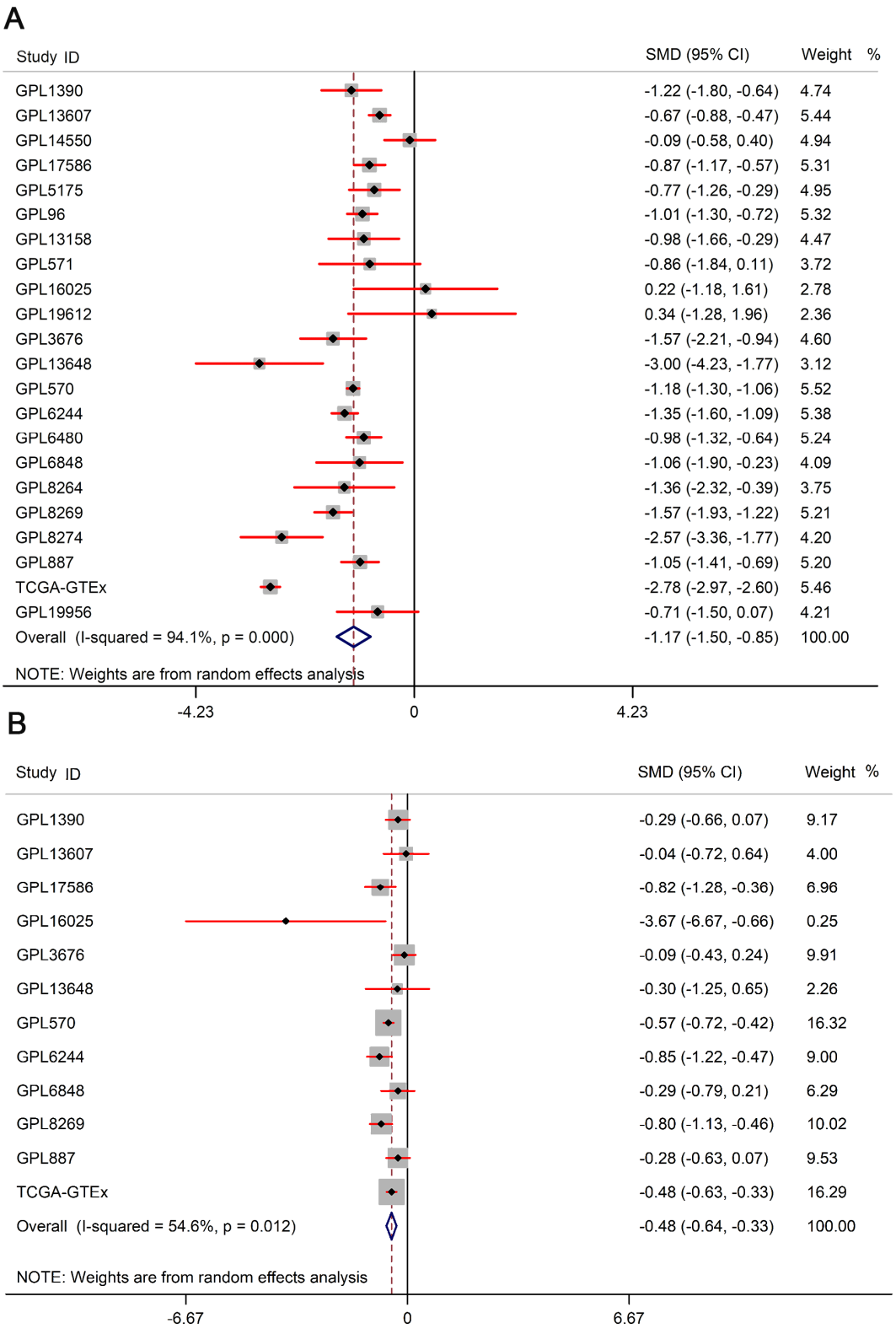

Figure 3. Overall expression trend of CDC14B mRNA in breast cancer. A: Compared to normal breast tissues, CDC14B mRNA is significantly downregulated in breast cancer tissues. B: Compared to triple-negative breast cancer (TNBC) tissues, CDC14B mRNA is significantly downregulated in non-TNBC tissues.

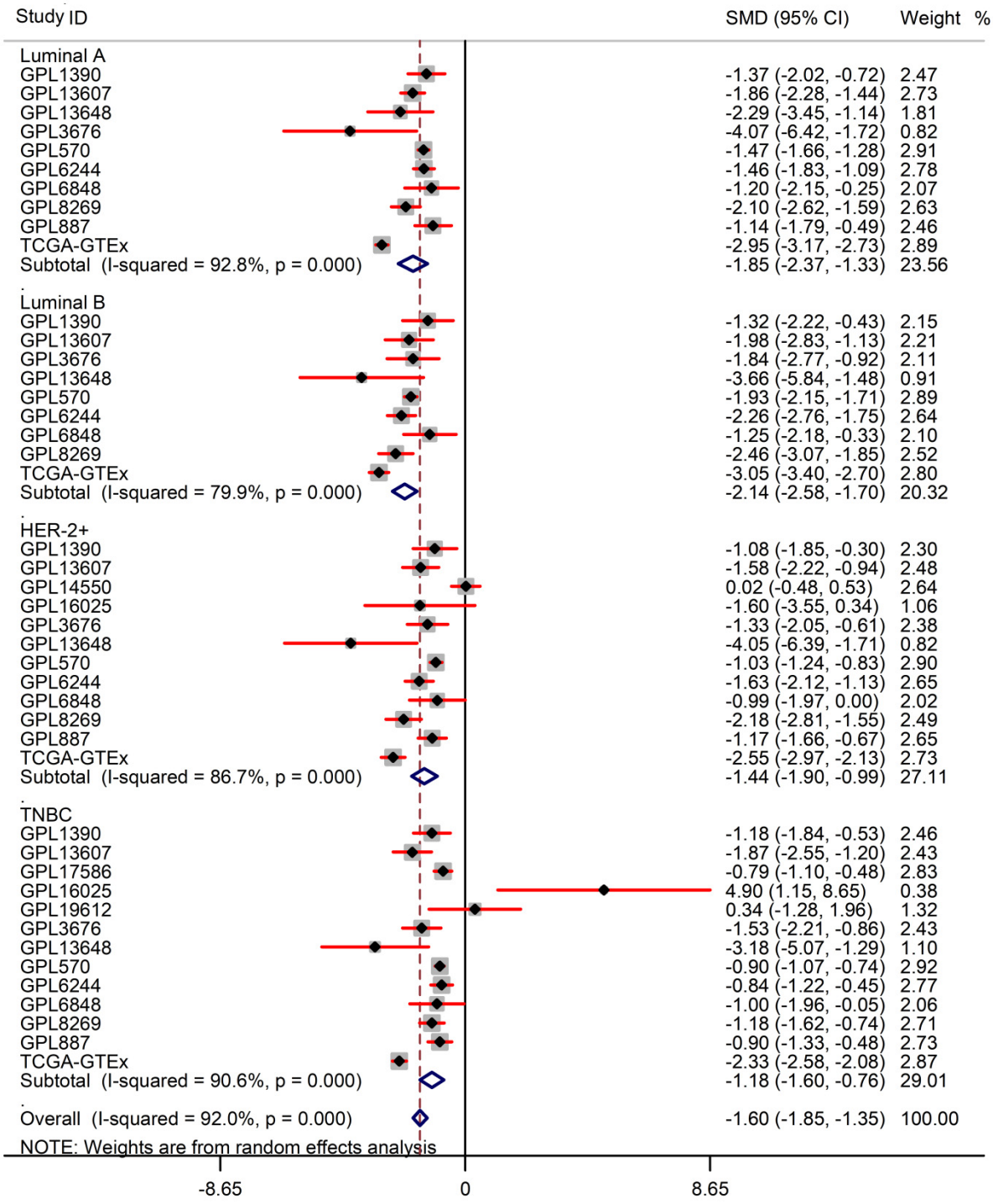

Figure 4. CDC14B mRNA expression levels in different molecular pathological classifications of breast cancer. CDC14B downregulation is a general phenomenon in luminal A, luminal B, HER-2+, and triple-negative breast cancer (TNBC). In addition, CDC14B mRNA level is significantly decreased in luminal B when compared to TNBC.

