

# High expression levels of centromere protein A plus upregulation of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling pathway affect chemotherapy response and prognosis in patients with breast cancer

SONGBO ZHANG\*, YANYAN XIE\*, TING TIAN, QIANRU YANG, YUTING ZHOU,  
JUANJUAN QIU, LI XU, NAN WEN, QING LV and ZHENGGUI DU

Department of Breast Surgery, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, P.R. China

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**Abstract.** Centromere proteins (CENPs) are involved in mitosis, and *CENP* gene expression levels are associated with chemotherapy responses in patients with breast cancer. The present study aimed to examine the roles and underlying mechanisms of the effects of *CENP* genes on chemotherapy responses and breast cancer prognosis. Using data obtained from the Gene Expression Omnibus (GEO) database, correlation and Cox multivariate regression analyses were used to determine the *CENP* genes associated with chemotherapy responses and survival in patients with breast cancer. Weighted gene co-expression network and correlation analyses were used to determine the gene modules co-expressed with the identified genes and the differential expression of gene modules associated with the pathological complete response (PCR) and residual disease (RD) subgroups. *CENPA*, *CENPE*, *CENPF*, *CENPI*, *CENPJ* and *CENPN* were associated with a high nuclear grade and low estrogen and progesterone receptor expression levels. In addition, *CENPA*, *CENPB*, *CENPC* and *CENPO* were independent factors affecting the distant relapse-free survival (DRFS) rates in patients with breast cancer. Patients with high expression levels of *CENPA* or *CENPO* exhibited poor prognoses, whereas those with high expression levels of *CENPB* or *CENPC* presented with favorable prognoses. For validation between databases, the Cancer Genome Atlas (TCGA) database analysis also revealed that *CENPA*, *CENPB* and *CENPO* exerted similar effects on

overall survival. However, according to the multivariate analyses, only *CENPA* was an independent risk factor associated with DRFS in GEO database. In addition, in the RD subgroup, patients with higher *CENPA* expression levels had a worse prognosis compared with those with lower *CENPA* expression levels. Among patients with high expression levels of *CENPA*, the PI3K/Akt/mTOR pathway was more likely to be activated in the RD compared with the PCR subgroup. The same trend was observed in TCGA data. These results suggested that high *CENPA* expression levels plus upregulation of the PI3K/Akt/mTOR signaling pathway may affect DRFS in patients with breast cancer.

## Introduction

Breast cancer is the most common cancer among women worldwide, with 2.1 million newly diagnosed cases in 2018 according to the World Health Organization (1). In the United States of America, 249,260 patients were diagnosed with breast cancer in 2016 (2). According to data released by the Cancer Center of China in 2014, the total incidence of breast cancer in China was 42.55 per 100,000 individuals, making breast cancer the most common malignant tumor in women (3).

The aim of chemotherapy is to interfere with the process of cell proliferation; therefore, most chemotherapeutic drugs exert major effects on proliferating cells, including cancer cells, which are characterized by abnormal and uncontrolled proliferation (4). Chemotherapy is currently the most effective method for systemic treatment of patients with breast cancer and prevents the division and proliferation of cancer cells by destroying cancer cell DNA and disrupting the intracellular components involved in mitosis (5). In addition, certain chemotherapeutic drugs kill cancer cells by inducing apoptosis (6).

Centromere proteins (CENPs) serve important roles in centromere function and mitosis; for example, *CENPA* encodes centromere protein A, which contains a variant of histone H3 that is specifically expressed on the centromere and kinetochore of the chromosome and is targeted by microfilaments during mitosis, resulting in the separation of centromeres during mitosis (7). *CENPB* supports kinetochore formation and contributes to the maintenance of chromosome

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*Correspondence to:* Dr Zhenggui Du, Department of Breast Surgery, West China Hospital, Sichuan University, 37 Guoxue Alley, Wuhou, Chengdu, Sichuan 610041, P.R. China  
E-mail: docduzg@163.com

\*Contributed equally

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segregation fidelity (8). CENPC serves as a scaffold for the specific recruitment of essential kinetochore proteins and links centromeres and kinetochores in mitosis (9). Thus, CENPs are important components of chromosomes during mitosis and can regulate the proliferation of tumor cells. Therefore, we hypothesized that the expression of *CENP* genes may be associated with the responses of breast tumor cells to chemotherapy and may affect patient survival.

The aim of the present study was to systematically explore the associations between 13 *CENP* genes and chemotherapy responses in patients with breast cancer and to identify genes that were most relevant to the patient prognosis.

## Materials and methods

**Gene datasets.** Five breast cancer mRNA expression profiles, namely GSE20194 (10,11), GSE20271 (12), GSE22093 (13), GSE23988 (13) and GSE25066 (14,15), were extracted from the Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>) database using the key words ‘breast cancer’, ‘neoadjuvant chemotherapy’ and ‘pre-operative chemotherapy’. Studies in which gene expression levels were analyzed in genome profiling data using pretreatment biopsies from patients who underwent neoadjuvant chemotherapy were included. The first four datasets (GSE20194, GSE20271, GSE22093 and GSE23988) were used as the training dataset (n=620 patients), and the fifth dataset (GSE25066) was used as the validation dataset (n=508 patients). The mRNA expression profiles of breast cancer from The Cancer Genome Atlas (TCGA; <https://portal.gdc.cancer.gov/>) database were used for validation. In the GEO database, the result of pathological response was classified into pathological complete response (PCR) and residual disease (RD). In TCGA database used for validation, the result of pathological response was classified into clinical complete response (CCR) and RD.

**Immunohistochemistry and molecular classification.** Immunohistochemistry and molecular classification results were extracted from the GEO database. The hormone receptor status of a tumor was defined as positive when the immunohistochemistry results were positive for either estrogen receptor (ER) or progesterone receptor (PR) in  $\geq 1\%$  of cells and as negative when both ER and PR were negative. According to global consensus (16), HER2 expression status was defined as negative when the immunohistochemistry results were negative or 1+, and as positive when the results were 3+. HER2 positivity was evaluated according to the results of fluorescent *in situ* hybridization if the immunohistochemistry results were 2+. Tumor stage was re-evaluated in accordance with the 8th edition of the American Joint Committee on Cancer system (17). Tumors were classified according to histological grade as well differentiated (G1), moderately differentiated (G2), poorly differentiated and undifferentiated (G3) or unknown.

