Downregulated ZNF132 Predicts Unfavorable Outcomes in Breast Cancer via Hypermethylation Modification

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Abstract

Background: Methylation modification of tumor suppressor gene is one of the most critical mechanisms in human breast cancer (BC). Some biomarkers, including HER2, ER and PR, have been used in clinical practice, however, the effective was limited in terms of high recurrence and mortality rate. Thus, a better diagnostic and therapeutic target for BC is urgent. To date, no study provide information on the status of ZNF132 in BC or analyze diagnosis and prognostic significance of ZNF132 in BC. Methods: In the present study, we investigated the expression and clinical signifcance of ZNF132 by TCGA database and clinical samples analysis. Besides, a further exploration was performed to assess the molecular mechanisms of ZNF132 using the multiple bioinformatic tools. Importantly, a MSP assay was executed to confirm the epigenetic alteration of ZNF132. Results: Our results showed that the mRNA expression of ZNF132 was significantly downregulated in BC tissues, the consistent results was obtained by immunohistochemistry assessment. Importantly, survial analysis revealed that the lower expression of ZNF132 was remarkably correlated to Relapse Free Survival (RFS), but not Overall Survival (OS). Besides, the ROC curve confirmed ZNF132 had powerful sensitivity and specificity to distinguish between BC and adjacent normal tissues. Finally, MSP analysis demonstrated that ZNF132 was hypermethylated in BC tissues. Interestingly, ZNF132 methylation level was negatively correlated with its gene expression. Conclusions: Our results revealed that hypermethylation of ZNF132 contributed to its downregulated expression and could be identified as a new diagnostic and prognostic marker in BC.

Background

Breast cancer (BC) is one of the most frequently malignant tumors in female and the fifth leading cause of cancer-associated mortality in the worldwide[1, 2]. Advancement in early detection and treatment have obviously improved 5 year-survial rates of BC patients[3-8]. Owing to tumor heterogeneous characteristics[9], BC has usually been classified several molecular subgroups: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2), normal, and basal-like based on immunohistochemical evaluation of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and proliferation marker Ki-67[10-13]. Although these biomarkers have been used as prognostic and therapeutic targets in clinical practice[14-16], the effective was limited in terms of high recurrence and mortality rate. Thus, a better understanding of the pathogenesis and molecular mechanisms of BC may provide us a new diagnostic and therapeutic strategies for BC.

Until now, multiple mechanisms were reported for breast carcinogenesis, including overactivation of oncogenes, overexpression of growth factors and receptors, as well as silence of tumor suppressor[17-19]. Besides, an increasing number of studies confirmed epigenetic alterations were also involved in tumor progression[18, 20], especially, aberrant DNA methylation of CpG islands was condidered to be a vital mechanism to silence anti-tumor genes[21, 22]. Simultaneously, promoter methylation was assessed as an important marker of tumor cells[23, 24]. Zinc Finger Protein 132 (ZNF132), located at chromosome 19q13.4, belongs to the zinc finger protein family. A critical C2H2 zinc finger motifs and Kruppel-associated box domain was considered to be a core component to exert function of transcriptional

repressor. To date, no study provide information on the status of ZNF132 in BC or analyze diagnosis and prognostic significance of ZNF132 in BC.

In the present study, we investigated the expression and clinical signifcance of ZNF132 by TCGA database and clinical samples analysis. Besides, a further exploration was performed to assess the status of ZNF132 using the cBioPortal tool. Importantly, we confirmed the epigenetic alteration of ZNF132 was associated with its downregulated expression. Collectively, our findings demonstrated ZNF132 could be a new potentially prognostic factors for BC and might serve as a promissing therapeutic target for BC patients.

Methods

Extraction of Multiple platforms data

To identify the expression of ZNF132 in BC, a total of 1104 BC tissue samples and 114 normal breast tissue samples that contained the expression of ZNF132 were collected from TCGA database (http://cancergenome.nih.gov/). Additional clinical variables such as age, gender, ER, PR, HER2, metastasis, and clinical stage were analyzed to assess the association between the expression of ZNF132 and these parameters. Other tools, including the Oncomine™ database (www.oncomine.org) and UALCAN platform (http://ualcan.path.uab.edu/) were also used to verify the correlation between ZNF132 expression and clinical outcome, including tissue type, gender, race, clinical stage, molecular pathological characteristics, menstrual status, and survival status.

Clinical samples and immunohistochemical analysis

To detect the expression of ZNF132 in protein level between BC tissue and adjacent normal tissue, we collected 19 cases clinical samples from the First Affiliated Hospital of Xi'an Jiaotong University. These patients did not receive any therapeutic intervention and signed an informed consent before surgery. All patients were finally histologically diagnosed by two pathologists. Ethical approval was provided by the First Affiliated Hospital of Xi'an Jiaotong University Ethics committee. All tissues were fixed in 4% formaldehyde at room temperature for 48h in preparation. The ZNF132 antibody (BIOSS, Beijing, China; cat. no. bs-7150R, 1:1000 dilution) was used for IHC detection. The staining intensity was defined according to the following criterias: staining intensity (negative=0, weak=1, moderate=2, strong=3). Positive staining ratio of ZNF132 (rare: <25%; middle: 25-75%; strong: >75%).

Diagnostic and prognostic significance of ZNF132

The Kaplan-Meier plotter (http://kmplot.com/) was used to assess the prognosis value of ZNF132 in BC patients, including Relapse Free Survival (RFS) and Overall Survival (OS). Besides, univariate and multivariate analysis based on a Cox proportional hazard regression model were performed to evaluate independent prognostic significance of ZNF132, clinical variables included age (≥60 years/<60 years), ER (positive/negative), PR (positive/negative), her2 (positive/negative), tumor size (T2-4/T1), lymph node

metastasis (N1-3/N0), distant metastasis (M1/M0), clinical stage (II-IV/I) and expression of ZNF132 (median value). Furthermore, a reciever operating characteristic (ROC) curve was plotted to determine whether the level of ZNF132 expression distinguish the difference between BC tissues and adjacent normal tissues.