3.2. Discriminating ability of CDC14B between breast cancer tissues with different PAM50 characteristics

Given the stable downregulation status of CDC14B in breast cancer, the authors sought to evaluate the discriminatory ability of CDC14B between different PAM50 subtypes of breast cancer. First, this study appraised the discrimination capability of breast cancer tissue and normal breast tissue. Among the 17 platforms mentioned in the 'Decreased expression levels of CDC14B in breast cancer patients' section, CDC14B displayed a strong discriminatory ability in three platforms, as follows: GPL8274, GPL13648, and TCGA-GTEx. In addition, a moderate discriminatory capability was detected in the other 14 platforms: GPL96, GPL570, GPL887, GPL1390, GPL3676, GPL5175, GPL6244, GPL6480, GPL6848, GPL8264, GPL8269, GPL13158, GPL13607, and GPL17586 (Figure 5). The summary receiver operating characteristic curve exhibited a moderate discriminating ability between breast cancer and healthy breast tissues (AUC=0.88, 95% CI: 0.85–0.90), with a sensitivity of 0.74 and a specificity of 0.85 (Figure S6A). Moreover, the values of LR＋, LR−, and their ratios showed a relatively high degree of accuracy (Figure S6B–D). Furthermore, the discrimination ability of CDC14B between TNBC and non-TNBC was subsequently studied. Although the discriminatory ability of CDC14B in TNBC and non-TNBC was weak (AUC = 0.69, 95% CI: 0.64–0.73; Figure S7A), it was found that CDC14B had a moderate sensitivity when differentiating TNBC from non-TNBC (sensitivity = 0.73, 95% CI: 0.58–0.82; LR＋ = 1.68, 95% CI: 1.40–2.01; LR− = 0.49, 95% CI: 0.37–0.66; Figure S7B–D).

3.3. Prognostic ability of CDC14B in breast cancer patients

The associations between CDC14B mRNA expression levels and clinicopathological information from the TCGA breast-cancer cohort were not significant (Table S1), which may be due to a relatively small sample size. When analysing survival data by integrating different breast cancer cohorts, it was found that downregulation of CDC14B was obviously correlated with poor RFS condition (Figure 6A–C; HR = 0.88, $p < 0.05$; n = 3951). Furthermore, the prognostic values of CDC14B across different intrinsic subtypes in breast cancer were explored. It was observed that CDC14B was a prognostic factor in the OS, RFS, and DMFS of TNBC patients. The result of the combined HR estimates indicates that CDC14B may serve as a prognostic hallmark in breast cancer patients because it is significantly correlated with the DFS conditions of breast cancer patients (Figure S9A–D).

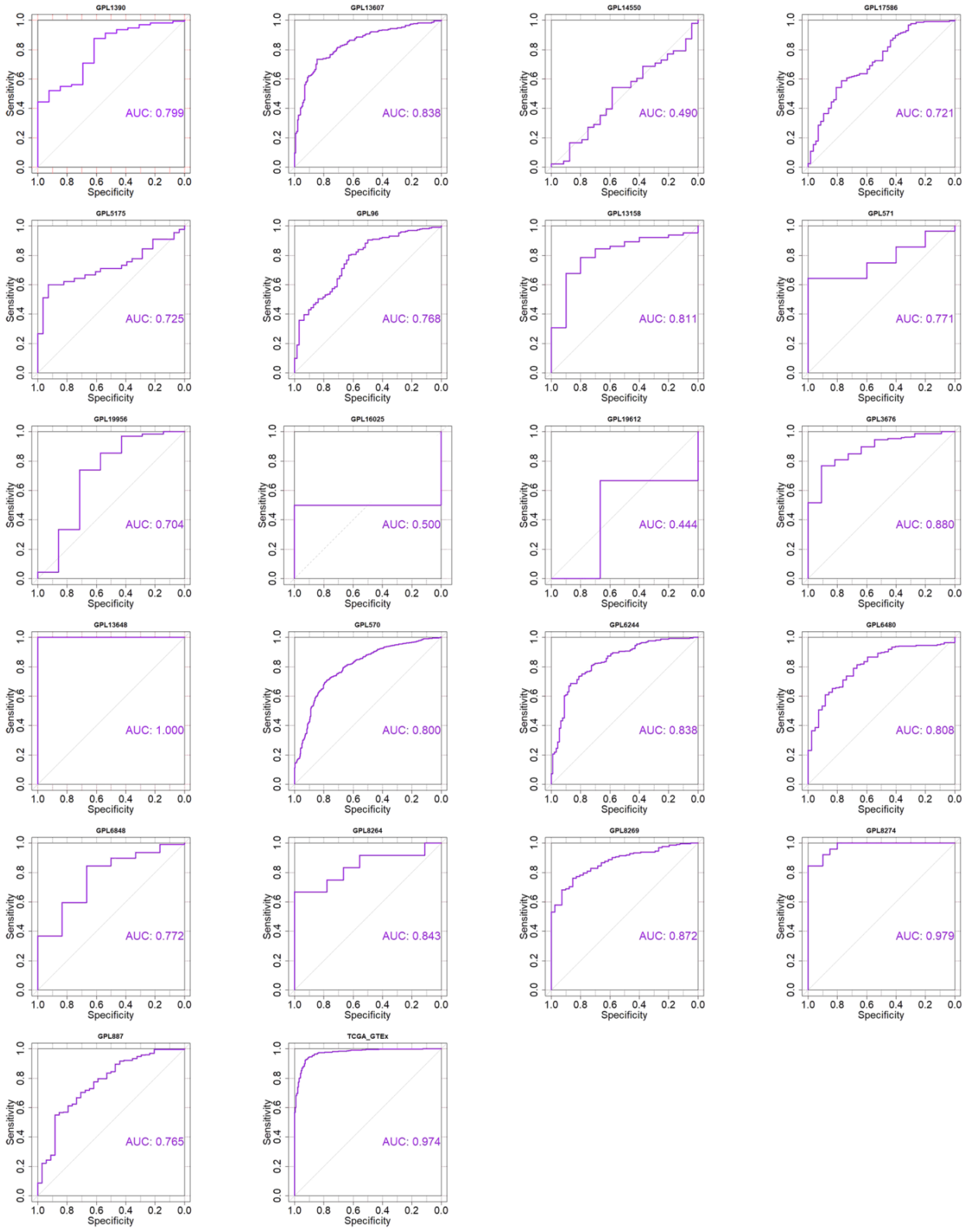

Figure 5. Discriminatory capacity of CDC14B between breast cancer and normal breast tissues. Receiver operating characteristic curves present a moderate capacity of CDC14B in distinguishing breast cancer patients from normal breast tissues.