**PAM50 subtype classification by ‘genefu’.** Data were extracted from the GEO database and, according to the PAM50 algorithm (18) using the ‘genefu’ package (19) of R software (version 3.5) (20), tumors were classified into basal-like, HER2-enriched, luminal A, luminal B, or normal breast-like molecular subtypes.

**Weighted correlation network analysis (WGCNA).** Data of this part were extracted from the GEO database publicly mentioned above. The ‘WGCNA’ package in the R software (21) was used to perform scale-free network topology analysis of microarray expression data of breast cancer samples. Standard WGCNA parameters were used for analysis. Using WGCNA, a co-expression module of genes associated with patient clinicopathological characteristics was extracted from the breast cancer data for subsequent analysis.

**Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of co-expression modules.** KEGG pathway analyses were performed to determine the biological functions of the genes in the green module identified by WGCNA to be highly correlated with *CENPA* using the DAVID Bioinformatics Tool (version 6.8; <https://david.ncifcrf.gov/home.jsp>).  $P < 0.05$  was considered to indicate a statistically significant result. A total of 12 records were extracted. Graphs were generated using the ‘ggplot2’ R package (22).

**Statistical analysis.** Data are presented as the mean, range, standard deviation or proportion. Statistical analyses were performed using R software (version 3.5). Univariate and multivariate survival analyses were performed using Cox regression models. The  $\chi^2$  test was used to analyze the associations between CENP expression levels and patient clinicopathological characteristics. Kaplan-Meier curves were used for survival analysis, and the Cramér-von Mises and log-rank tests were used for the curves with and without crossovers, respectively. Comparisons of correlations were performed using the ‘correlation coefficients’ module in MedCalc software (Version 19.6.4; MedCalc Software, Ltd.). For comparisons of means, continuous variables between groups were analyzed using the independent-samples Student's t-test or one-way ANOVA. Comparisons of rates were performed using the  $\chi^2$  test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Basic characteristics of the study population.** In the present study, five datasets including a total of 1,128 patients were evaluated. The specific characteristics of each study are presented in Table I. The cancer grades were primarily II and III, and the nuclear grades were mainly G2 and G3. All patients in the present study received neoadjuvant chemotherapy, and the PCR rate was  $\sim 22.4\%$  for all datasets combined. Data of 1,025 patients were extracted from TCGA; all of these patients were evaluated for overall survival rate, and 175 patients in this cohort with chemotherapeutic response were evaluated for the gene expression levels of *PI3K*, *AKT* and *MTOR*.

**Associations between CENP gene expression levels and the clinicopathological characteristics of patients with breast cancer.** In the training dataset, the associations between 13 *CENP* genes and patient clinicopathological characteristics related to breast cancer were analyzed (Fig. 1A). The results demonstrated that *CENPA* was significantly associated with pathological response, nuclear grade and PAM50 subtype. In addition, high *CENPA* expression levels were associated

Table I. Basic characteristics of the study population.

Characteristic	GSE20194	GSE20271	GSE22093	GSE23988	GSE25066
Total, n	278	178	103	61	508
Age, years (mean $\pm$ SD)	52.0 $\pm$ 10.8	51.0 $\pm$ 10.7	49.0 $\pm$ 11.1	48.7 $\pm$ 9.1	49.8 $\pm$ 10.5
Ethnicity					
White	176	81	0	0	0
Black	29	13	0	0	0
Asian	18	1	0	0	0
Hispanic	42	83	0	0	0
Mixed	3	0	0	0	0
Unknown	10	0	103	61	508
T stage, n					
T0	3	2	1	0	3
T1	23	11	2	1	30
T2	147	76	51	20	255
T3	50	37	26	40	145
T4	53	51	18	0	75
Unknown	2	1	5	0	0
N stage, n					
N0	79	59	21	21	157
N1	125	71	16	32	244
N2	31	38	10	5	66
N3	42	9	4	3	41
Unknown	1	1	52	0	0
Clinical AJCC stage, n					
I	6	2	0	0	8
II	145	82	26	34	272
III	126	92	28	27	228
Unknown	1	2	49	0	0
ER IMH, n					
Negative	114	80	56	29	205
Positive	164	98	42	32	297
Unknown	0	0	0	5	6
PR IMH, n					
Negative	157	95	0	0	258
Positive	121	83	0	0	243
Unknown	0	0	103	61	7
HER2, n					
Negative	219	152	0	0	489
Positive	59	26	0	0	6
Unknown	0	0	103	61	13
Nuclear grade, n					
G1	13	15	3	1	32
G2	104	61	29	19	180
G3	150	72	47	37	259
Unknown	11	30	24	4	37
PAM50 subtype, n					
LumA	73	41	25	10	143
LumB	89	61	28	17	147
HER2-enriched	35	21	3	2	41
Basal	69	42	44	30	160
Normal	12	13	3	2	17
Unknown	0	0	0	0	0

Table I. Continued.

Characteristic	GSE20194	GSE20271	GSE22093	GSE23988	GSE25066
Pathologic response, n					
RD	222	152	69	41	389
PCR	56	26	28	20	99
Unknown	0	0	0	6	20

T, tumor; N, node; AJCC, American Joint Committee on Cancer; ER, estrogen receptor; PR, progesterone receptor; IMH, immunohistochemistry; LumA, luminal subtype A; LumB, luminal subtype B; Basal, basal-like subtype; Normal, normal breast-like subtype; RD, residual disease; PCR, pathological complete response.