Bioinformatic exploration

To further investigate the molecular mechanisms of ZNF132 in BC, we firstly evaluated the status of ZNF132 that included ZNF132 alteration and its impact on the prognosis of BC patients by the cBioPortal OncoPrint (http://www.cBioPortal.org/index.do). Importantly, Gene Set Enrichment Analysis (GSEA, http://www.linkedomics.org/) was used to predict potential biological processes and pathways. In addition, DNA methylation expression and different methylation sites analysis from the TCGA database were evaluated to identify the downregulated mechanisms of ZNF132 in BC.

Methylation analysis

To identify the epigenetic effect of ZNF132, 6 human BC cell lines, including MDA-MB-231, MCF7, MDA-MB-453, HCC1937, T47D and DU4475 were used in our study. Each cell line was authenticated by STR analysis in Genesky Co.Ltd (Shanghai, China) and were excluded the mycoplasma contamination using One-step Quickcolor Mycoplasma Detection Kit (Shanghai Yise Medical Technology Co., Ltd.). Then, 2 subgroups were entered into practice, test group was treated with 5μM DNA methyltransferase (DNMT) inhibitor 5-aza-2′-deoxycytidine (5-Aza-dC) (Sigma-Aldrich) and control group was treated with the vehicle. When the cell density was up to 80%, RNA was extracted using TRIzol® protocol. Next, the levels of ZNF132 between test and control group were detected by SYBR®Green FAST (Kapa Biosystems, Inc., Wimington, MA, USA) using Bio-Rad CFX Manager detection system. The PCR protocol followed the parameters: 95˚C for 30 sec, 38 cycles of 5 sec at 95˚C and 30 sec at 55˚C. The primers used are as follow: ZNF132: forward: 5′- CCACAGTGTGATGCTGGAAAACC-3′, reverse: 5′-GCTTTCTTGGTGGAAGGATCTGC-3′; 18s rRNA: forward: 5′-CGCCGCTAGAGGTGAAATTC-3′, reverse: 5′-CTTTCGCTCTGGTCCGTCTT-3′.

In addition, genomic DNA from 6 BC cell lines were extracted according to standard phenol/chloroform protocol. Then, DNA were treated using sodium bisulfite. Briefly, a mixture, including 4μg genomic DNA, 10μg salmon sperm DNA, and 0.3M NaOH, was collected. Next, supply a certain volume of water to a final volume of 20 μl , incubated at 50°C for 20min to denature the DNA. Finally, transfer the mixture into 500μl of solution containing 3M sodium bisulfite and 10mM hydroquinone (Sigma, Saint Louis, MO), incubated at 70°C for 4h. DNA was subsequently purified using the Wizard DNA Clean-Up System (Promega Corp., Madison, WI) and dissovled in distilled water. Importantly, a methylation-specific PCR (MSP) was performed in our study. The PCR procedure was as follows: 4 min denaturation at 95°C, then 45s denaturation at 95°C, 45s anneal at 55°C, and 45s extension at 72°C, repeat this step with 35 cycles, finally an extension at 72°C for 5 min. The reaction products were presented on a 1.2% agarose gel and visualized under UV illumination using an ethidium bromide stain along with a positive control and negative control.

Statistical analysis

All statistical analyses were performed using SPSS 18.0 (IBM Corp., Armonk, NY, USA) and Graphpad Prism 5.0 software. The association between ZNF132 expression and clinical characteristics was analysed using the Chi-square test. Univariate and Multivariate analyses based on COX regression model were performed to detect connection between clinical variables and the prognosis of BC. Moreover, the ROC curve was identified to evaluate the diagnostic capability between BC and adjacent normal tissue. Student's t-test was used to assess methylation differences of CpG island sites between BC and adjacent normal tissue. P value <0.05 was considered to indicate a statistically significant difference.

Results

Low expression of ZNF132 in BC

The analysis based on mRNA expression from TCGA database indicated that ZNF132 was downregulated in BC compared of adjacent normal tissues (P <0.0001, Fig.1A). 114 matched pairs of BC and adjacent normal tissues also showed a similar trend (P <0.0001,Fig.1B). Meaningfully, data from the Oncomine 4.5 database also revealed that mRNA expression of ZNF132 was significantly reduced in BC tissues than in normal tissues (P ≤0.01). In addition, ZNF132 was remarkably downregulated in different molecular types of BC, including Luminal, HER2 positive and triple negative (P <0.0001, Fig.1C). Importantly, immunohistochemistry analysis of clinical samples showed ZNF132 was overexpressed in normal breast tissues (Fig.1D). Besides, the c^2 test showed ZNF132 expression was associated with clinicopathological variables including HER2 (P =0.001), tumor size (P =0.006), lymph node metastasis (P =0.003) and clinical stage (P =0.006) status (Table 1).

ZNF132 was a prognostic factor and diagnostic marker in BC

The result from Kaplan-Meier plotter revealed low ZNF132 expression was significantly associated with a shorter RFS in BC（Fig.2A）. Besides, univariate COX regression analysis confirmed that some clinical features, including age, tumor size, lymph node metastasis, distant metastasis and clinical stage were significantly associated with OS in BC patients (Table2). However, there was no statistical significance between the expression of ZNF132 and the prognosis of BC (HR=1.129, P =0.587) using multivariate analysis (Table3). Moreover, an area under the curve (AUC, representing accuracy of differentiation) of 0.887 suggested that the level of ZNF132 has sufficient sensitivity and specificity to identify difference between BC and adjacent normal tissues (Figure 2B).