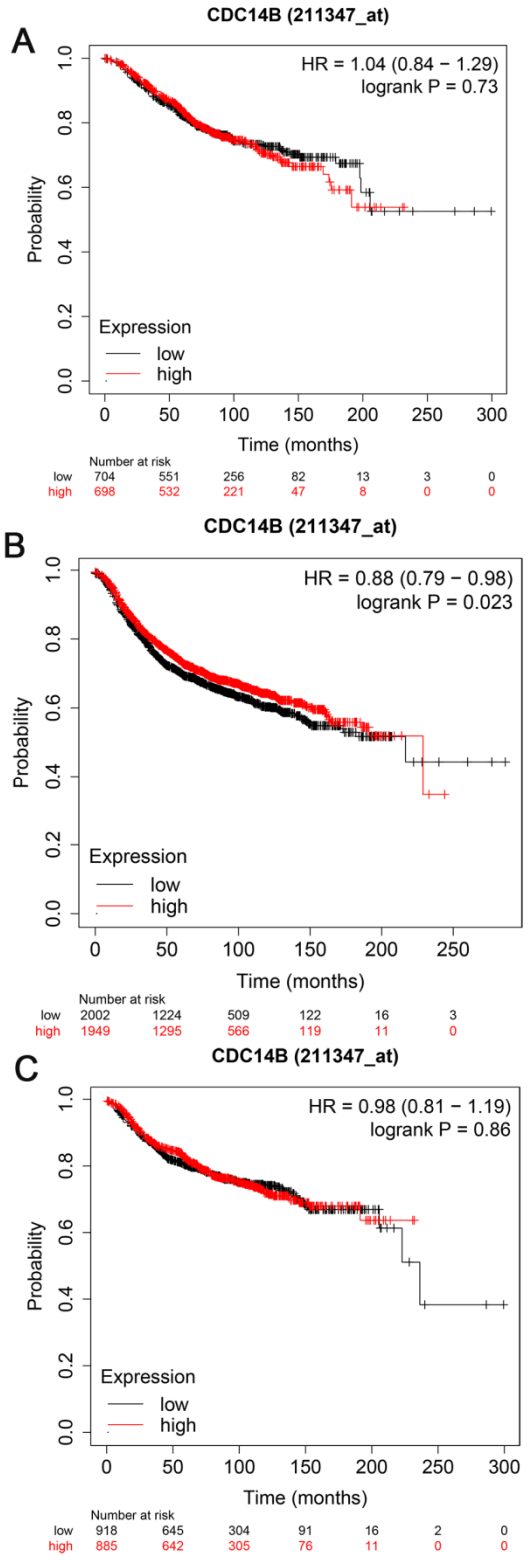

Figure 6. Prognostic value of CDC14B mRNA expression level in breast cancer achieved from Kaplan-Meier plotter. The prognostic value of CBC14B in breast cancer patients: overall survival (n = 1402, A); relapse-free survival (RFS, n = 3951, B); and distal-metastasis-free survival (n = 1746, C). Decreased CDC14B significantly correlated with poorer RFS condition.

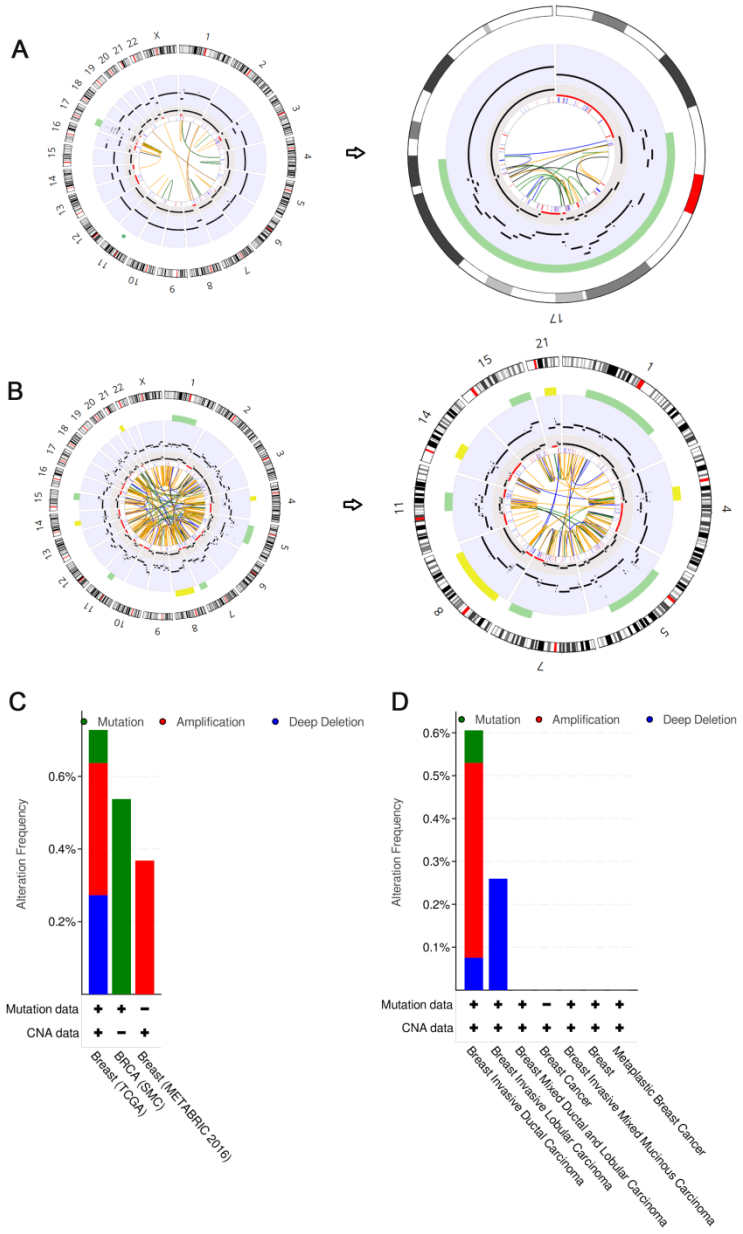

Figure 7. Mutation statuses of breast cancer patients. A–B: Circos plot based on special mutation types of ductal carcinoma in situ and lobular carcinoma, respectively. From outside to inside, track 1: hg19 cytobands; track 2: green dots present the intermutation distance for pathogenic indels; yellow and green bar presents high-confidence and low-confidence chromothripsis regions, respectively; track 3: black lines show the total copy number values; track 4: red regions present loss-of-heterozygosity; track 5. red and blue lines present oncogenes and tumor suppressors, respectively; track 6. blue, orange, black, and green lines stand for duplication-like structure variations, deletion-like structure variations, head-to-head inversions, and tail-to-tail inversions, respectively. C: Amplification and mutation account for most of the alterations in breast cancer patients; D: Amplification and mutation mainly appear in invasive ductal breast carcinoma and invasive lobular breast cancer. These results are according to Chromothripsis Explorer and cBioPortal.