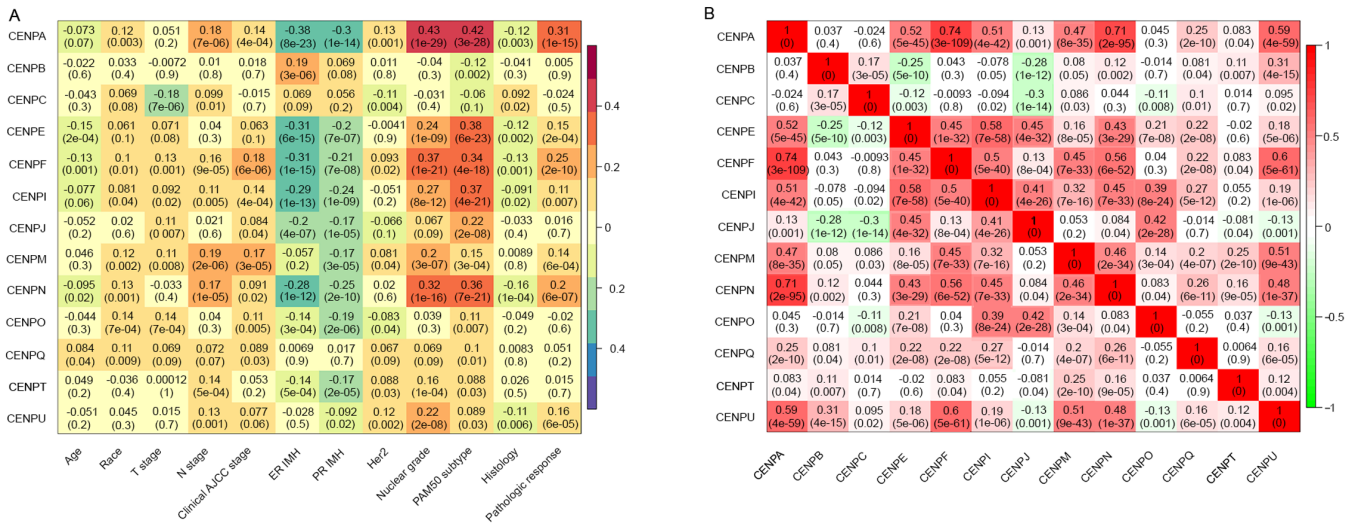


Figure 1. *CENP* gene expression and co-expression levels. (A) Association between *CENP* gene expression levels and clinicopathological characteristics of patients in the training dataset. (B) Co-expression analysis of *CENP* genes. *CENP*, centromere protein; T, tumor; N, node; AJCC, American Joint Committee on Cancer; ER, estrogen receptor; PR, progesterone receptor; IMH, immunohistochemistry.

with low levels of ER and PR expression. Similar results were observed for *CENPE*, *CENPF*, *CENPI*, *CENPJ* and *CENPN*. Therefore, we hypothesized that *CENPA*, *CENPE*, *CENPF*, *CENPI*, *CENPJ* and *CENPN* were likely to be co-expressed. By contrast, the associations between *CENPB*, *CENPC*, *CENPM*, *CENPO*, *CENPQ*, *CENPT* and *CENPU* and patient clinicopathological characteristics were less notable.

**Co-expression analysis of CENPs.** To assess the potential co-expression relationships among the 13 CENP genes, the co-expression of 13 CENP genes was analyzed (Fig. 1B). The results demonstrated that *CENPA* expression levels were positively correlated with those of *CENPE*, *CENPF*, *CENPI*, *CENPN* and *CENPU*. Additionally, a rectangular positive correlation cluster was observed from *CENPE* to *CENPQ*. Significant positive correlations were also identified among the expression levels of *CENPU*, *CENPF* and *CENPM*.

**Univariate and multivariate analyses of CENP gene expression levels.** Univariate and multivariate analyses of all CENP genes associated with survival were next performed in the validation dataset (Table II). In the univariate analysis, with the exception of *CENPM*, *CENPQ*, *CENPT* and *CENPU*, the expression levels of CENP genes significantly affected patient

survival. However, in the multivariate analysis, only *CENPA*, *CENPB*, *CENPC* and *CENPO* were independent prognostic factors for survival. In addition, compared with the low CENP gene expression groups, the DRFS rates were decreased when the expression levels of *CENPA* and *CENPO* were high, and increased when the levels of *CENPB* and *CENPC* were high (Fig. 2A and C), suggesting that *CENPB* and *CENPC* may serve protective roles in breast cancer.

To further determine the associations between the *CENPA*, *CENPB*, *CENPC* and *CENPO* gene expression levels and the clinicopathological characteristics of patients in the training and validation datasets, correlation analysis was performed (Fig. 2B). The results demonstrated that the associations between the *CENPA*, *CENPB*, *CENPC* and *CENPO* gene expression levels and patient clinicopathological characteristics were similar in the training and validation datasets. Although survival data in the training dataset were lacking, based on these findings, the effects of *CENPA*, *CENPB*, *CENPC* and *CENPO* on survival were estimated to be similar between the training and validation datasets, since the survival data in the validation dataset were clear. The survival analysis was further validated using TCGA database, which revealed that *CENPA*, *CENPB* and *CENPO* exerted the same effects on patient survival rates as those observed in the GEO validation



Table II. Univariate and multivariate analysis of the association between patient survival and *CENP* gene expression in the validation dataset.

Gene	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
<i>CENPA</i>	1.926 (1.426-2.600)	<0.001	2.041 (1.494-2.788)	<0.001
<i>CENPB</i>	0.470 (0.277-0.796)	0.005	0.459 (0.272-0.773)	0.003
<i>CENPC</i>	0.317 (0.131-0.762)	0.010	0.347 (0.143-0.842)	0.019
<i>CENPE</i>	1.975 (1.373-2.839)	<0.001		
<i>CENPF</i>	2.094 (1.396-3.140)	<0.001		
<i>CENPI</i>	3.917 (1.943-7.896)	<0.001		
<i>CENPJ</i>	3.694 (1.851-7.373)	<0.001		
<i>CENPM</i>	1.432 (0.942-2.175)	0.093		
<i>CENPN</i>	2.031 (1.327-3.111)	0.001		
<i>CENPO</i>	3.321 (1.343-8.212)	0.009	3.363 (1.307-8.653)	0.012
<i>CENPQ</i>	1.118 (0.377-3.322)	0.840		
<i>CENPT</i>	0.902 (0.560-1.453)	0.671		
<i>CENPU</i>	1.099 (0.925-1.307)	0.284		

*CENP*, centromere protein; OR, odds ratio; CI, confidence interval.

dataset (Fig. 2C). Notably, in TCGA database, the results for *CENPC* did not match those in the GEO data; however, this difference was not statistically significant.

**Univariate and multivariate analysis of *CENP* gene expression levels and patient clinicopathological characteristics.** As a number of *CENP* genes were significantly associated with patient clinicopathological characteristics, these characteristics were included in the univariate and multivariate analyses in order to elucidate whether they may replace the indicative function of *CENP* genes and affect patient survival, and to confirm whether the identified *CENP* genes were independent predictors of survival. First, patient clinicopathological characteristics were analyzed (Table III), and multivariate analysis these characteristics and *CENP* gene expression levels was subsequently performed (Table IV). The results demonstrated that only the node (N) stage, PAM50 subtype, pathological response and *CENPA* expression levels were independent factors affecting the survival of patients with breast cancer.