Bioinformatic analysis of ZNF132

The result from cBioPortal revealed that 6% (58/960) of BC exhibited ZNF132 alteration, including missense mutation (2/960), amplification (19/960), deep deletion (6/960), mRNA upregulation (13/960) and mRNA downregulation (18/960) (Fig.3A). Besides, a prognosis analyses were performed to explore the influences with and without ZNF132 alteration. The result showed a statistically significant difference

existed for OS, but not for DFS (Fig.3B). Subsequently, the analysis from GSEA demonstrated that ZNF132 participated in mediating multiple biological processes, including cilium organization, cilium or flagellum-dependent cell motility, synaptic transmission, glutamatergic, microtubule bundle formation, mitochondrial gene expression, mitochondrial respiratory chain complex assembly, ribonucleoprotein complex biogenesis, translational initiation etc (Fig.3C). The biological pathways of ZNF132 contained the regulation of cell cycle, glycolysis, cholesterol biosynthesis, ubiquitin proteasome pathway, TCA cycle (Fig.3D). Importantly, ZNF132 was negatively associated the cyclin E1（Fig. 4A）and ENO1 (Fig. 4B) level.

Hypermethylation of ZNF132 in BC

We firstly analyzed the expression of DNMT1, DNMT3A and DNMT3B in the BC tissues. Compared to $ZNF132^{high}$, the 3 DNA methyltransferases showed significantly higher expression in $ZNF132^{low}$ group (Fig.5A). Importantly, the result from MethHC (http://methhc.mbc.nctu.edu.tw/php/index.php) also demonstrated that the methylation level of ZNF132 in BC tissues was significantly higher than the normal sample (Fig.5B). Besides, the analysis of different CpG island methylation sites based on TCGA database also revealed the same trend (Fig. 5C). Together, ZNF132 methylation level was negatively correlated with its gene expression (Fig. 5D). Finally, the MSP assessment showed complete or partial ZNF132 methylation was found in six BC cell lines. To further explore whether ZNF132 was inactivated by DNA methylation, these cells were treated with the DNMT inhibitor 5-Aza-dC, As expected, 5-Aza-dC treatment increased ZNF132 expression in two cell lines (Fig.5F).

ZNF132 methylation was correlated with prognosis and clinicopathological features of BC

The investigation from MethSurv (https://biit.cs.ut.ee/methsurv/) showed that BC patients with higher ZNF132 methylation had a shorter survival time (Fig.6A; P =1.802E-04). Importantly, survival analyses of different methylated regions also demonstrated a similar trends (Fig.6A; cg12042695, P =0.00038; cg19776201, P =0.041; cg24366702, P =0.029; cg00868383, P =0.0023; cg03735888, P =0.00024). Besides, UALCAN was used to evaluate the impact of aberrant methylation on the clinicopathological features of BC patients. Compared to the patients without or lower methylation of ZNF132, the BC patients with higher methylation of ZNF132 were associated with age, gender, ethnic and tumor stage (Fig.6B).

Discussion

Breast cancer is an aggressive malignancy tumor in female, the common metastasis locations in clinical included the lung, bone and brain, leading to approximately 522,000 deaths yearly [1]. Until now, the causes of BC were mainly involved in a variety of events, including genetics and epigenetics modification, especially for epigenetic change, such as DNA promoter methylation, gene mutation and deletion in tumorigenesis. In the past decades, DNA methylation has been demonstrated to be a promising early diagnostic biomarker for BC, however, useful markers in practise have not been identified.

ZNF132, a member of zinc finger protein family, was only reported to be downregulated by promoter methylation in ECSC and PC[25, 26]. However, its diagnostic and prognostic value have not been elucidated in BC until now. The present study, to the best of our knowledge, is the first one to systematically explore the clinical significance of ZNF132 in BC.

In agreement with the study of ZNF132 in ECSC and PC, our results indicated that ZNF132 had a signifcantly lower expression in BC tissues than adjacent normal tissues both in mRNA and protein level, which implied that ZNF132 might serve as a tumor suppressor in BC. Without a doubt, The larger samples need to be collected to provide more powerful evidence to verify the role of ZNF132 in BC. In addition, the ROC curve revealed that ZNF132 displayed a signifcant diagnostic value for BC (AUC=0.887, P 0.001). Signifcantly, reduced ZNF132 expression was correlated to the worse prognosis of BC. Consequently, ZNF132 might be served as a promissing diagnostic and prognostic marker for BC. However, as demonstrated in Table 2 and Table 3, univariate and multivariate analysis did not provide independent prognostic information for ZNF132 expression. The possible reasons we summarized are as follows: a. A serious of mixed factors were involved in the prognosis of BC, the common facters were only listed in present study. b. Samples in our study based on TCGA database were not enough to clarify the correlation between ZNF132 expression and prognostic value, so large-scale prospective study would be necessary to confrm the value of ZNF132 in future. Moreover, analysis from Kaplan-Meier plotter revealed that low ZNF132 expression was significantly associated with a shorter RFS for patients of BC, but not with OS. Therefore, we speculated that ZNF132 expression can detect BC recurrence. Besides, analysis based on TCGA database revealed that downregulated ZNF132 was significantly correlated to malignant phenotype of BC, including positive HER2 status, larger tumor sizes, distant metastasis and advanced clinical stage, which suggested that ZNF132 might inhibit the progression of BC by inhibitting the growth, invasion and metastasis of tumor cells.

To recognize the potential mechanisms of ZNF132 in BC, the first investigation from cBioPortal showed that approximately 6% BC patients exhibited ZNF132 alterations, among which, the mRNA downregulation was the predominant type of alteration, which could contributed to the downregulation of ZNF132 in BC. In addition, the analysis based on GSEA demonstrated that ZNF132 participated in a variety of important biological processes and pathways. Significantly, ZNF132 expression was negatively correlated with CCNE1 and ENO1. Cyclin E1 (CCNE1), belongs to the highly conserved cyclin family, forms a complex with CDK2, whose activity is required for cell cycle G1/S transition. CCNE1 has been reported to upregulated in various human cancer, including breast[27], bladder[28] and ovarian[29], by mediating premature S-phase entry, ineffective DNA replication, and genomic instability. Alpha-enolase (ENO1), as a prominent glycolytic enzyme, was upregulated in multiple cancers and its overexpression was involved in tumor cell proliferation and metastasis, such as glioma[30], gastric[31], pancreatic[32], colorectal[33], BC[34]. So we speculated that ZNF132 might inhibit the progression of BC by regulating the expression of ENO1 and CCNE1.