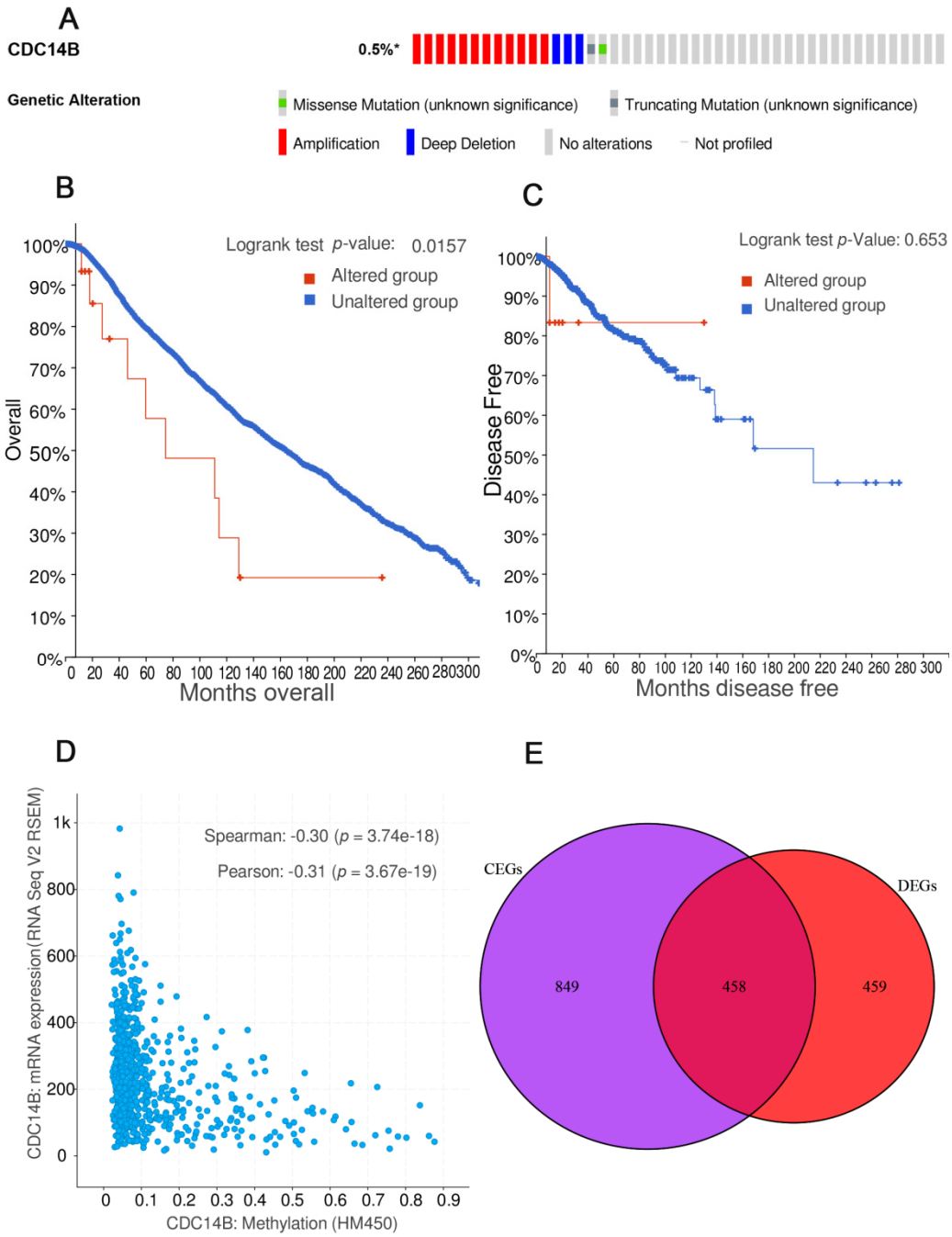

Figure 8. Genetic alterations of CDC14B in breast cancer patients. A: Amplification accounts for most of the alterations of CDC14B in breast cancer patients. B–C: Altered CDC14B correlated with poorer overall survival condition of breast cancer patients. No significant relevance is determined between altered CDC14B and disease-free survival. D: CDC14B mRNA expression levels correlated with CDC14B DNA methylation. E: Venn plot shows the intersection of downregulated genes in breast cancer and co-expressed genes of CDC14B.

3.4. Genetic alterations of CDC14B and its prognosis in breast cancer patients

As is shown in the circos plot, the mutation statuses varied from subtypes to subtypes in breast cancer tissue (Figure 7A–B). Interestingly, patients with lobular breast carcinoma had more chromothripsis regions and structural variations than DCIS. For example, chromothripsis regions were frequently found in chromosomes 1, 4, 5, 7, 8, 11, 14, 15, and 21 of patients with lobular breast carcinoma. However, in patients with DCIS, chromothripsis regions only appeared in chromosome 17. In addition, structural variations, such as duplication-like or deletion-like structure variations or head-to-head or tail-to-tail inversions, were more common in lobular breast carcinoma. When comparing the alterations of CDC14B in various histological types of breast cancer, it was found that amplification, deep deletion, and mutation accounted for most of the alterations in breast cancer patients. Moreover, amplifications and deep deletions were detected to be the most common types of CDC14B alterations in invasive ductal breast carcinoma and invasive lobular breast cancer, respectively (Figure 7C–D). The oncoprint schematic from cBioPortal indicated that CDC14B was altered in 17 (<0.1%) of all 5604 patients (5863 samples), including 12 cases of amplification, three cases of deep deletion, one case of missense mutation and one case of truncating mutation (Figure 8A). The relations between altered CDC14B and clinical information of breast cancer patients are illuminated in Table 2. Surprisingly, CDC14B alterations were significantly correlated with menopausal status, metastatic tumor indicator, ER status by immunohistochemistry, and lymph node stage. The result of the OS analysis exhibited that CDC14B alteration was significantly associated with poor condition of breast cancer patients (Figure 8B). Nevertheless, there was no significant correlations between the alterations of CBC14B and DFS of the breast cancer patients (Figure 8C). Furthermore, it was noticed that downregulation of CDC14B mRNA was inversely related to the DNA methylation status of CDC14B (Spearman's relation coefficient = −0.30; Figure 8D).

3.5. Construction of the regulatory gene network based on differentially expressed genes and co-expressed genes of CDC14B in breast cancer

Differentially expressed genes and co-expressed genes relating to CDC14B were identified and collected from each included dataset (Figure S10A–D). A total of 458 dysregulated genes co-expressed with CDC14B were obtained after intersecting 1307 co-expressed genes of CDC14B and 917 differentially expressed genes in breast cancer cells. In terms of GO, consistent with CDC14B's roles in cell cycle, the intersection genes that co-expressed with CDC14B were dominantly clustered in mitotic-nuclear division, DNA packaging complex, collagen-containing extracellular matrix, and protein heterodimerization activity (Figure 9 and Table S2). In addition, cell cycle and tyrosine metabolism were significant KEGG pathways enriched by these genes (Figure 10A and Table S3). What aroused the interests of the authors was that some pathways, mainly the peroxisome proliferators-activated receptor (PPAR) signalling pathway and the AMP-activated protein kinase (AMPK) signalling pathway were closely linked to the development of cancer. The result of the DO enrichment also shed light on the associations between such intersected genes and malignant tumors of urinary system cancer, renal carcinoma, germ cell cancer, embryonal cancer, non-small cell lung carcinoma, and breast cancer (p.adjusted < 0.05; Figure 10B and Table S4). Moreover, it was found that condensation of prophase chromosomes, signalling by nuclear receptors, DNA methylation, polycomb repressive complex 2 methylates histones and DNA, RNA polymerase I promoter opening, cell cycle checkpoints, and M phase were prominent Reactome pathways (Figure 10C and Table S5). These Reactome pathways also showed the relationship between CDC14B and the cell cycle process,

as well as the DNA methylation status. PPI networks were established and a total of 10 hub genes (i.e., LPL, FABP4, ADIPOQ, CCNE2, CCNB2, CCNB1, SLC2A4, LEP, PPARG, EGFR) were identified from the networks of PPAR signaling pathway (Figure 11A), cell cycle (Figure 11B), AMPK signaling pathway (Figure 11C), and breast cancer disease (Figure 11D). Oncoplot signified that ACACB was the most mutated gene in such pivotal pathways (Figure 12).

