PARPBP is a prognostic marker and confers chemotherapeutic resistance to breast cancer

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Abstract

Background: PARPBP (PARP1 Binding Protein) is an important homologous recombination inhibitor of human cells during DNA repair. But the expression and function of PARPBP in breast cancer remain unclear.

Methods: We evaluated the expression and prognostic value of PARPBP in breast cancer by immunohistochemical analysis in our patients (n=137) and public available datasets. Multiple in vitro and in vivo animal models characterized its functions.

Results: In the present study, we demonstrated that PARPBP was significantly upregulated in breast cancer tissues compared with normal breast tissues. PARPBP high expression group had poor overall survival (OS) compared to the PARPBP low expression group. Similar results got in TCGA and KM Plotter overall survival analysis. Knockdown of PARPBP suppressed breast cancer cell growth proliferation and colony formation. Furthermore, we found that high expression of PARPBP related with chemotherapy resistance in breast cancer. Depletion of PARPBP increases breast cancer cell apoptosis and DNA damage caused by chemotherapy drugs. Transcription factor forkhead box M1(FOXM1) activated PARPBP expression by directly binding to the promoter of PARPBP. Moreover, tumor xenograft experiments further demonstrated that PARPBP was involved in breast cancer chemoresistance.

Conclusions: Taken together, our results highlight PARPBP is a prognostic marker and confers chemotherapeutic resistance to breast cancer.

Background

Breast cancer is one of the most common malignancies whose management is complicated by its high molecular heterogeneity [1,2].Chemotherapy is a conventional and essential treatment which has reduced the death rate for breast cancer patients[3]. However, resistance to chemotherapeutic agents is a major obstacle for the effective treatment in breast cancer. Chemotherapy-refractory breast cancer patients recurs within months to years after treatment and leads to subsequent death. Therefore, there is a critical need to elucidate the mechanisms of resistance to chemotherapy and develop new chemosensitizers.

Induction of DNA damage is a predominant anti-tumor mechanism for many chemotherapy drugs[4]. Platinum drugs induce DNA damages by binding to DNA therefore creating inter- or intra-strand cross links. Anthracycline antibiotics cause DNA damage by embedding between the DNA double-stranded bases. However, some tumor cells can develop drug resistance through repair mechanisms that counteract the DNA damage. Research has shown that enhancing the DNA repair capability of tumor cells results in intrinsic and therapy-induced chemoresistance.

PARPBP (PARP1 Binding Protein), also named PARI or C12orf48, is an important homologous recombination inhibitor of human cells during DNA repair[5]. Previous studies have shown that PARPBP abnormally expressed in a variety of tumors and interacts directly with some regulators of DNA repair,

including PARP-1, PCNA and RAD51[6,7]. Downregulation of PARPBP could preserve genomic stability and improve homologous recombination. A previously study by Pitroda et al. developed a Recombination Proficiency Score (RPS) which calculated based on the expression levels for four genes including PARPBP[8]. They showed that RPS provides predictive characterization of individual breast cancers. Low RPS breast tumors simultaneously exhibit a heightened sensitivity to DNA-damaging therapy[9]. However, the expression and function of PARPBP in breast cancer remain unclear.

In this study, we provided evidence that PARPBP is up-regulated in breast cancer and correlate with prognosis of breast cancer patients. Knockdown of PARPBP suppressed breast cancer cell growth proliferation and colony formation. Furthermore, we found that high expression of PARPBP related with chemotherapy resistance in breast cancer. Depletion of PARPBP increases breast cancer cell apoptosis and DNA damage caused by chemotherapy drugs. Transcription factor forkhead box M1(FOXM1) activated PARPBP expression by directly binding to the promoter of PARPBP. Moreover, tumor xenograft experiments further demonstrated that PARPBP was involved in breast cancer chemoresistance. Taken together, our results highlight PARPBP is a prognostic marker and confers chemotherapeutic resistance to breast cancer.

Materials And Methods

Bioinformatics analysis

PARPBP expression levels in various types of cancers and normal tissues were identified in the Oncomine database (www.oncomine.org)[10].The threshold was set at a 1.5-fold difference in expression between cancers and normal tissues with a P-value < 0.0001. The Cancer Genome Atlas (TCGA)[11] and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC)[12] datasets were analyzed and the figures were generated using Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancerpku.cn/index.html)[13] and the cBio Cancer Genomics Portal (http://cbioportal.org)[14,15]. All TCGA data included in this manuscript are following the TCGA publication guidelines. The KM Plotter Online Tool (http://www.kmplot.com) is a public database that combined survival analysis across multiple microarray datasets[2,16].

Patients and tissue specimens

In total, 162 breast cancer tissues (cohorts 1, 2) were obtained from the Sun Yat-Sen University Cancer Centre and prepared as paraffin blocks. The Ethics Committee of Sun Yat-Sen University Cancer Centre Health Authority approved this study. All samples were collected in accordance with Health Insurance Portability and Accountability Act guidelines and the Declaration of Helsinki. Cohorts 1: a total of 137 breast cancer tissues used for survival analysis were collected between March 2005 and September 2011. Using the formalin-fixed paraffin-embedded (FFPE), a tissue microarray was built for immunohistochemistry (IHC) studies. Cohorts 2: a total of 25 patients received anthracycline-based neoadjuvant chemotherapy. Tissue specimens were collected between March 2017 and September 2018

before patients received neoadjuvant chemotherapy. Progressive disease or stable disease defined as chemo resistant. Complete response or complete response defined as chemo sensitive.