As expected, methylation expression of the ZNF132 was significantly higher in BC than in adjacent normal tissues according to the TCGA database. Besides, 3 DNA methyltransferases were also

overexpressed in ZNF132^{low} group. Finally, methylation analysis in 10 CpG island sites, including cg169294963, cg11618529, cg07878486, cg00868383, cg24366702, cg00547077, cg13877915, cg03735888, cg12042659 and cg19776201, indicated that these sites were hypermethylated in BC sample, suggesting that DNA methylation in these sites might inactivate ZNF132 gene transcription. Importantly, clinical samples analysis provided us a strong evidence between promoter methylation status and expression of ZNF132 in BC. Therefore, ZNF132 hypermethylation may act as an independent risk factor in BC patients.

Conclusions

In conclusion, our research firstly demonstrated the expression, diagnostic ability and prognostic significance of ZNF132 based on TCGA database. Importantly, aberrant hypermethylation of ZNF132 mediated its silence in BC. Therefore, ZNF132 could be used as a potential target for diagnosis and prognostic evaluation in BC.

Declarations

Ethics approval and consent to participate

The present study was approved by the First Affiliated Hospital of Xi'an Jiaotong University Ethics committee. Written informed consent was obtained from all patients.

Consent for Publication

All patients consented to the publication of data and any associated images.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared that they had no competing interest

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Authors' contributions

All authors have read and approved the manuscript. ZL,JL, and PX conceived and designed the experiments; WFZ and XHZ performed the experiments; MX analyzed the data. In addition, PX provided BC specimens, as well as giving final approval of the version to be published.

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Not applicable.

Abbreviations

TCGA: The Cancer Genome Atlas; ZNF132: Zinc Finger Protein 132; AUC: An area under curve; ROC: receiver operating characteristic; OS: Overall survival; RFS: Relapse-free survial; DFS: Disease-free survival; BC: BC; GSEA: Gene Set Enrichment Analysis; MSP: Methylation-specific PCR;

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Tables

Table1. Clinical association between ZNF132 expression and clinicopathological variables in BRCA patients

		ZNF132 expression		c^2 test	Correlation	
Variable	Number	Low	High	P- value	*r*	P-value
Age						
≤60	611	289	321	0.053	-0.046	0.123
>60	493	263	231			
Her2						
Negative	652	292	360	0.001	-0.135	0.000
Positive	114	70	44			
ER						
Negative	179	111	68	0.000	0.209	0.000
Positive	601	266	335			
PR						
Negative	255	157	98	0.000	0.236	0.000
Positive	522	216	306			
Tumor size						
T1	282	121	161	0.006	-0.125	0.000
T2-T4	819	429	390			
Lymph node metastasis						
No	516	420	465	0.003	-0.056	0.064
Yes	568	118	81			
Distant metastasis						
No	964	464	450	0.233	-0.044	0.170
Yes	22	14	8			
Clinical stage						
I	183	80	103	0.058	-0.084	0.006
II--IV	899	462	437			

Table 2. Univariate analysis of prognostic factors of BRAC

Variable	Hazard ratio	95%CI	P-value
Age(>60/≤60)	1.928	(1.402,2.652)	0.000
Her2(Positive / Negative)	1.402	(0.592,1.834)	0.886
ER(Positive/Negative)	1.018	(0.659,1.573)	0.937
PR(Positive/Negative)	0.938	(0.637,1.379)	0.744
Tumor size(T2-T4/T1)	1.500	(1.020,2.206)	0.040
Lymph Node metastasis(Yes/No)	2.200	(1.542,3.140)	0.000
Distant metastasis(Yes/No)	4.749	(2.840,7.940)	0.000
Clinical stage(II-IV/I)	2.249	(1.337,3.783)	0.002
ZNF132 expression(High/Low)	0.829	(0.602,1.143)	0.252

Table 3. Multivariate analysis of prognostic factors of BRAC

Variable	Hazard ratio	95%CI	P-value
Age(□60/≤60)	2.278	(1.453,3.571)	0.000
Her2(Positive / Negative)	0.804	(0.405,1.597)	0.533
ER(Positive/Negative)	1.015	□0.507,2.032□	0.966
PR(Positive/Negative)	0.618	□0.330,1.154□	0.131
Tumor size(T2-T4/T1)	1.238	(0.548,2.800)	0.608
Lymph Node metastasis(Yes/No)	1.630	(0.959,2.770)	0.071
Distant metastasis(Yes/No)	3.427	(1.665,7.055)	0.001
Clinical stage(II-IV/I)	1.140	(0.386,3.369)	0.813
ZNF132 expression(High/Low)	1.129	(0.728,1.753)	0.587