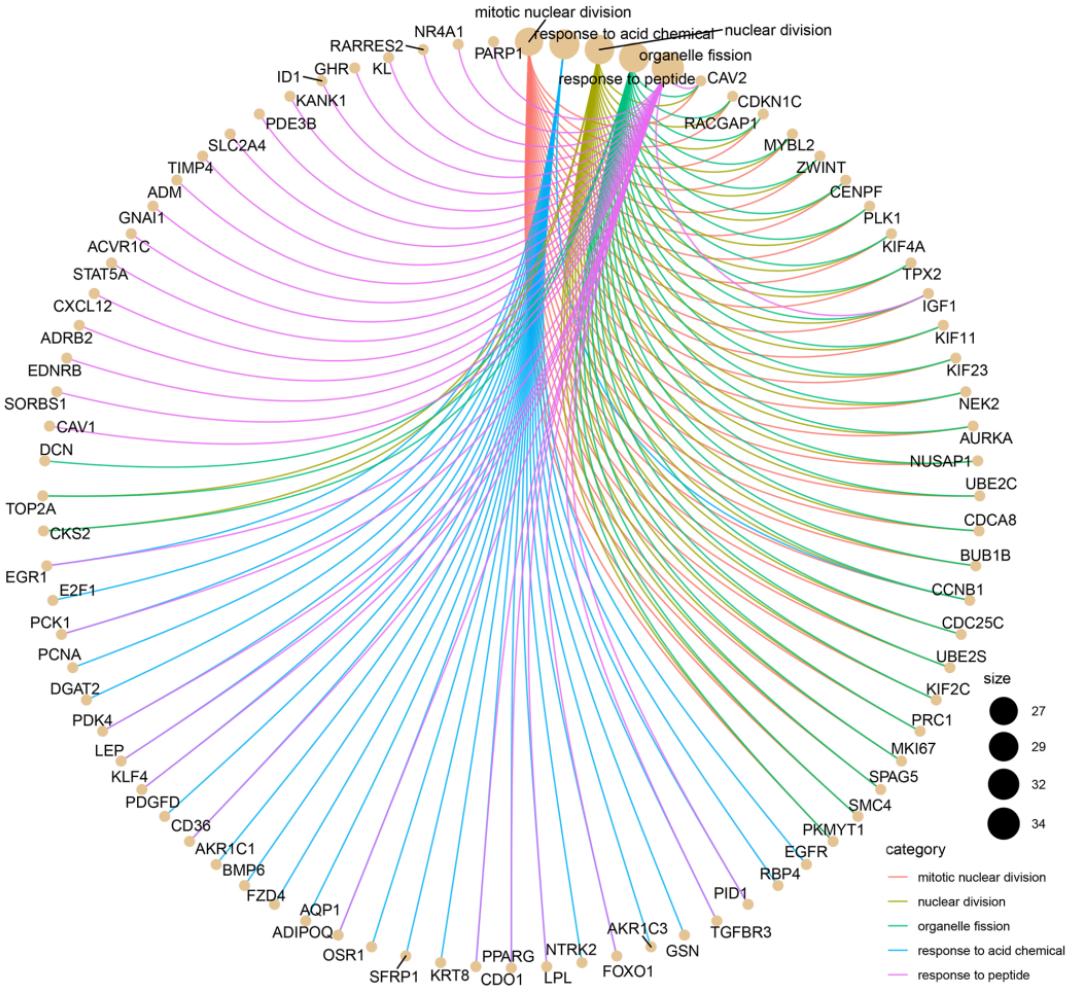

Figure 9. Gene Ontology analysis based on intersection of downregulated genes in breast cancer and co-expressed genes of CDC14B. Mitotic nuclear division is the most clustered biological process.

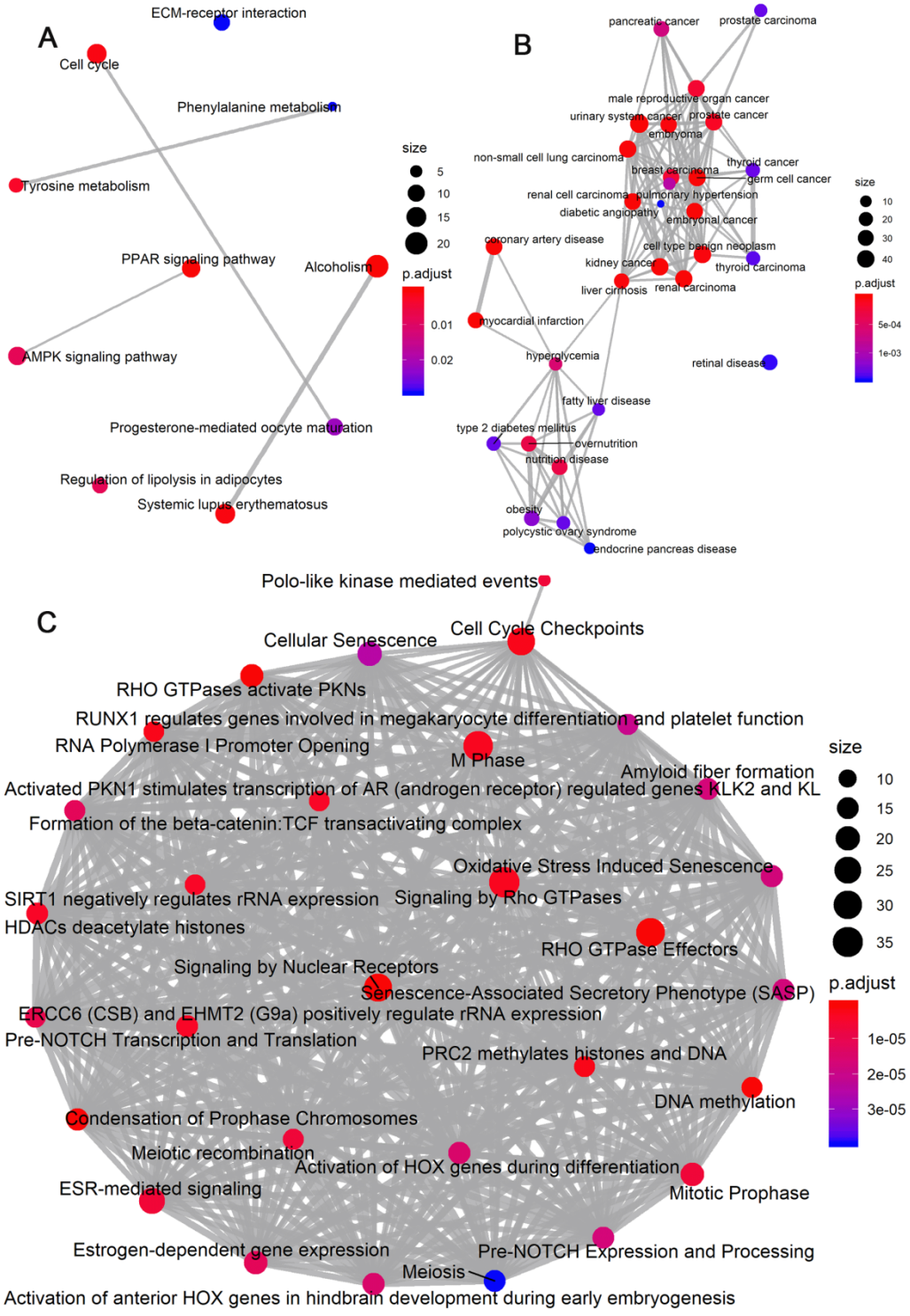

Figure 10. Pathway and disease enrichment analyses based on intersection of downregulated genes in breast cancer and co-expressed genes of CDC14B. A: PPAR signaling pathway, cell cycle, and AMPK signaling pathway are significantly enriched Kyoto Encyclopedia of Genes and Genomes pathways. B: Breast cancer is detected to be one of the most significantly associated diseases. C: DNA methylation, RNA polymerase I promoter opening, and cell cycle checkpoints are significantly clustered Reactome pathways.