Cell lines and culture conditions

Human breast cancer cell lines (MDA-MB-231, BT549, HCC38, T47D, MDA-MB-468, BT474, Skbr-3 and MCF-7) and normal mammary epithelial cell lines (MCF-10A) were obtained from the American Type Culture Collection (Manassas, VA, USA). The drug resistance cell subline of MCF-7 (MCF-7/ EPI) was derived from the parental cells by using low concentration epirubicin stepwise incremental method[17]. All cells were passaged in our laboratory for fewer than 6 months on receipt and were tested mycoplasma free.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The Nano Drop ND-1000 Spectrophotometer (Nano Drop, Waltham, MA, USA) was used to evaluate RNA quality. Complementary DNA was synthesized using the PrimeScript RT reagent kit (Takara Bio Inc., Dalian, China). RT-PCR were performed using SYBR Premix Ex Taq (Takara Bio Inc., Dalian, China). Each reaction was performed in triplicate. The primer sequences are given in Supplementary Table S1. The values were normalized to internal controls and fold changes were calculated through relative quantification ($2^{-\Delta\Delta Ct}$).

CCK8 assay

Cell viability was assessed by cell counting kit-8 assay (CCK-8; Dojindo Laboratories, Kumamoto, Japan). The cells (1×10^3) were seeded into 96-well plates. After 2 h of CCK-8 solution (10 μL) incubation at 37 °C, the absorbance at 450 nM was measured using a microtiter plate reader (Bio-Tek EPOCH2, BioTek Instrument, Inc., USA)[18].

Colony formation assay

At 48 h after transfection, cells were cultured with or without EPI at the indicated concentrations for 3h. Then, the cells were harvested, seeded 500 cells per well into six-well plates and cultured for an additional 2 weeks. For scoring the colony-forming units, plates were stained with crystal violet (crystal violet 0.5%, ethanol 2%) and photographed.

Western blot analysis

Western blot analysis was performed as previously described[19]. The following antibodies were used in western blot analysis: anti-PARPBP (1:500, Abcam, USA), anti- FOXM1 (1:1000, Cell Signaling Technology, MA, USA) and β-actin (1:5000, Cell Signaling Technology, MA, USA). The membranes were further incubated in a secondary antibody (1:5000 dilution) and ECL reagents (New England Biolabs, Ipswich, MA, USA) were used to detect the protein.

Apoptosis assay

Cell apoptosis was detected using an Andy Fluor 488 Annexin V and PI Apoptosis Kit (GeneCopoeia, Rockville, MD, USA). After breast cancer cells were treated with or without EPI at the indicated concentrations for 48 h, they were harvested and resuspended in 400 ml of binding buffer. Next, 5 μl of Annexin V-FITC and 2 μl of PI were added to the suspensions, and the cells were incubated in the dark at 4 °C for 15 min. Ten thousand events were considered for the analysis.

Immunohistochemistry (IHC) analysis

Immunohistochemistry staining was performed as described previously[20]. Staining intensity scored as (0, negative staining; 1, weak staining; 2, moderate staining; 3, strong staining) and the extent of staining scored as the percentage of positive cells (1,0～25%; 2, 26～50%; 3, 51～75%; 4, 76～100%). The final quantitation of each staining was obtained by multiplying the two scores. PARPBP expression was classified into two groups: high expression group (score was higher than 2.0) and low expression group (score was 2.0 or less).

RNA interference and plasmid

Sequences of siRNAs were listed in Supplementary Table S2. Expression construct pcDNA3.1-PARPBP-HA, pcDNA3.1-FOXM1 and shFOXM1 were obtained from GeneCopoeia, USA. The lentivirus knocking down PARPBP (shPARPBP) were packaged and purchased from GenePharma, Shanghai using siPARPBP1 corresponding sequences. The cell transfection was performed using Lipofectamine 2000 (Invitrogen, USA).

Promoter Reporters and Dual-luciferase Assay

The PARPBP promoter region (–1500, +76) was amplified and the fragment was cloned into the luciferase reporter plasmids pGL3-basic vector (Promega, USA), designated as pGL3-PARPBP. Mutant construct pGL3-PARPBP-MU was generated by site-directed mutagenesis. Luciferase assay was performed as described previously[19]. Each experiment analysis was repeated three times.

Chromatin immunoprecipitation assay

The chromatin immunoprecipitation (ChIP) assay was performed using a Zymo-Spin ChIP kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. Chromatin was mechanically sheared using sonication after cells were collected and cross-linked by formaldehyde. Protein-DNA complexes were precipitated by control immunoglobulin G and anti-FOXM1 antibody respectively, followed by eluting the complex from the antibody. The amount of DNA was further assessed by quantitative real-time PCR, using the primers specific for PARPBP promoter and SYBR Select Master Mix (Applied Biosystems, Grand Island, NY, USA).

Tumor xenograft experiments

Cells were collected and suspended in 200 μl of PBS at a concentration of 5×10^6 cells per ml, then injected into the mammary fat pads of six-week-old female BALB/c nude mice. Ten days after injection, the mice were then intraperitoneally injected with 5 mg/kg EPI (once per 2 days) for another 2 weeks. The xenograft tumors were harvested after 4 weeks. Tumor tissues were also processed and sectioned for histological evaluation. Animal experimentation was conducted in accordance with the guidelines of the local institutional animal care and use committee.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 software (SPSS, Chicago, IL, USA). Student's t-test was used to make a statistical comparison between groups. The Chi-squared test and Fisher's exact test were used to investigate the significance of the correlation of PARPBP expression with clinicopathological features in breast cancer patients. Survival curves were calculated by the Kaplan-Meier method and compared with the log-rank test. A P value <0.05 was considered significant.