**Subgroup analysis.** Since *CENPA* was identified as an independent factor for patient survival, further analysis of its expression levels was performed in patient subgroups categorized by the other three independent factors (Fig. 3A). Similar results were obtained in the training and validation datasets. As the N stage increased, *CENPA* expression levels significantly increased. Among the PAM50 subtypes, the *CENPA* expression levels in luminal B, HER2-enriched and basal-like subtypes were significantly higher compared with those in luminal A and normal breast-like subtypes. In the pathological response subgroups, the expression levels of *CENPA* in the PCR group were higher compared with those in the RD group.

The specific subgroups that affected the survival of patients with breast cancer according to *CENPA* expression levels were determined by performing additional subgroup

analysis (Fig. 3B). The results demonstrated that *CENPA* expression levels significantly affected the survival of patients with chemotherapy responses in the RD group, but not in the PCR group (Fig. 3C).

**Co-expression analysis of *CENPA*.** To determine how *CENPA* affected the survival of patients with RD after chemotherapy, co-expression analysis of *CENPA* was performed using WGCNA (Fig. 4). The results demonstrated that the brown module in the PCR and RD groups was positively correlated with *CENPA* expression levels. However, no significant differences were observed in the correlations between the brown module and *CENPA* expression levels in the two groups (Table V). By contrast, the green module (Supplementary data) was significantly negatively correlated with *CENPA* levels in the PCR group and slightly negatively correlated with those in the RD group; the correlation between the two groups was significant ( $P=0.0001$ ; Table V). In addition, the green-yellow, pink and red modules also exhibited significant differences between the two groups ( $P<0.01$ ; Table V).

**Enrichment analysis.** To further explain how *CENPA* affected the survival of patients who underwent chemotherapy, enrichment analysis of signaling pathways was performed using the green module (Fig. 5A). The results revealed that the green module was mainly enriched in three pathways: 'ECM-receptor interaction', 'focal adhesion' and 'PI3K-Akt signaling pathway'. Therefore, the present study focused on three key genes in this pathway: PI3K catalytic subunit  $\alpha$  (*PIK3CA*), *AKT1* and *MTOR* (23) (Fig. 5B). In the PCR and RD groups, the median *CENPA* expression levels were used to define whether *CENPA* was highly or lowly expressed. The results demonstrated that when *CENPA* was highly expressed, the expression levels of *PIK3CA* were also high in the PCR and RD groups. However, compared with the low *CENPA* expression group, *MTOR* expression levels were significantly decreased in the PCR