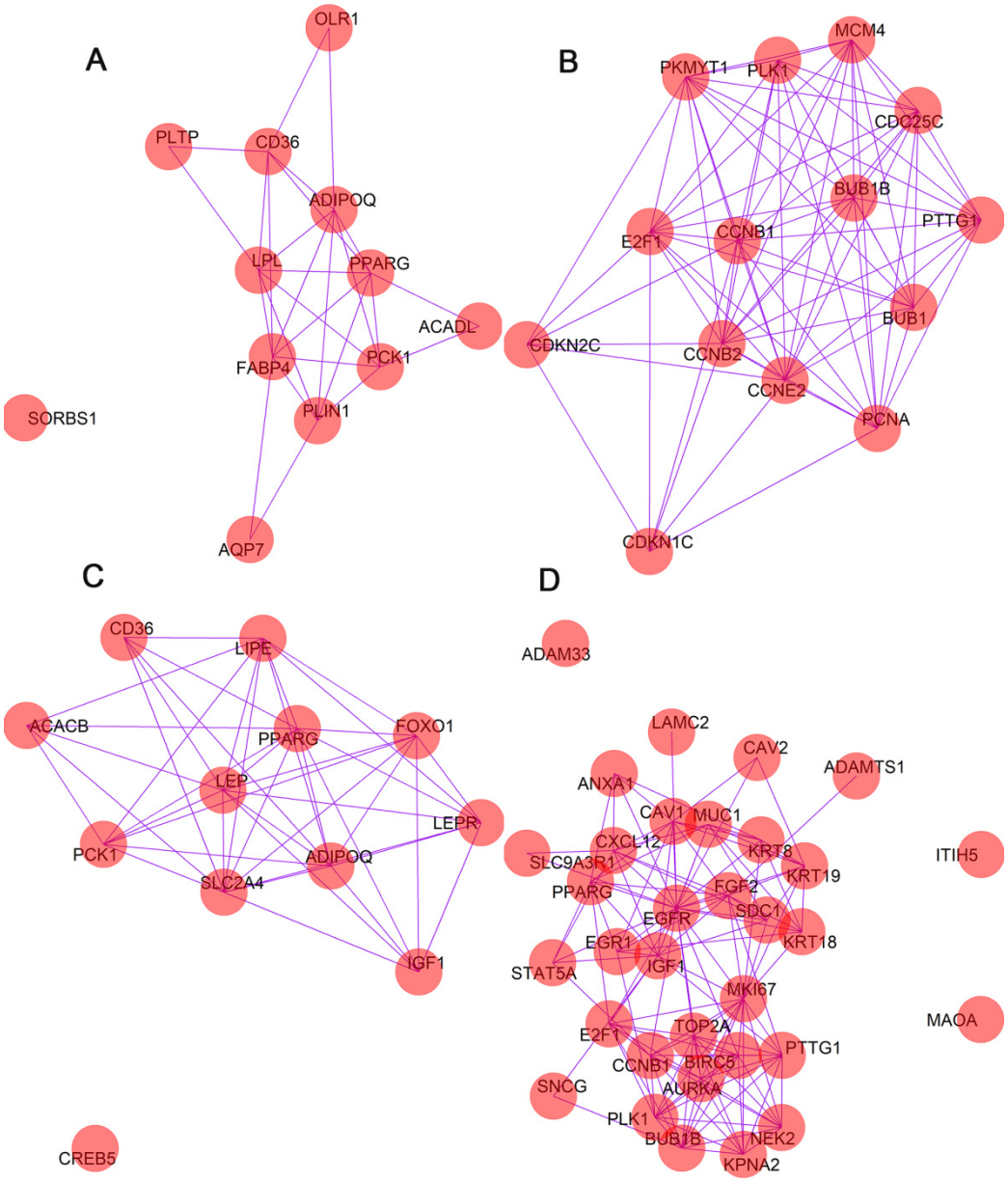

Figure 11. Protein-to-protein interaction internets of genes clustered in: A: PPAR signaling pathway; B: cell cycle; C: AMPK signaling pathway; and D: breast cancer disease.