Results

PARPBP expression is up-regulated in breast cancer and correlate with prognosis of breast cancer patients

We explored PARPBP mRNA level in common types of human cancer and normal tissues by using GEPIA database. The PARPBP mRNA expression was significantly upregulated in breast invasive carcinoma (BRCA) and other17 common types of cancer tissues compared with adjacent normal tissues (Figure 1A). Similarly, Oncomine database analysis revealed that PARPBP mRNA expression of breast cancer increased in 10 data sets compared to the normal tissues (Figure 1B). Then, the expression of PARPBP at protein level was further validated by immunohistochemistry (IHC) on breast cancer tissue microarray (all subtypes, n =137). While the PARPBP staining in adjacent normal breast tissues was usually not detected, high proportion of the breast cancer tissues displayed strong (47/137), moderate (37/137) or low (41/137) PARPBP staining and only 12 patients not detected (Figure 1C). These results suggested that PARPBP was significantly upregulated both at the mRNA and at the protein levels in breast cancer tissues compared with normal breast tissues. Based on the final quantitation of each breast cancer tissue IHC staining (multiplying the staining intensity score and the extent of staining score), 137 patients were classified into two groups: PARPBP high expression group (score > 2.0; n=84) and PARPBP low expression group (score≤2.0; n=53). As shown in Table 1, the expression of PARPBP was positively correlated with the tumor status, lymph node status and TNM stage of breast cancer patients. Furthermore, we found that PARPBP high expression group had poor overall survival (OS) compared to the PARPBP low expression group (Figure 1D). Similar results got in TCGA (Figure 1E) and KM Plotter overall survival analysis (Figure 1F).

PARPBP promotes breast cancer cell proliferation

We tested PARPBP mRNA levels in MCF-10A and 9 human breast cancer cell lines. As shown in Figure 2A, PARPBP mRNA levels are up-regulated in 6 human breast cancer cell lines, including MCF-7, MCF-7/EPI, TD47, MDA-MB-468, BT474 and Skbr-3. Then, we investigated the function of PARPBP in breast cancer. Two specific small interference RNAs and negative control were transiently transfected into MCF-7 or MCF-7/EPI, respectively. The efficiency of PARPBP knockdown was analyzed by qRT-PCR. In cell viability assays, both MCF-7 and MCF-7/EPI cells transfected with si-PARPBP1/2 slowed cell growth and proliferation compared with the negative control group (Figure 2B). For colony formation assays, two lentivirus particles containing shNC and shPARPB were packaged and infected into MCF-7 and MCF-7/EPI cells. The results showed that PARPBP knockdown also decreased the ability of colony formation of breast cancer cells (Figure 2C). Conversely, the proliferation and colony formation ability of PARPBP-overexpressed MDA-MB-231 and BT549 cells was increased significantly relative to the control group (Figure 2D-E). These observations suggested that PARPBP has a positive effect on breast cancer cell growth in vitro.

High expression of PARPBP related with chemotherapy resistance in breast cancer

To assess the possible role of PARPBP in chemoresistance, we performed immunohistochemical staining for PARPBP in 25 cases of human breast cancer treated with anthracyclines-based neoadjuvant chemotherapy. The results showed that the expression of PARPBP in chemo resistant tumors were significantly higher than that in nonresistant tumors (Figure 3A and 3B). Meanwhile, ectopic PARPBP expression significantly increased cell viability in MDA-MB-231 and BT549 cells with EPI treatment (Fig. 3C and 3D). Our above results indicated that PARPBP might attenuate breast cancer cell sensitivity to chemotherapy drugs.

Depletion of PARPBP increases breast cancer cell apoptosis and DNA damage caused by chemotherapy drugs

Next, we further examined cell growth and apoptosis induced by EPI in MCF-7 and MCF-7/EPI cells with PARPBP inhibition. In colony formation assay, we showed knockdown of PARPBP enhanced anti-tumor efficacy of EPI in MCF-7 and MCF-7/EPI cells (Figure 4A and 4B). After EPI treatment, the apoptosis proportion of cells transfected with PARPBP shRNA was significantly increased relative to cells treated with negative control (Figure 4C and 4D). Meanwhile, PARPBP depletion significantly increased EPI induced level of γH2AX but decreased BRCA1 protein levels (Figure 4E and 4F).

PARPBP promotes breast cancer chemoresistance in vivo

To investigated the functional role of PARPBP in regulating the drug resistance of breast cancer in vivo, cells were injected into the mammary fat pads of female BALB/c nude mice followed by treatment with EPI (Figure 5A). The volume and weight of tumors in PARPBP overexpressed group (PARPBP/231) were obviously higher than that in the control group (Figure 5B to 5F). That means EPI effectively inhibited tumor growth in the mice with control tumors but not in the mice with PARPBP-overexpressing tumors.

FOXM1 directly binds to the PARPBP promoter and regulates its activity

We used TCGA and METABRAC datasets to identify the putative co-expression genes of PARPBP in human breast cancer. FOXM1 and BURB are the intersection of the top 20 co-expression genes in the two datasets (Figure 6A). Then, we focus on FOXM1 which is a transcriptional activator and a critical mediator of epirubicin and paclitaxel resistance in breast cancer (Figure 6B and 6C). We transfected FOXM1 shRNA into MCF-7 and MCF-7/EPI cells (Figure 6D). The results indicated that the mRNA level and protein level of PARPBP were significantly decreased with FOXM1 knockdown (Figure 6D and 6E). Similarly, MCF-7 and MCF-7/EPI cells treated with FoxM1 inhibitor thiostrepton (THR) at concentrations of 4 and 8 μM depressed PARPBP levels (Figure 6F). It was increasing with the mRNA level of PARPBP, when MDA-MB-231 and BT549 cells were transfected with the increasing dosage of FoxM1(Figure 6G). Then, we explored whether FOXM1 regulates PARPBP promoter activity. We scanned PARPBP gene promoter region with the canonical binding DNA motifs of FOXM1 (5'-TAAaCa-3') and a putative binding DNA motif were found (Figure 6H). Then, we also generated a mutated PARPBP promoter construct (Figure 6H). The effect of FOXM1 expression on PARPBP promoter activity significantly attenuated by disruption of the FOXM1 binding site (Figure 6I). Additionally, we performed chromatin immunoprecipitation assay and verified the binding of FOXM1 to the endogenous PARPBP promoter region (Figure 6J). Collectively, our findings supported that FoxM1 could transcriptionally activate PARPBP by interacting with predicted binding sites.