Figures

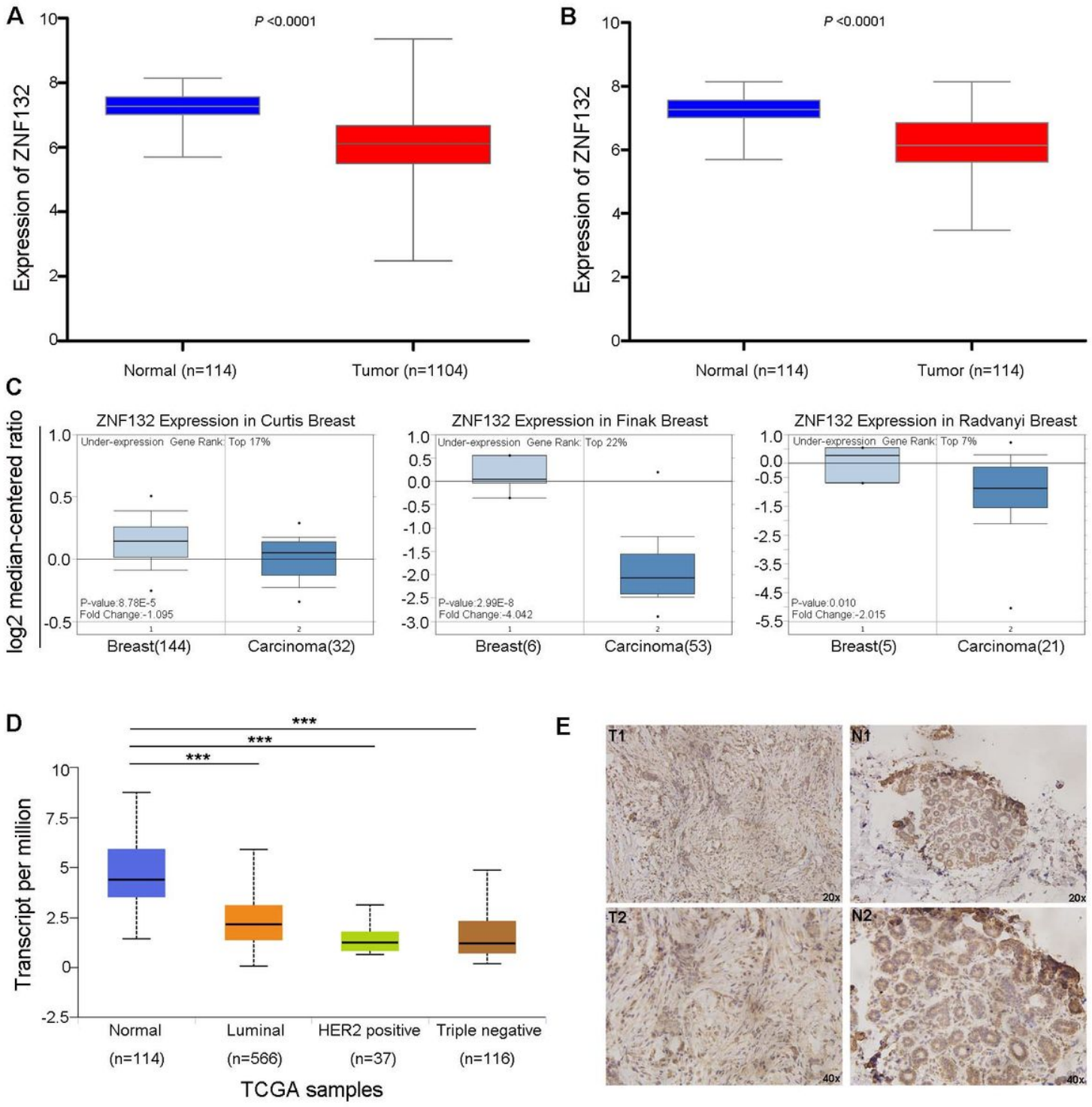

Figure 1

Expression of ZNF132 based on TCGA database. (A) ZNF132 was downregulated in 1104 BC tissues compared with 114 adjacent normal tissues. (B) 114 matched pairs of BC and adjacent normal tissues showed a similar trend. (C) Data from the Oncomine 4.5 database also revealed that mRNA expression of ZNF132 was significantly reduced in BC tissues than in normal tissues ($P \leq 0.01$). (D) ZNF132 expression was reduced in molecular subgroups, including luminal A, luminal B, Her2-positive, Triple negative BC. (E)

Immunohistochemistry analysis in BC and adjacent normal tissues. N1 (×20)/N2 (×40): normal tissue, T1 (×20)/T2 (×40): tumor tissue.