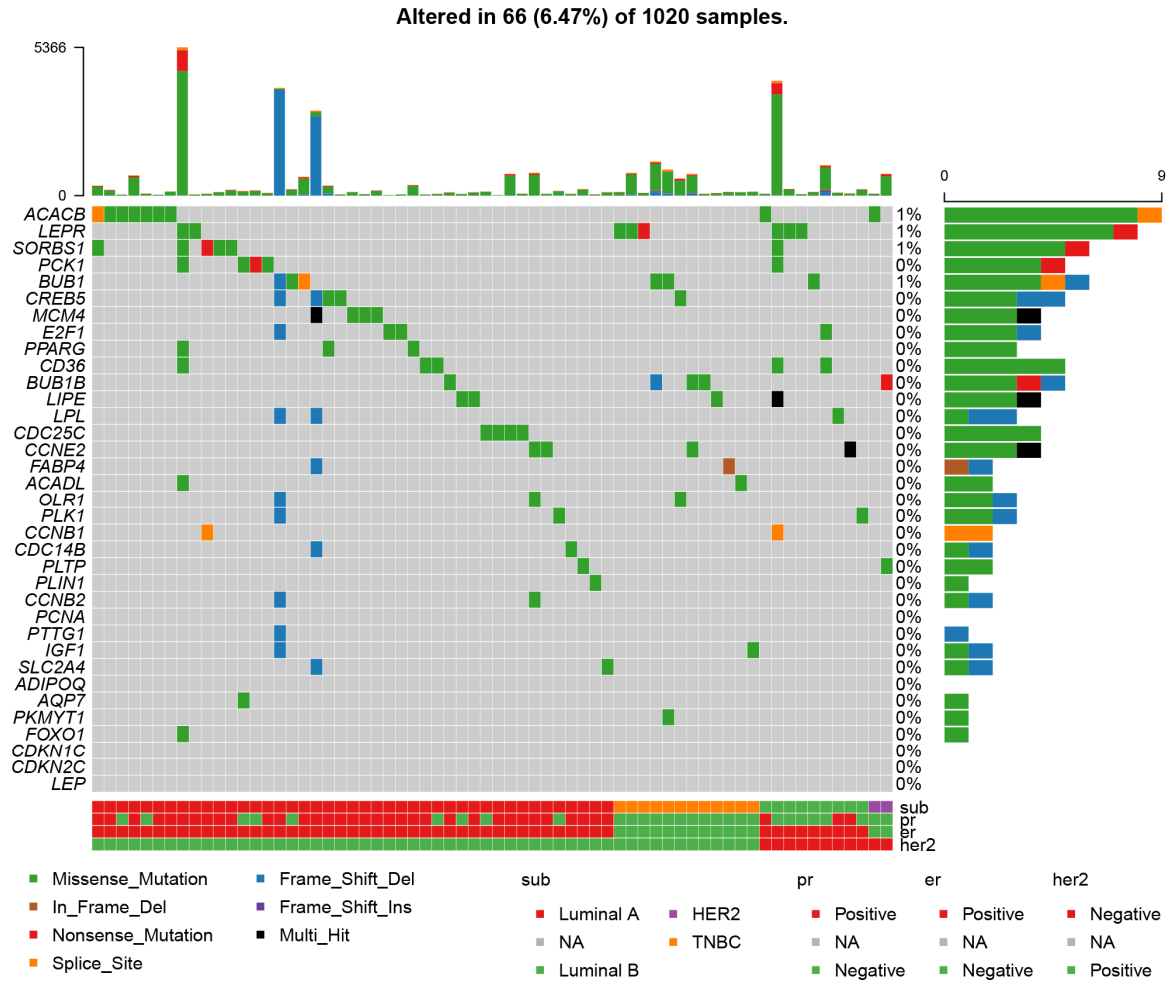

Figure 12. Oncoplot based on the predominant signaling pathways. ACACB and LEPR are the top two most mutated genes in PPAR signaling pathway, cell cycle, and AMPK signaling pathway.

3.6. Prognostic panels based on prognosis-related hub genes in the regulatory gene network

Among the hub genes identified after PPI networks were established, ADIPOQ and CCNE2 were identified as two prognostic factors in breast cancer through Cox regression analysis (Figure 13A). The distinct expression patterns of ADIPOQ and CCNE2 in breast cancer are depicted in Figure 13B. The discriminatory ability of these two genes in breast cancer and normal breast tissues were compared to that of CDC14B, and CDC14B was detected to be the most accurate biomarker (Figure 13C). A prognostic model was established based on ADIPOQ and CCNE2 from the integrated cohort of breast cancer patients (n = 3951). Surprisingly, both ADIPOQ (also named ACDC) and CCNE2 showed a significant ability in forecasting the prognosis of breast cancer patients. Nonetheless, the two prognosticators were completely different in the prognosis of breast cancer patients—expression of ADIPOQ indicated better outcomes of OS, RFS, and DMFS (HR < 1, $p < 0.05$; Figure S10A–C) while expression of CCNE2 indicated worse outcomes of OS, RFS, and DMFS (HR > 1, $p < 0.05$; Figure S11D–F).

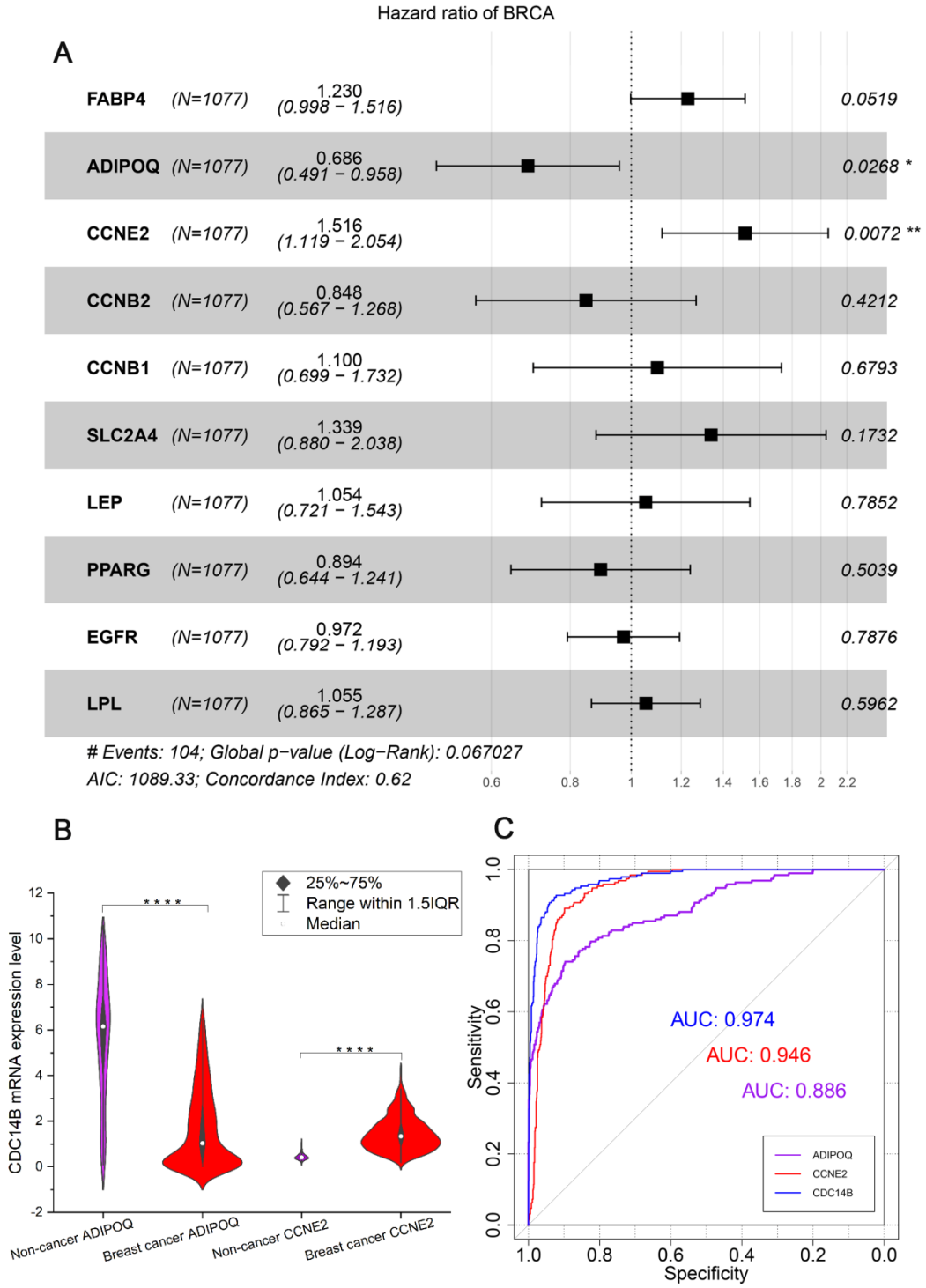

Figure 13. The prognostic value of hub genes in breast cancer. A: ADIPOQ is identified as a protective factor for breast cancer patients. CCNE2 is identified as a risk factor for breast cancer patients. B: ADIPOQ is significantly downregulated in breast cancer tissues in comparison with normal breast tissues. CCNE2 is significantly upregulated in breast cancer tissues in comparison with normal breast tissues. C: ADIPOQ, CCNE2, and CDC14B have a discriminatory ability between breast cancer tissues and normal breast tissues.