Discussion

Breast cancer persists as a leading cause of cancer death in women worldwide[21]. Although systemic chemotherapy is effective in early and advanced breast cancer, the high rate of recurrence and resistance are still the major challenges in breast cancer treatment. In the present study, we revealed a role for PARPBP in breast cancer prognostic and chemoresistance.

Previous studies have reported that PARPBP overexpression associated hyperproliferation and severe clinical outcomes in lung, gastric, pancreatic, cervical cancers, hepatocellular carcinoma and myeloid leukemia[22-25]. Here, we found that PARPBP was significantly upregulated in breast cancer tissues both at mRNA and protein levels compared with that in normal breast tissues. The expression of PARPBP was positively correlated with the tumor status, lymph node status and TNM stage of breast cancer patients. In addition, high expression of PARPBP has shown to be associated with poor prognosis in breast cancer patients, which suggested that PARPBP may be a promising prognostic biomarker.

In current study, we observed that PARPBP upregulated in breast cancer and had a relatively high expression in MCF7, MCF7/EPI and T47D cells with ER-positive and a relatively low expression in triple negative breast cancer cells (MDA-MB-231 and BT549 cells). PARPBP knockdown decreased cell proliferation and colony formation in breast cancer. Conversely, the proliferation and colony formation ability of PARPBP-overexpressed breast cancer cells were increased significantly relative to the control group. PARPBP is highly expression in ER-positive breast cancers which are usually shown to be

insensitive to chemotherapy. DNA repair capacity is critical for survival of cancer cells upon therapeutic DNA damage and thus is an important determinant of susceptibility to chemotherapy in cancer patients[26]. PARPBP is an element of the homologous recombination pathway of DNA repair. PARPBP downregulation improves genomic stability and homologous recombination in HR-deficient Fanconi Anemia/BRCA pathway in activated cancer cells[6]. Meanwhile, we found the expression of PARPBP in chemo resistant tumors were significantly higher than that in nonresistant tumors. Therefore, we raised a possibility that PARPBP contributed chemotherapy resistance.

Epirubicin (EPI), a representative anthracycline antibiotic, is the mainstay chemotherapy drug in the treatment for breast cancer. Epirubicin intercalated into DNA, which could have caused DNA damage and observed necrosis of cancer cells. In this study, knockdown of PARPBP increased the apoptosis of breast cancer cells caused by EPI. Moreover, we also showed depletion of PARPBP increased DNA damage induced by EPI. Histone variant H2AX phosphorylation in response to DNA damage is the major signal for recruitment of DNA damage-response proteins to the regions of damaged chromatin[27]. Usually, γH2AX levels are used as an indicator of the degree of DNA damage[28]. We found that depletion of PARPBP attenuated intracellular γH2AX level in the cancer cells treated by EPI. In vivo experiments, we also found PARPBP enhanced breast cancer cell chemoresistance.

Through the analysis of the TCGA and METABRIC databases, we found that the expression of PARPBP was positively correlated with the expression of FOXM1. FOXM1 is a proliferation-associated transcription factor with important functions in cell proliferation, differentiation and apoptosis[29]. FOXM1 is generally highly expressed in several aggressive human carcinomas and related to oncogenesis in many tissue types, including breast cancer[30]. Investigators have also shown that FOXM1 might be involved in resistance to chemotherapy drugs (cisplatin, paclitaxel and epirubicin)[31,32]. Here, we found a putative binding site of FOXM1 in PARPBP promoter and confirmed PARPBP transcription is modulated by FOXM1 directly. Our findings additionally identified an important role for FOXM1 in breast cancer chemoresistance by regulating PARPBP expression.

Conclusion

In conclusion, we show that PARPBP is upregulated in breast cancer and that it might regulate by FOXM1. High PARPBP expression levels are associated with poor overall survival in breast cancer patients. We also demonstrate that downregulation of PARPBP can effectively promote EPI sensitivity in breast cancer cells. PARPBP might be an attractive possibility for the treatment of breast tumors that were resistant to chemotherapy. Our findings may help to understand the tumor development and provide a new promising therapy target for breast cancer.

List Of Abbreviations

BRCA	Breast invasive carcinoma

ChIP Chromatin immunoprecipitation

FFPE Formalin-fixed paraffin-embedded

FOXM1 Transcription factor forkhead box M1

GEPIA Gene Expression Profiling Interactive Analysis

IHC Immunohistochemistry

METABRIC Molecular Taxonomy of Breast Cancer International Consortium

OS Overall survival

PARPBP PARP1 Binding Protein

qRT-PCR Quantitative reverse transcription polymerase chain reaction

RPS Recombination Proficiency Score

TCGA The Cancer Genome Atlas

Declarations

Ethics approval and consent to participate

This study had been approved by the Ethics Committee of Guangdong Provincial People's Hospital and The Ethics Committee of Sun Yat-Sen University Cancer Centre Health Authority. All patients provided written informed consent for translational research. All samples were collected in accordance with Health Insurance Portability and Accountability Act guidelines and the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusion of this article are included within the article and its additional files. Please contact the author for additional reasonable data requests.

Competing interests

No conflicts of interest.

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Authors' contributions

BC, NL and HT designed the experiment and critically revised the paper; BC, JL and DD performed most of the experiments; DD and RC contributed to collected the patients data; BC, JL and DD performed the statistical and bioinformatic analysis; HT cultured cells and built animal models; BC wrote the manuscript. All authors read and approved the final manuscript.

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Authors' information

Not applicable.