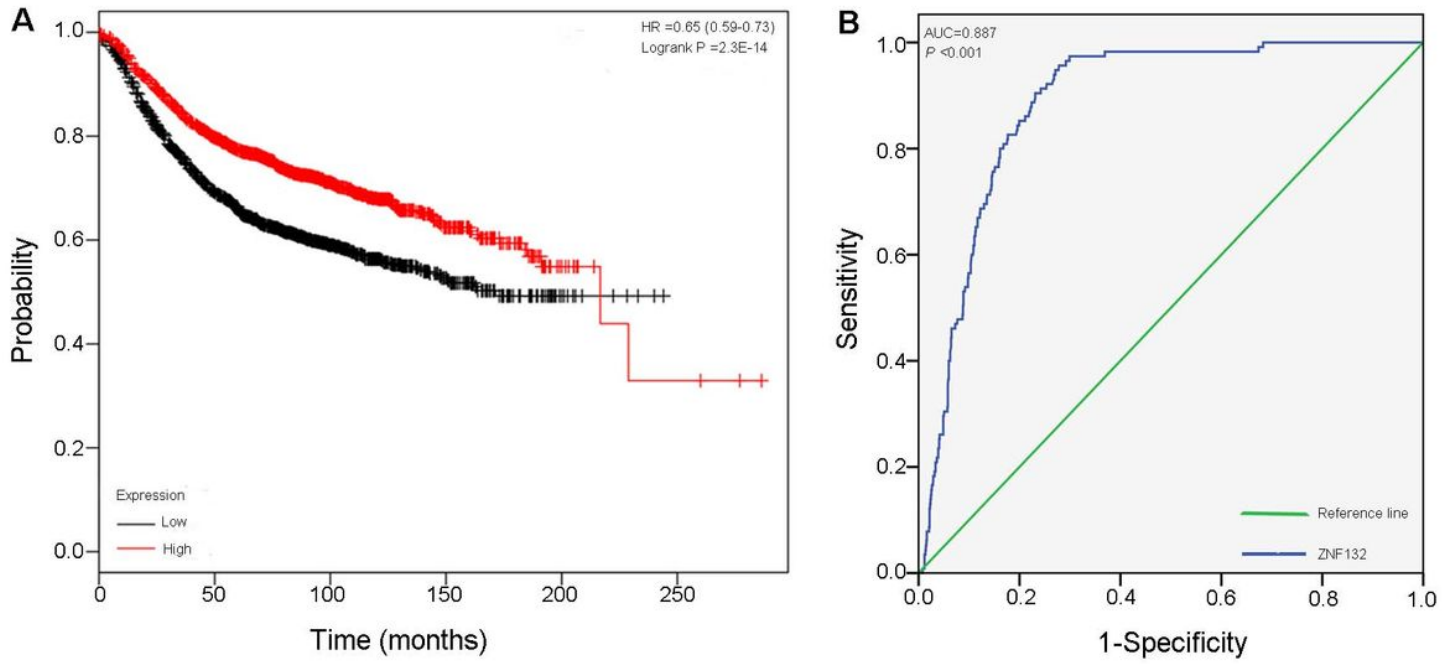

Figure 2

Prognostic and diagnostic value of ZNF132. (A) Survival analysis of ZNF132 in BC demonstrated that low ZNF132 expression was significantly associated with a reduced RFS using Kaplan-Meier Plotter. (B) The ROC curve indicated that ZNF132 possessed an adequate diagnostic ability for BC (AUC =0.887, P <0.001).

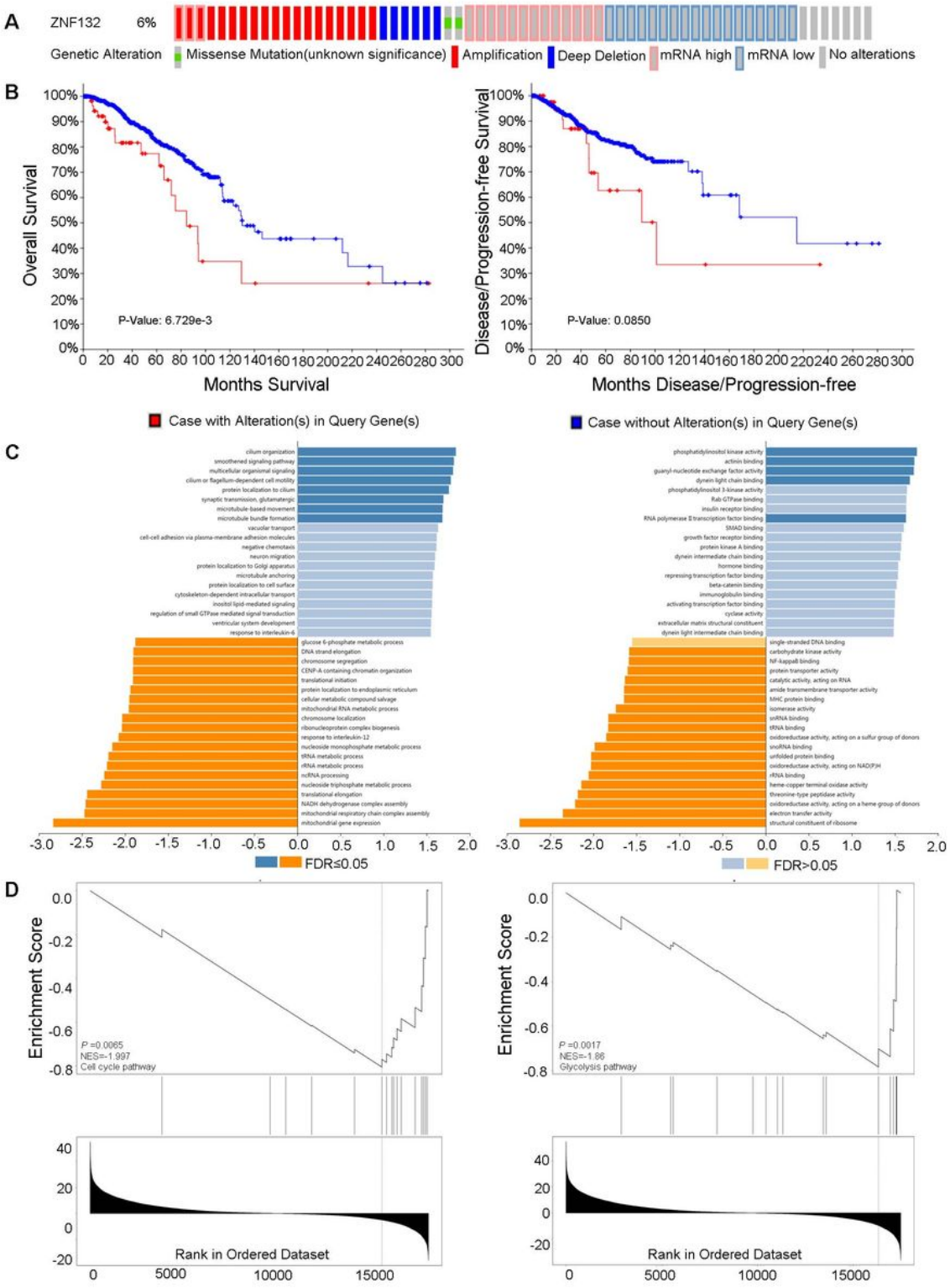

Figure 3

Bioinformatic analysis of ZNF132. (A) A total of 6% (58/960) of GC cases exhibited ZNF132 alteration. (B) OS analysis of BC patients with and without ZNF132 alteration. (C) Potential biological processes and biological pathways in BC were identified by GSEA analysis. (D) ZNF132 expression was negatively correlated with cell cycle and glycolysis pathway.