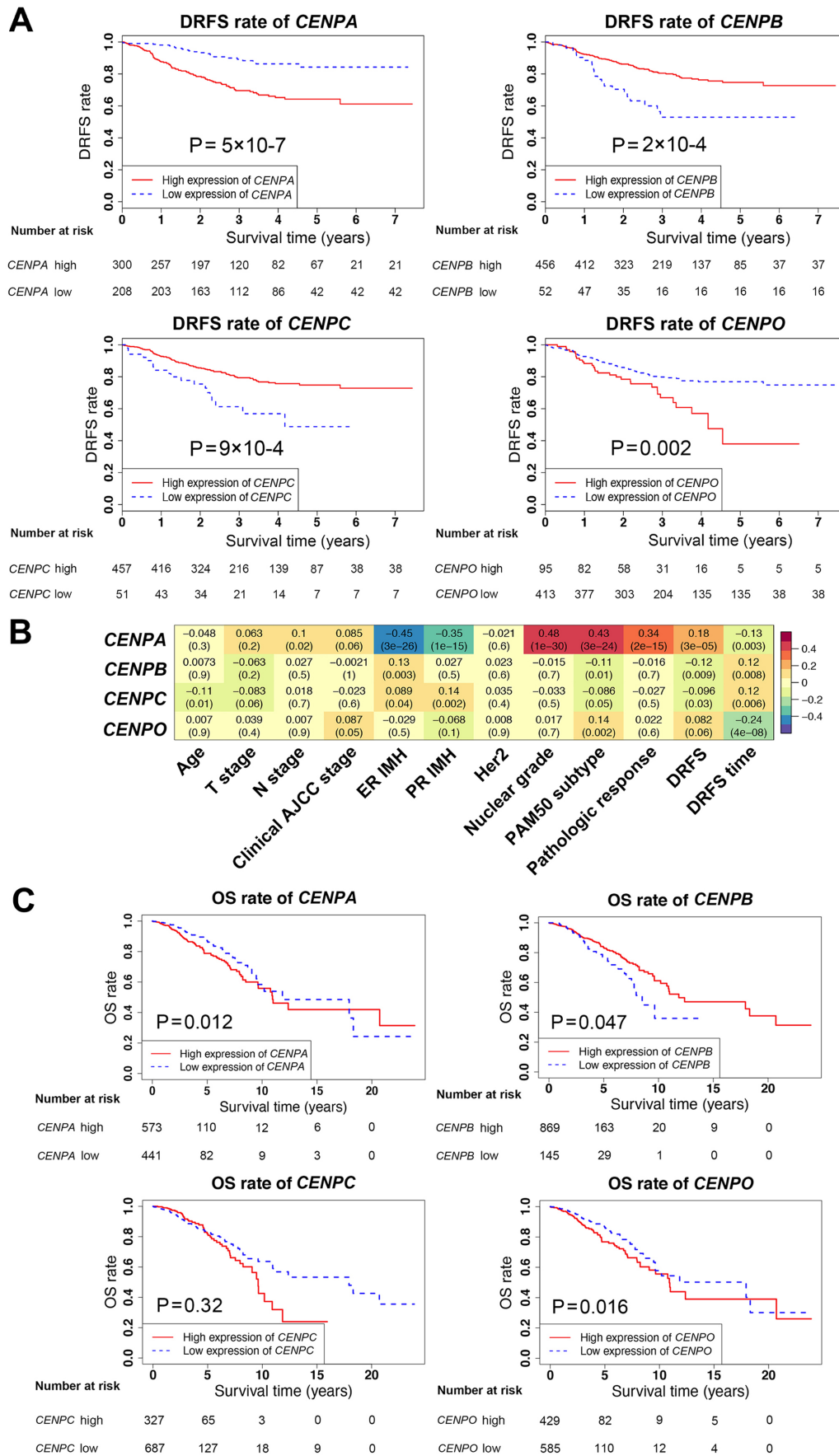


Figure 2. Survival analysis and associations between *CENPA*, *CENPB*, *CENPC* and *CENPO* expression levels and patient clinicopathological characteristics. (A) DRFS rates in patients with high or low expression of *CENPA*, *CENPB*, *CENPC* and *CENPO* using Kaplan-Meier analysis in the validation dataset. (B) Associations between *CENPA*, *CENPB*, *CENPC* and *CENPO* expression levels and clinicopathological characteristics in the validation dataset. (C) OS rates in patients with high or low expression levels of *CENPA*, *CENPB*, *CENPC* and *CENPO* using Kaplan-Meier analysis in The Cancer Genome Atlas database. DRFS, distant relapse-free survival; *CENP*, centromere protein; OS, overall survival.

Table III. Univariate analysis of the association between patient clinicopathological characteristics and survival in the validation dataset.

Characteristic	OR (95% CI)	P-value
Age	0.998 (0.981-1.016)	0.860
T stage		
T0	1 (ref)	<0.001
T1	0.139 (0.027-0.723)	0.019
T2	0.149 (0.036-0.617)	0.009
T3	0.204 (0.049-0.856)	0.030
T4	0.440 (0.105-1.850)	0.263
N stage		
N0	1 (ref)	<0.001
N1	2.378 (1.381-4.095)	0.002
N2	4.432 (2.364-8.311)	<0.001
N3	4.548 (2.265-9.133)	<0.001
Clinical AJCC stage		
I	1 (ref)	<0.001
II	0.643 (0.155-2.669)	0.544
III	1.562 (0.381-6.405)	0.536
ER IMH	0.344 (0.234-0.507)	<0.001
PR IMH	0.380 (0.252-0.571)	<0.001
HER2	1.752 (0.432-7.107)	0.433
Nuclear grade		
G1	1 (ref)	0.038
G2	7.018 (0.961-51.232)	0.055
G3	9.466 (1.313-68.249)	0.026
PAM50 subtype		
LumA	1 (ref)	<0.001
LumB	1.434 (0.691-2.977)	0.333
HER2-enriched	3.280 (1.579-6.812)	0.001
Basal	3.975 (2.356-6.706)	<0.001
Normal	0.828 (0.280-2.449)	0.734
Pathologic response	0.257 (0.120-0.554)	0.001

T, tumor; N, node; AJCC, American Joint Committee on Cancer; ER, estrogen receptor; PR, progesterone receptor; IMH, immunohistochemistry; LumA, luminal subtype A; LumB, luminal subtype B; Basal, basal-like subtype; Normal, normal breast-like subtype; OR, odds ratio; CI, confidence interval.

group but not altered in the RD group when CENPA was highly expressed; this result was also validated using TCGA data (Fig. 5C). Since the CCR group in TCGA validation dataset included not only patients with PCR but also with RD, the difference was not statistically significant. In the RD group in TCGA data, due to the limited number of patient samples (n=18), the results did not exhibit accordance with those in the GEO datasets; however, a similar trend was observed.

## Discussion

The crucial factor affecting the success of breast cancer treatment is systemic therapy, particularly chemotherapy.

Table IV. Multivariate analysis of patient clinicopathological characteristics and CENPA expression levels in the validation dataset.

Characteristic	OR (95% CI)	P-value
N stage		
N0	1 (ref)	<0.001
N1	2.848 (1.550-5.236)	0.001
N2	4.995 (2.508-9.948)	<0.001
N3	4.016 (1.868-8.636)	<0.001
PAM50 subtype		
LumA	1 (ref)	<0.001
LumB	1.169 (0.525-2.603)	0.703
HER2-enriched	2.105 (0.880-5.038)	0.095
Basal	3.665 (1.884-7.128)	<0.001
Normal	0.968 (0.322-2.907)	0.094
Pathologic response	0.094 (0.037-0.237)	<0.001
CENPA	1.605 (1.000-2.576)	0.050

N, node; LumA, luminal subtype A; LumB, luminal subtype B; Basal, basal-like subtype; Normal, normal breast-like subtype; CENPA, centromere protein A; OR, odds ratio; CI, confidence interval.

Table V. Comparative analysis of the correlation between each module and expression of centromere protein A in the pathological complete response and residual disease groups in the training and validation datasets.

Module	Z statistic	P-value
Blue	-2.2523	0.0243
Brown	-0.2285	0.8193
Magenta	-2.0805	0.0375
Black	-0.4904	0.6239
Turquoise	-2.0475	0.0406
Green	3.9366	0.0001
Green-yellow	2.7879	0.0053
Tan	1.1540	0.2485
Pink	3.1028	0.0019
Purple	2.3559	0.0185
Red	3.0159	0.0026
Yellow	2.1615	0.0307
Gray	1.1922	0.2332

CENPs are proteins that facilitate centromere formation during mitosis; the majority of chemotherapeutic drugs kill cancer cells by affecting or disrupting mitosis (24). Therefore, the expression of specific CENP genes may affect the chemotherapy responses and patient prognosis. Among patients with ER-positive breast cancer who received no systemic therapy or tamoxifen, high levels of CENPA were associated with low 5-year survival rates and were positively correlated with Ki-67 expression levels (25). In addition, CENPO is required for bipolar mitotic spindle assembly and segregation