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Tables

Table 1. Relationship between PARPBP expression and clinicopathologic factors of breast cancer patients

Variables	n=137	PARPBP				P value
		high	No. (%)	low	No. (%)	
Age (years)						1
<50	75	46	54.76%	29	54.72%	
≥50	62	38	45.24%	24	45.28%	
Menopause						0.857
yes	52	31	36.90%	21	39.62%	
no	85	53	63.10%	32	60.38%	
Tumor status (T)						**<0.001**
T1	35	11	13.10%	24	45.28%	
T2+T3+T4	102	73	86.90%	29	54.72%	
Lymph node status (N)						**<0.001**
N0	53	18	21.43%	35	66.04%	
N1	37	22	26.19%	15	28.30%	
N2	22	20	23.81%	2	3.77%	
N3	25	24	28.57%	1	1.89%	
Histological grade						0.295
G1+G2	106	62	73.81%	44	83.02%	
G3	31	22	26.19%	9	16.98%	
TNM stage						**<0.001**
I-II	87	38	45.24%	49	92.45%	
III-IV	50	46	54.76%	4	7.55%	
Subtype						0.861
non-TNBC	114	69	82.14%	45	84.91%	
TNBC	23	15	17.86%	8	15.09%	

Supplementary Table Legends

Supplementary Table S1. The list of primer sequences.

Supplementary Table S2. The list of siRNAs sequences.

Figures

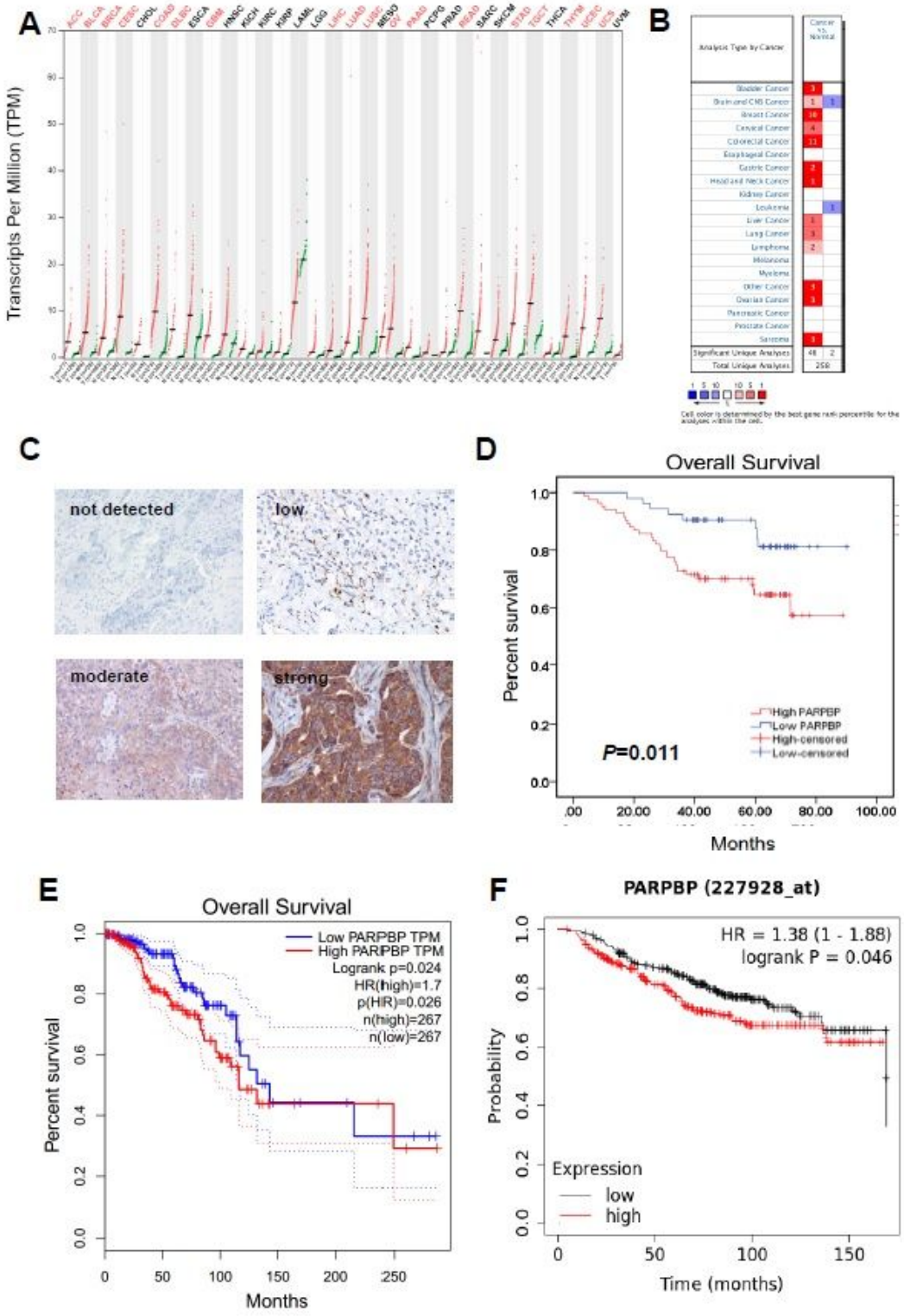

Figure 2

PARPBP expression is up-regulated in breast cancer and correlate with prognosis of breast cancer patients (A) PARPBP mRNA expression in different normal human tissues and cancer tissues from GEPIA and (B) Oncomine database. (C) Representative IHC images of PARPBP in breast cancer tissues. High levels of PARPBP correlate with poor prognosis. The OS curves for (D) our cohorts1,(E)TCGA and (F) KM Plotter Online Tool.