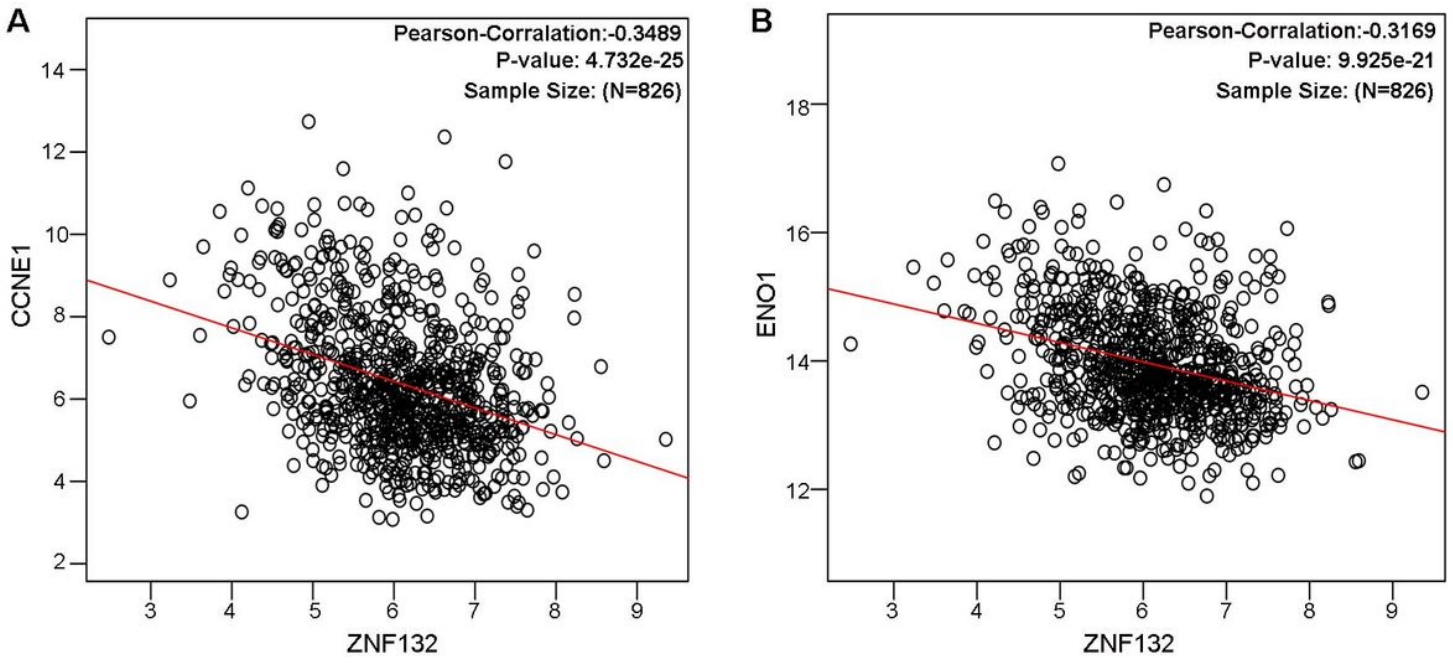

Figure 4

Genes involved in the cell cycle and glycolysis were associated with ZNF132 expression. (A) ZNF132 was negativly correlated with the expression of CCNE1 in cell cycle and (B) the expression of ENO1 in glycolysis.