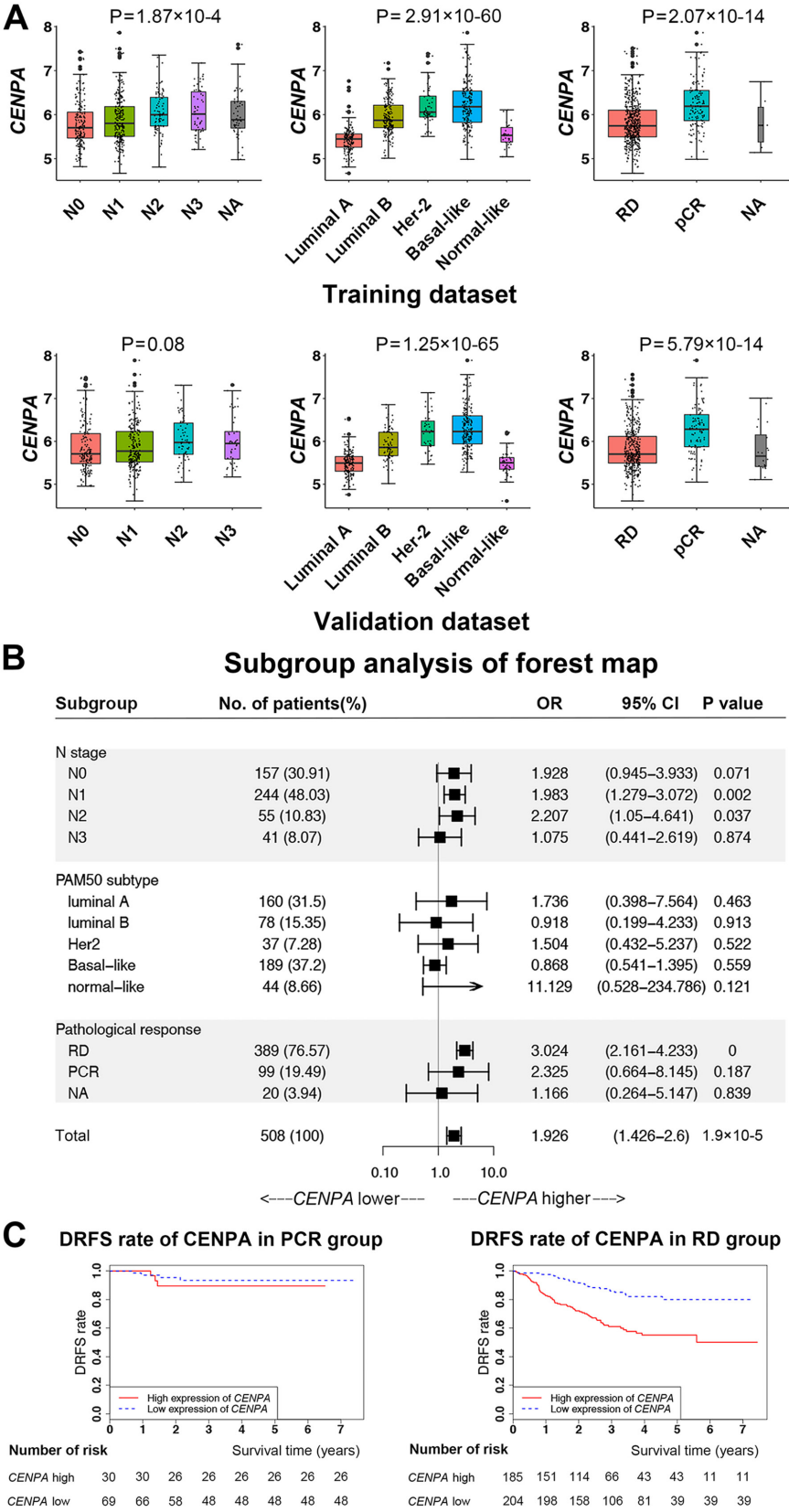


Figure 3. *CENPA* expression in patient subgroups and survival analysis. (A) *CENPA* expression in patient subgroups according to N stage, PAM50 subtype and pathological response in the training and validation datasets. (B) Subgroup analysis of survival by forest plot. (C) Kaplan-Meier survival analysis according to *CENPA* expression levels in the RD and PCR subgroups. *CENPA*, centromere protein A; PCR, pathological complete response; RD, residual disease; N, node; DRFS, distant relapse-free survival; NA, not available.

of chromosome during mitosis; CENPO expression regulates gastric cancer cell proliferation and is associated with

poor patient prognosis (26,27). By contrast, certain genes in the *CENP* family encode proteins associated with mitosis



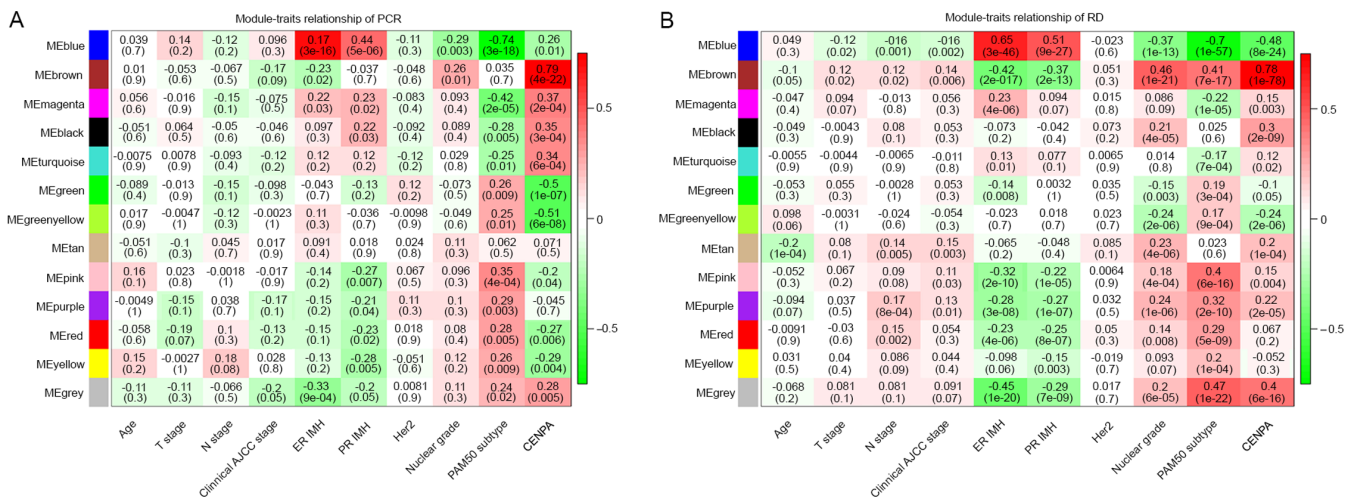


Figure 4. Weighted correlation network analysis plot. (A) Associations between gene modules and clinicopathological characteristics of patients in the PCR group. (B) Associations between gene modules and clinicopathological characteristics of patients in the RD group. *CENP*, centromere protein; RD, PCR, pathological complete response; RD, residual disease; T, tumor; N, node; AJCC, American Joint Committee on Cancer; ER, estrogen receptor; PR, progesterone receptor; IMH, immunohistochemistry.

stabilization and may therefore improve patient prognosis. *CENPB* supports kinetochore formation and contributes to the maintenance of chromosome segregation fidelity (8). In mitosis, *CENPC* serves as a scaffold for the specific recruitment of essential kinetochore proteins and links centromeres and kinetochores (9). The results of the present study demonstrated that high expression levels of *CENPB* and *CENPC* were associated with a favorable prognosis in patients with breast cancer.