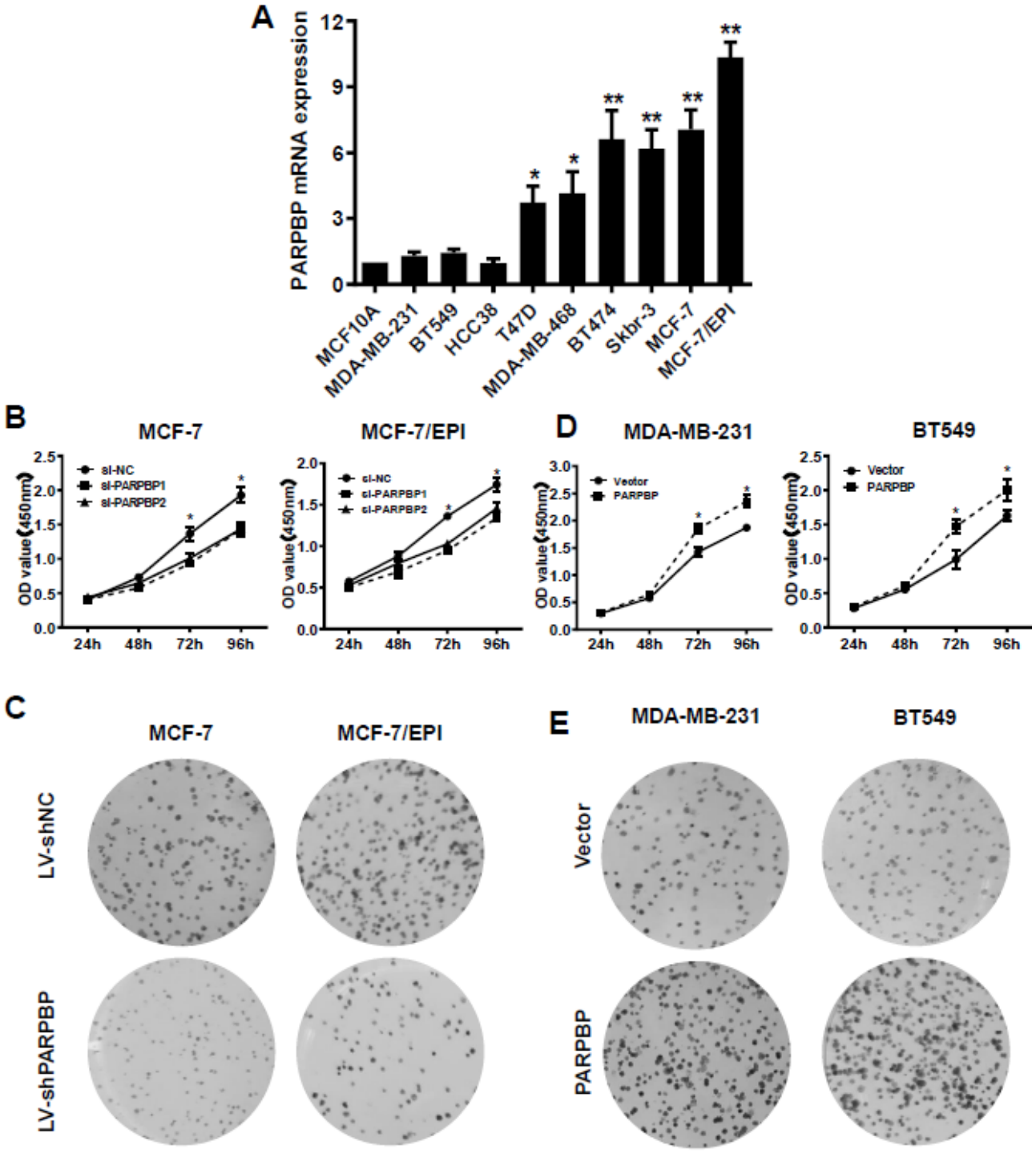

Figure 4

PARPBP promotes breast cancer cell proliferation (A) PARPBP mRNA levels determined by qRT-PCR in MCF-10A and 9 human breast cancer cell lines. (B) The growth of MCF-7 and MCF-7/EPI cells infected with si-PARPBP1/2 or si-NC was assayed by CCK8. *P < 0.05. (C) Colony formation assays performed on MCF-7 and MCF-7/EPI cells transfected with shPARPB or shNC. (D) The growth of MDA-MB-231 and BT549 cells transfected with PARPBP overexpressing or control vector was assayed by CCK8. *P < 0.05.

Colony formation assays performed on MDA-MB-231 and BT549 cells transfected with PARPBP overexpressing or control vector.