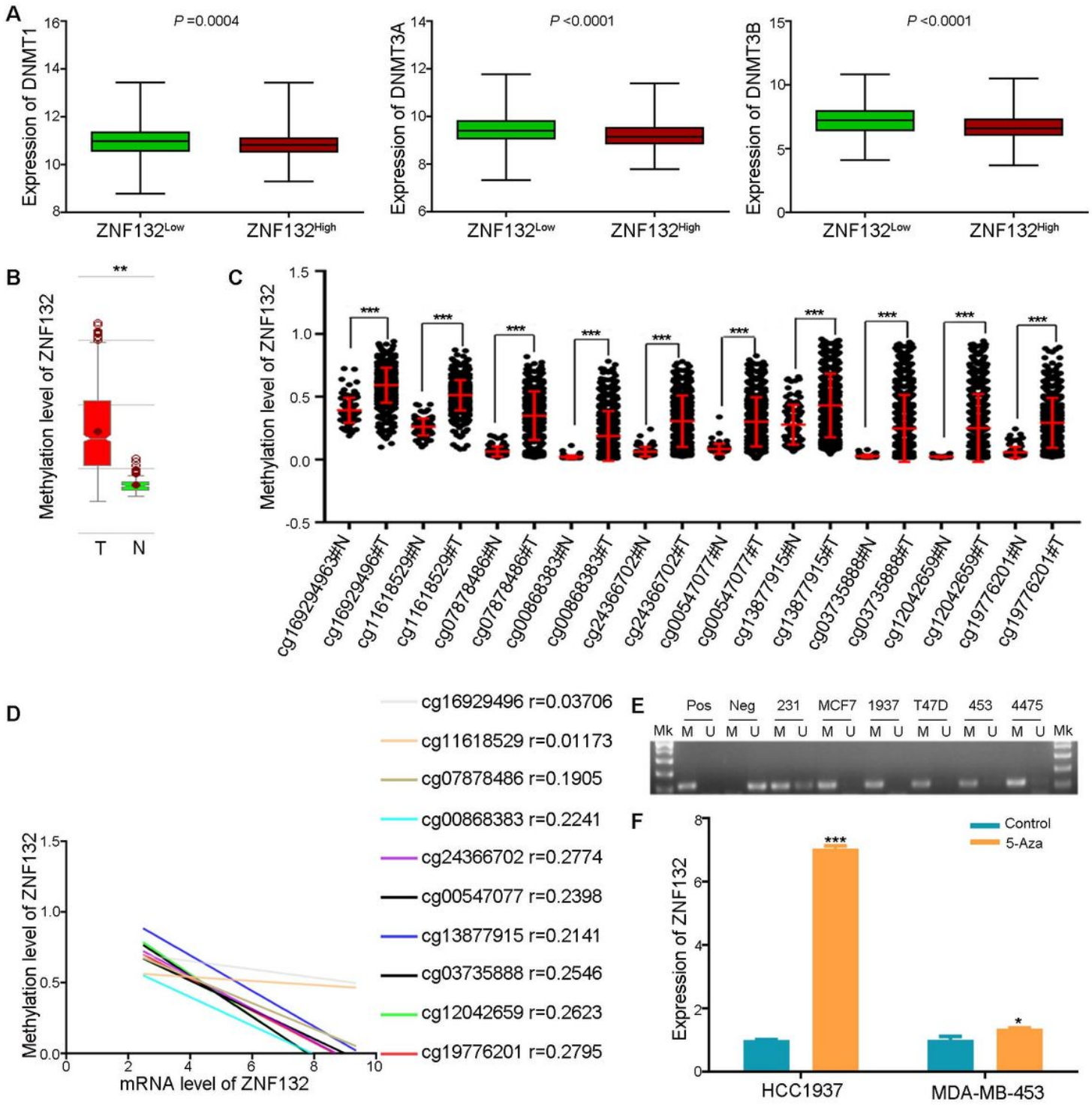

Figure 5

Aberrant methylation of ZNF132 in BC. (A) Expression of 3 DNA methyltransferases (DNMT1, DNMT3A and DNMT3B) in ZNF132High and ZNF132Low group. (B) MethHC anlysis demonstrated that the methylation level of ZNF132 in BC tissues was significantly higher than the normal sample. (C) Methylation level of ZNF132 in different CpG sites between BC tissues and matched normal tissues. (D) ZNF132 methylation level was negatively correlated with its gene expression. (E) Promoter methylation of

ZNF132 in BC cell lines was determined by the MSP assay. In vitro methylated DNA as positive control for methylated gene (Pos); Bisulfite-modified normal leukocyte DNA as positive control for unmethylated gene (Neg); Mk, DNA marker; M, methylated gene; U, unmethylated gene; 231, MDA-MB-231; 1937, HCC1937; 453, MDA-MB-453; 4475, DU4475. (F) mRNA level of ZNF132 was partially restored by 5-Aza-dC in HCC1937 and MDA-MB-453 cell line. Ctr, Control; 5-Aza, 5-Aza-Dc. Statistically significant differences were indicated: *P <0.01, **P <0.001, ***P <0.0001.

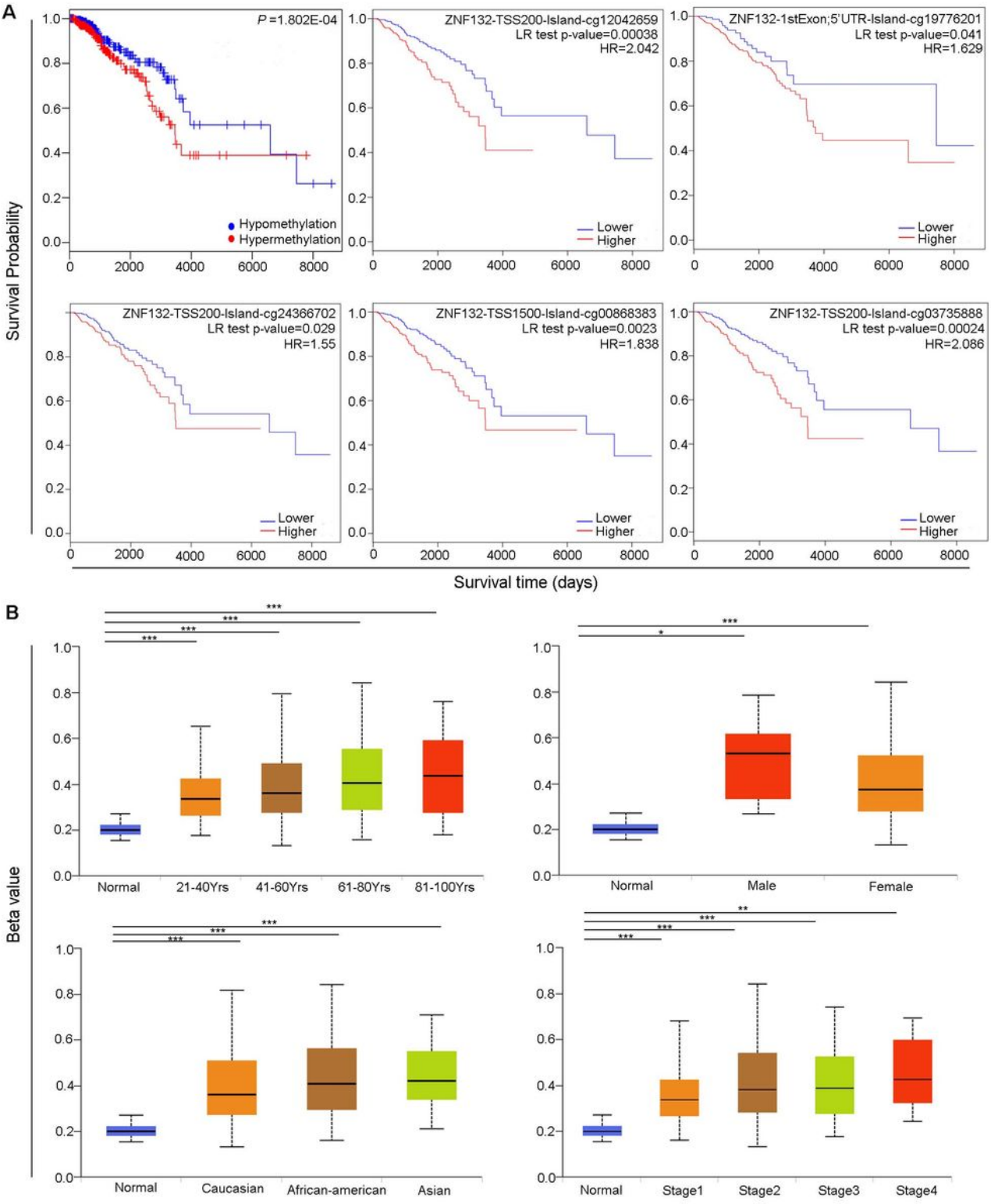

Figure 6

The effect of ZNF132 methylation on prognosis and clinicopathological features. (A) High methylation level of ZNF132 was negatively with overall survial in BC patients. (B) UALCAN was used to evaluate the impact of aberrant methylation on the clinicopathological features of BC patients.