4. Discussions

The absence of comprehensive studies exploring the expression and clinical usages of CDC14B in breast cancer indicates a need to conduct more research into this possibility. In this first study of its kind, an all-databases search was performed before combining the gene microarrays with RNA sequencing data to access and compare the expression levels of CDC14B in 5218 breast cancer tissues and 1176 normal breast tissues. It was found that CDC14B may serve as a hallmark in the prognosis of breast cancer patients and in distinguishing between TNBC and non-TNBC. A moderate discriminatory ability was detected between breast cancer and non-cancer, while a weak discrimination ability was exhibited between TNBC and non-TNBC. In addition, this is the first study to unveil the pathogenic alteration patterns of CDC14B and its relations to the prognosis and other clinical features of breast cancer patients. Moreover, this study explored the mechanisms of CDC14B underlying breast cancer and raise a possible avenue for future breast cancer research. ADIPOQ and CCNE2, as two pivotal genes in the gene regulatory network, were identified as promising prognosticators in breast cancer.

Through this dataset integration and expression study, decreased CDC14B was found to be a potential biomarker to discriminate between TNBC and non-TNBC. This study explored the expression levels of CDC14B in 5218 breast cancer tissue samples using 1176 normal breast tissue samples as controls. During this exploration, a downregulated trend was seen in breast cancer tissue but not in normal breast tissue. Interestingly, to better comprehend the downregulated patterns of CDC14B, the authors subsequently compared the decreased levels of CDC14B in different PAM50 subtypes. While CDC14B was significantly downregulated in various molecular-subtype classifications of breast cancer, a significantly downregulated level was detected in non-TNBC, especially the luminal B subtype, when compared to TNBC.

Given the differentially expressed trends of CDC14B among PAM50 subtypes, the authors further appraised the discrimination ability of CDC14B between TNBC and non-TNBC; however, the result signified only a weak discriminatory ability. Although many targeted therapies have been evaluated for the treatment of TNBC, there are few specific strategies for the disease [32,33]. Therefore, this study may provide a novel hallmark in distinguishing TNBC from non-TNBC. Further research must be conducted to validate the potential of CDC14B in developing novel agents, especially those with a high specificity, for the treatment of TNBC patients.

The further prognostic analysis of this study revealed a relationship between CDC14B downregulation and poor outcomes in breast cancer patients. It was found that lower CDC14B was apparently correlated with poorer RFS condition in 3951 breast cancer patients. In addition, combined HRs of DFS outcomes inferred that CDC14B may be an independent protective prognostic factor for breast cancer patients experiencing disease-free intervals. It has been noted in previous research that 5-hydroxymethylcytosine beyond 5-methylcytosine at the CDC14B gene is a potential prognostic factor for cervical cancer patients [34]. Nonetheless, the clinical value of CDC14B in cancer is not well understood thus far, and much less is understood about its prognostic implication in breast cancer patients. Thus, this first study to evaluate the prognosis of CDC14B in breast cancer is a much-needed addition to cancer research.

This study also revealed the alteration landscape of CDC14B in breast cancer for the first time.

Surprisingly, CDC14B amplification was found to be the most common genetic alteration, whereas CDC14B expression was significantly downregulated in breast cancer. Furthermore, CDC14B mRNA expression was inversely correlated to the DNA methylation of CDC14B. Considered together, it can be inferred that although intracellular CDC14B DNA is amplified, its transcription and translation, as a result of aberrant DNA methylation modification, cannot increase accordingly, thus leading to the downregulation of extracellular CDC14B mRNA and protein. This study provides a possible reason for the downregulation of CDC14B in breast cancer; however, more evidence is needed to support this hypothesis.

In addition, this study prospectively compared chromothripsis and structural variation statuses in various breast cancers. The authors noted that patients with lobular breast carcinoma had more chromothripsis regions and structural variations than DCIS. The research of chromothripsis in cancers, characterized by intensive chromosomal rearrangements in a short time, has gained popularity in recent years [35–37]. The appearance of chromothripsis updates our understanding of tumorigenesis because malignancies induced by chromothripsis can arise abruptly without gradual accumulation [38–40]. Therefore, the chromothripsis status and its resulting structural variations may contribute to the mechanisms for tumorigenesis of breast cancer through extensive activation of oncogenes and the inactivation of tumor suppressor genes. This study indicates a new direction for genetic alteration research in breast cancer. Since the clinical impact that chromothripsis has on breast cancer patients is unknown, further studies are necessary.

This study's functional annotation of the dysregulated genes co-expressed with CDC14B shed light on the mechanisms of CDC14B underlying the development of breast cancer. Besides the previously known processes of cell cycle, tyrosine metabolism, and mitotic-nuclear division, this study revealed two other novel signalling pathways—PPAR and AMPK signalling cascades—in which CDC14B may participate. Both PPAR and AMPK pathways are pivotal in physiological metabolisms and energetic homeostasis [41]. In addition, these pathways also play roles in various cancers [42–44]. In breast cancer, PPAR-pathway-related genes were illuminated as be possible diagnostic biomarkers for DCIS [45]. An in vitro experiment demonstrated that a PPAR agonist—WY-14643—was able to activate PPARα and improve the expression activity of cytochrome P450 family 1 subfamily B member 1, which eventually mediated the deterioration of breast cancer [46]. Likewise, AMPK inactivation was reported to induce the carcinogenesis and progression of breast cancer [47]. Interestingly, considering the impacts of PPAR and AMPK signalling pathways in breast cancer, several therapeutic targets have been indicated for TNBC patients [48–52]. From the results above, the authors hypothesise that CDC14B could play a part in the formation and progression of breast cancer by synergistically interacting with its dysregulated co-expressed genes through the PPAR and AMPK pathways. Finally, two hub genes, ADIPOQ and CCNE2, were found to exhibit promising prognostic values in the OS, RFS, and DMFS of breast cancer patients. This finding was consistent with previous evidence [53–56]; however, more experiments are needed for verification.

Furthermore, the validity of the findings in this study deserves consideration. As is known, a balance between internal and external validity is essential to minimize the influence of potential confounders and to enhance the generalizability [53]. In this study, several strategies have been used to promote such a balance, covering strict eligibility standards [54], multidimensional subgroup

analysis [55], and normal breast tissues control condition. Inevitably, there may be another internal and external threats to the validity of this study. For example, several confounding factors are potential internal threats to the validity, such as menstrual status, fertility, and living habits. In addition, a lack of representativeness of breast cancer tissue samples and the limitations of gene expression measurement tools may be primary external factors influencing the validity of this study. In the future, more studies must be carried out from different perspectives to apply the findings of this study to a real-life setting.

Despite its many findings, there were still some limitations to this study. First, although this was a retrospective study to probe the clinical utility of CDC14B in breast cancer patients by comprehensively integrating gene microarrays, RNA sequencing datasets, and clinicopathological information, the natural values of CDC14B in the prognosis of breast cancer have not been certified with a randomized controlled trial. Therefore, further clinical experiments must be carried out to better evaluate its implication value. Second, experimental research on the methylation of CDC14B in breast cancer had not been conducted to verify the proposal that CDC14B downregulation may be a result of the abnormal methylation of CDC14B DNA. However, this possibility could provide a new direction for the future study on the underlying causes of CDC14B downregulation. Third, the molecular functions of CDC14B in breast cancer have not been addressed through experimental methods. Therefore, further exploration of the roles of CDC14B in breast cancer should occur, including in-depth in vitro and in vivo experiments.

5. Conclusions

CDC14B was significantly downregulated in breast cancer and may be a promising hallmark in triple-negative breast cancer patients. The dysregulated genes co-expressed with CDC14B may play an important role in the development of breast cancer through PPAR and AMPK signalling pathways. Moreover, ADIPOQ and CCNE2 may be two promising prognostic factors in breast cancer.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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