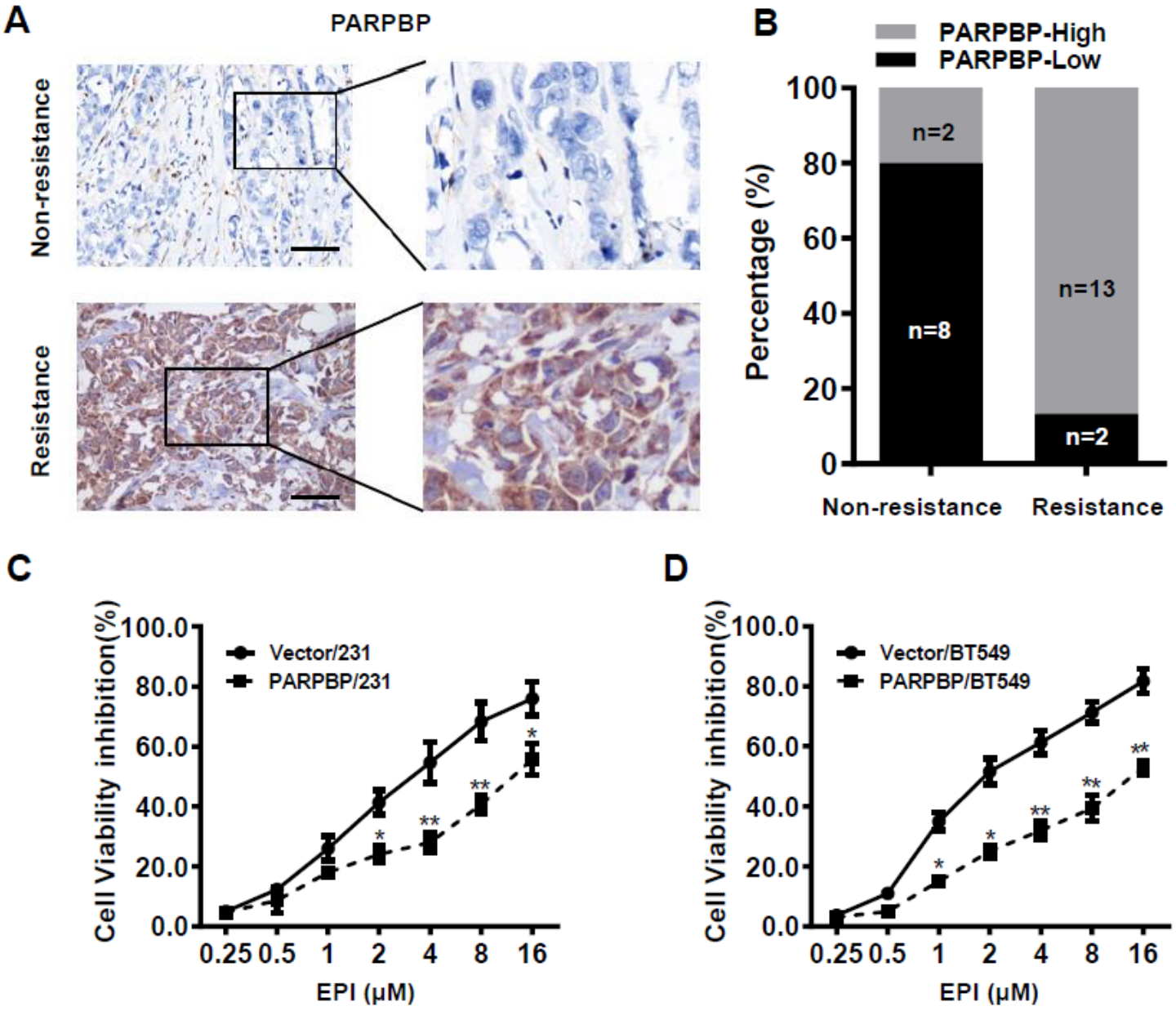

Figure 5

High expression of PARPBP related with chemotherapy resistance in breast cancer (A) Representative images of immunohistochemical staining of PARPBP in chemo-resistant and non-resistant tumors are shown. Scale bars, 50 μm. (B) Expression of PARPBP in chemo resistant tumors were significantly higher than that in non-resistant breast tumors. (C) PARPBP/231 and Vector/231 cells were treated with different concentrations of EPI for 48 h, respectively. (D) PARPBP/BT549 and Vector/BT549 cells were treated with different concentrations of EPI for 48 h, respectively. *P < 0.05, **P < 0.01.