In the present study, high PCR rates of patients with breast cancer were observed when the majority of *CENP* genes were highly expressed. In addition, *CENPA* was identified as an independent factor affecting survival, particularly in patients presenting with RD following chemotherapy. Notably, genes co-expressed with *CENPA* were mainly associated with cell division. However, in the PCR and RD groups, no significant differences were observed in the correlation between *CENPA* expression levels and the cell division pathway, suggesting that *CENPA* affected survival through a different pathway. Furthermore, the correlations between the green module and *CENPA* expression levels in the PCR and RD groups were significantly different. Additionally, since the 'PI3K-Akt signaling pathway' was among the main enriched pathways in the green module, we hypothesized that the chemotherapy response and prognosis may be associated with the combination of *CENPA* expression and the PI3K/Akt/mTOR pathway.

Enhanced centromere function and mitosis are associated with high expression levels of *CENPA*, which is particularly notable in human cancer cells (7). Studies using RNA sequence data from the Oncomine database have revealed a significant positive correlation between high *CENPA* expression and chemotherapy response (using taxane as the main drug) (28). In addition, a previous study has demonstrated a strong correlation between high levels of *CENPA* expression and a positive oncolytic response to chemotherapy using Oncomine, which indicates that high *CENPA* expression levels may be used as a predictive biomarker for a positive outcome of

taxane-based chemotherapy for breast cancer (29). The results of the present study also demonstrated that when *CENPA* was highly expressed, the PCR rates increased, suggesting that the response to chemotherapy may have been enhanced. Thus, we hypothesized that high levels of mitotic activity in response to high *CENPA* expression levels may lead to active cell proliferation, which may enhance the chemotherapy response of tumors.

Since chemotherapy functions by inhibiting mitosis (3), in which *CENPA* serves a crucial role, we hypothesized that tumors may be highly sensitive to chemotherapy when *CENPA* is expressed at high levels, and the survival rate of patients with high *CENPA* expression levels may also be higher compared with that of patients with low *CENPA* levels. A previous study has demonstrated that high *CENPA* expression levels in colon cancer is associated with a favorable relapse-free survival rate (29). By contrast, the results of the present study demonstrated that the survival rates were lower in patients with high *CENPA* expression levels compared with those in patients with low *CENPA* levels. Similar results have been observed in other types of cancers, including osteosarcoma (30,31), lung adenocarcinoma (32) and ovarian cancer (33). Specifically, the survival times of patients in the high *CENPA* expression group were significantly lower compared with those in patients in the low *CENPA* expression group in patients with osteosarcoma. Additionally, the survival time of patients with lung adenocarcinoma in the high *CENPA* expression group was <120 months (32). Another study demonstrated that *CENPA* was highly expressed in ovarian epithelial cancer and was associated with poor survival (33). In addition, in a study of breast cancer, high expression levels of *CENPA* were also associated with poor survival in patients with triple-negative breast cancer (34). To further validate these results, the present study divided the patients into groups based on RD or PCR following chemotherapy. The results of this analysis demonstrated that in the RD group, the DRFS rate was low when *CENPA* was highly expressed; by contrast, *CENPA* expression levels exerted no significant effects on the survival of patients in the PCR group.

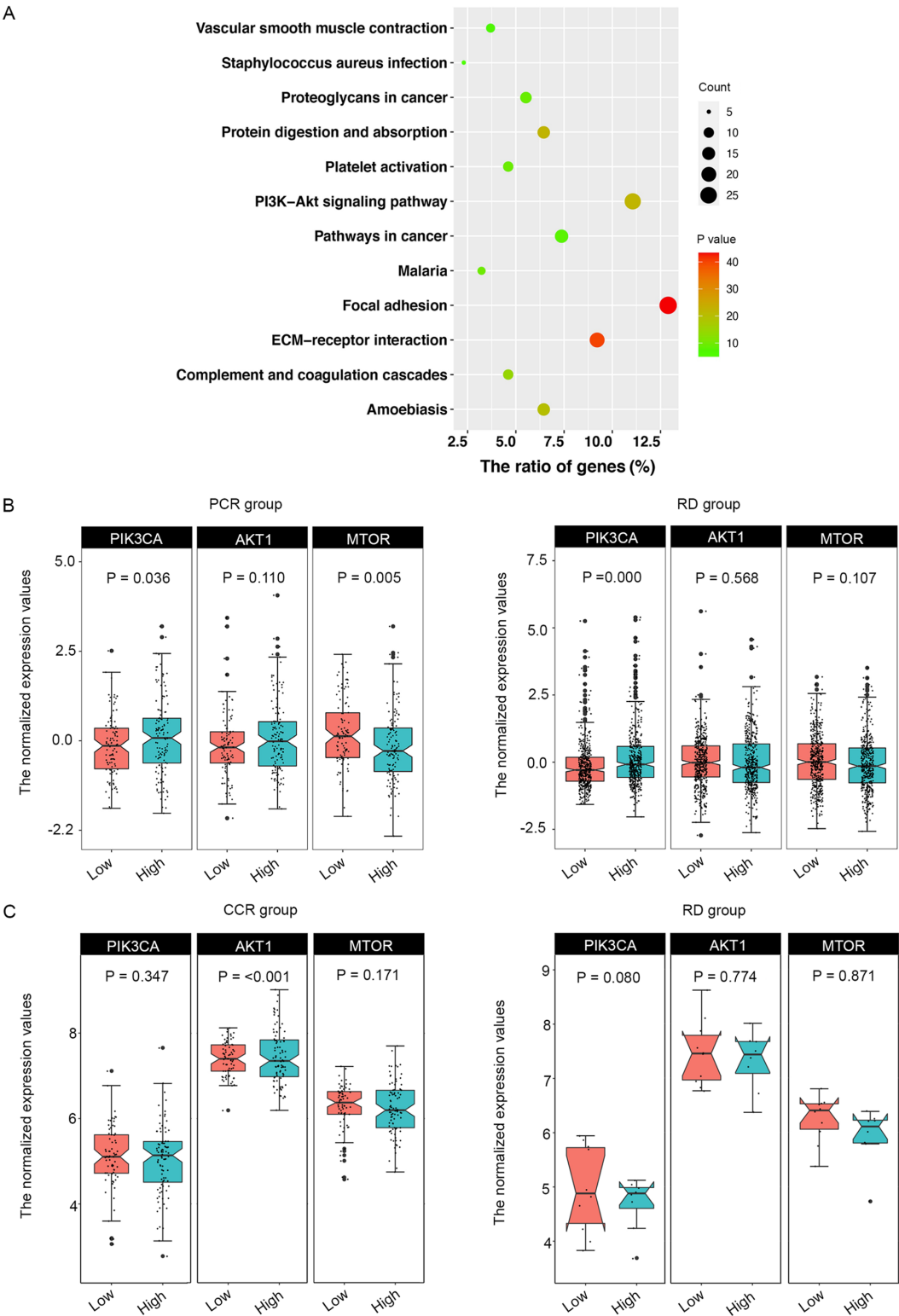


Figure 5. KEGG enrichment analysis and expression levels of *PIK3CA*, *AKT1* and *MTOR*. (A) KEGG pathway enrichment analysis of the green module. (B) Gene expression levels of *PIK3CA*, *AKT1* and *MTOR* based on *CENPA* expression in the PCR and RD groups in the Gene Expression Omnibus data. (C) Gene expression levels of *PIK3CA*, *AKT1* and *MTOR* based on *CENPA* expression in the CCR and RD groups in data from The Cancer Genome Atlas. KEGG, Kyoto Encyclopedia of Genes and Genomes; *PIK3CA*, PI3K catalytic subunit  $\alpha$ ; *CENPA*, centromere protein A; PCR, pathological complete response; RD, residual disease; CCR, clinical complete response.

Therefore, other mechanisms may mediate the decreased survival when *CENPA* is highly expressed in patients whose tumors present with PCR following chemotherapy.

In the present study, WGCNA was performed to analyze the genes associated with *CENPA* expression in the PCR

and RD groups. The KEGG results demonstrated that genes co-expressed with *CENPA* were mainly enriched in cell cycle-associated pathways, including cell division cycle, nuclear division cycle, and cell cycle phase transition (35). These results suggested that the mitosis pathway may be the

key to determining the effects of *CENPA* on survival. There were no significant differences in the correlations between the mitosis pathways and the RD group, which indicated that *CENPA* did not affect survival by synergizing with the hub genes in this module. The green module and *CENPA* were significantly negatively correlated in the PCR group, and there was a statistically significant difference between the PCR and RD groups, suggesting that genes in the green module may be associated with tumor progression.

Further analysis in the present study revealed that the main pathways enriched in the green module genes were 'focal adhesion', 'ECM-receptor interaction' and 'PI3K-Akt signaling pathway'. The focal adhesion pathway is primarily associated with cancer cell migration (36). Focal adhesion kinase is a cytoplasmic tyrosine kinase and a key regulator of the focal adhesion complex, which mediates various intracellular processes, such as cell motility, invasiveness, proliferation and viability (36,37). Signals of the extracellular matrix (ECM) modulate cell behavior, cell interactions in forming tissues and homeostasis (38). Various components of the ECM provide cells with a scaffold that controls and determines cell shape, mobility, proliferation, viability and differentiation (38). Previous studies have demonstrated that dysregulation of the ECM components causes cancer cell invasion and progression (39-41).

The PI3K/Akt/mTOR pathway serves important roles in the development and treatment of breast cancer (42). It is associated with the cell cycle and affects cell proliferation, viability, differentiation and proliferation (43). Upregulation of the PI3K/Akt/mTOR signaling pathway promotes cell proliferation, migration, survival and angiogenesis (44). In addition, in response to a variety of intracellular and extracellular stimuli, such as metabolic molecules, growth factors and hypoxia, the PI3K/Akt/mTOR pathway regulates intracellular metabolism, cell cycle progression, angiogenesis and tumor aggressiveness (45). Therefore, a large number of breast cancer cases are associated with the activation of the PI3K/Akt/mTOR pathway, which promotes stable survival and invasion of breast cancer cells, leading to metastasis (46). Currently, inhibitors of the PI3K/Akt/mTOR pathway, such as sirolimus, temsirolimus and everolimus, demonstrate promising preclinical activity for the treatment of breast cancer (47,48). Therefore, we hypothesize that in patients presenting with RD, tumor cell mitosis may be activated when *CENPA* is expressed at high levels, and upregulation of the PI3K/Akt/mTOR pathway may promote stable survival in activated tumor cells without being affected by chemotherapy drugs, leading to poor survival. Additionally, the results of the present study demonstrated that in the PCR group, despite the high expression levels of *CENPA*, the PI3K/Akt/mTOR pathway was not significantly activated, resulting in sufficient sensitivity to chemotherapy.

One limitation of the present study was that the chemotherapy response or resistance were not evaluated in cultured cells in order to verify that the effects of *CENPA* synergized with the PI3K/Akt/mTOR pathway. However, the present study used multiple databases for validation and demonstrated that the *CENPA* levels and chemotherapy responses may be promising indicators to predict the survival of patients with breast cancer.

In summary, the results of the present study demonstrated that *CENPA* affected the chemotherapy responses and prognosis of patients with breast cancer. In patients whose tumors presented with RD following chemotherapy, the DRFS rate was significantly decreased when *CENPA* was expressed at high levels. These effects may be associated with the upregulation of the PI3K/Akt/mTOR pathway in these patients.

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#### Availability of data and materials

Not applicable.

#### Authors' contributions

ZD and QL proposed the study concept and design. SZ and YX performed the experiments and acquired the data. SZ and NW analyzed and interpreted the data. SZ wrote the first draft of the manuscript and critically revised it for important intellectual content. YZ, JQ, QY, TT and LX analyzed the data and produced the figures. SZ supervised the study. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

Not applicable.

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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