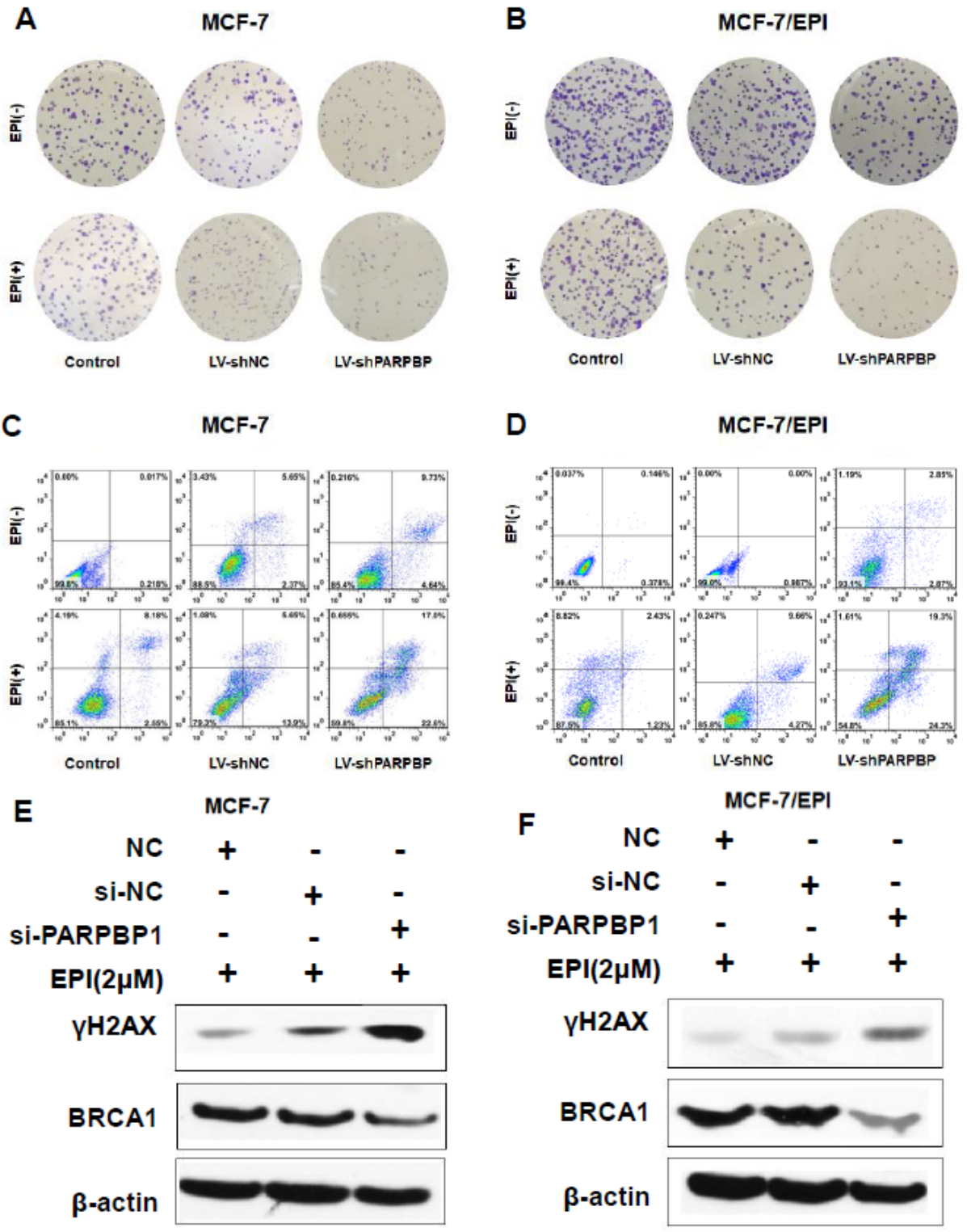

Figure 7

Depletion of PARPBP increases breast cancer cell apoptosis and DNA damage caused by chemotherapy drugs Colony-forming ability of the Control, LV-shNC transfected and LV-shPARPBP-transfected (A) MCF-7 and (B)MCF-7/EPI cells in the absence or presence of EPI (2 μM) for 48 h. The apoptotic rates of (C) MCF-7 and (D)MCF-7/EPI cells transfected with LV-shNC and LV-shPARPBP in the absence or presence EPI (2 μM) for 48 h were visualized by flow cytometry. Western blot analysis of γH2AX and BRCA1

expression in(E) MCF-7 and (F)MCF-7/EPI cells and cells transfected with LV-shNC and LV-shPARPBP after EPI treatment (2 μM) removal.

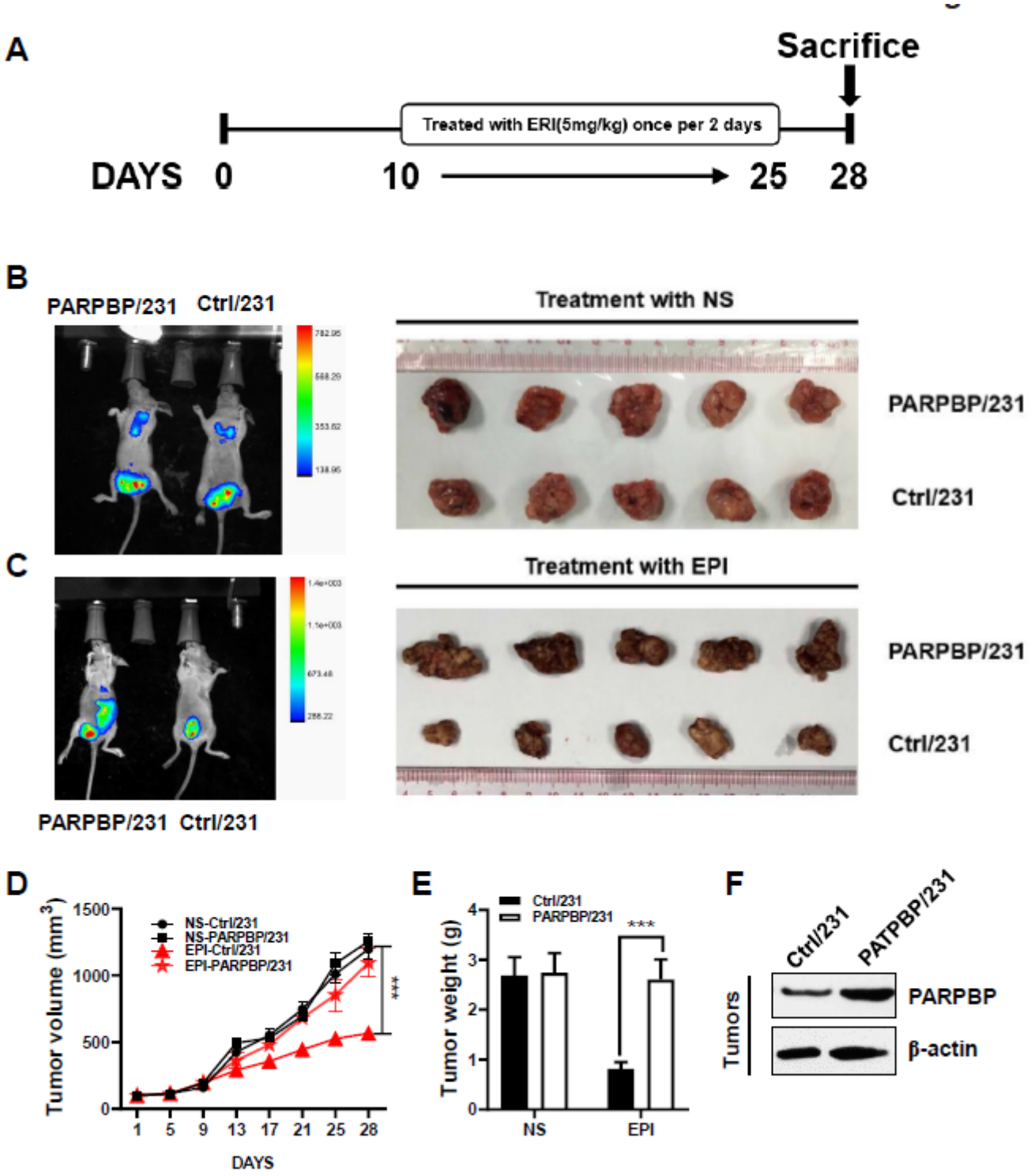

Figure 10

PARPBP promotes breast cancer chemoresistance in vivo (A) PARPBP/231 and Ctrl/231 cells were respectively injected into the mammary fat pads of nude mice (n = 5). Tumor development was allowed for 10 days, and then the mice were intraperitoneally injected with 5 mg/kg EPI or normal saline for another 2 weeks. Tumors from PARPBP/231 and Ctrl/231 mice that were, respectively, treated with

normal saline(B) and EPI (C)are shown. (D) The growth curves of the tumors are plotted. (E)The weights of the xenograft tumors are summarized. ***P < 0.001 vs respective control in Student's t test. (F) Expression of PARPBP in breast cancer xenografts was examined by western blotting.

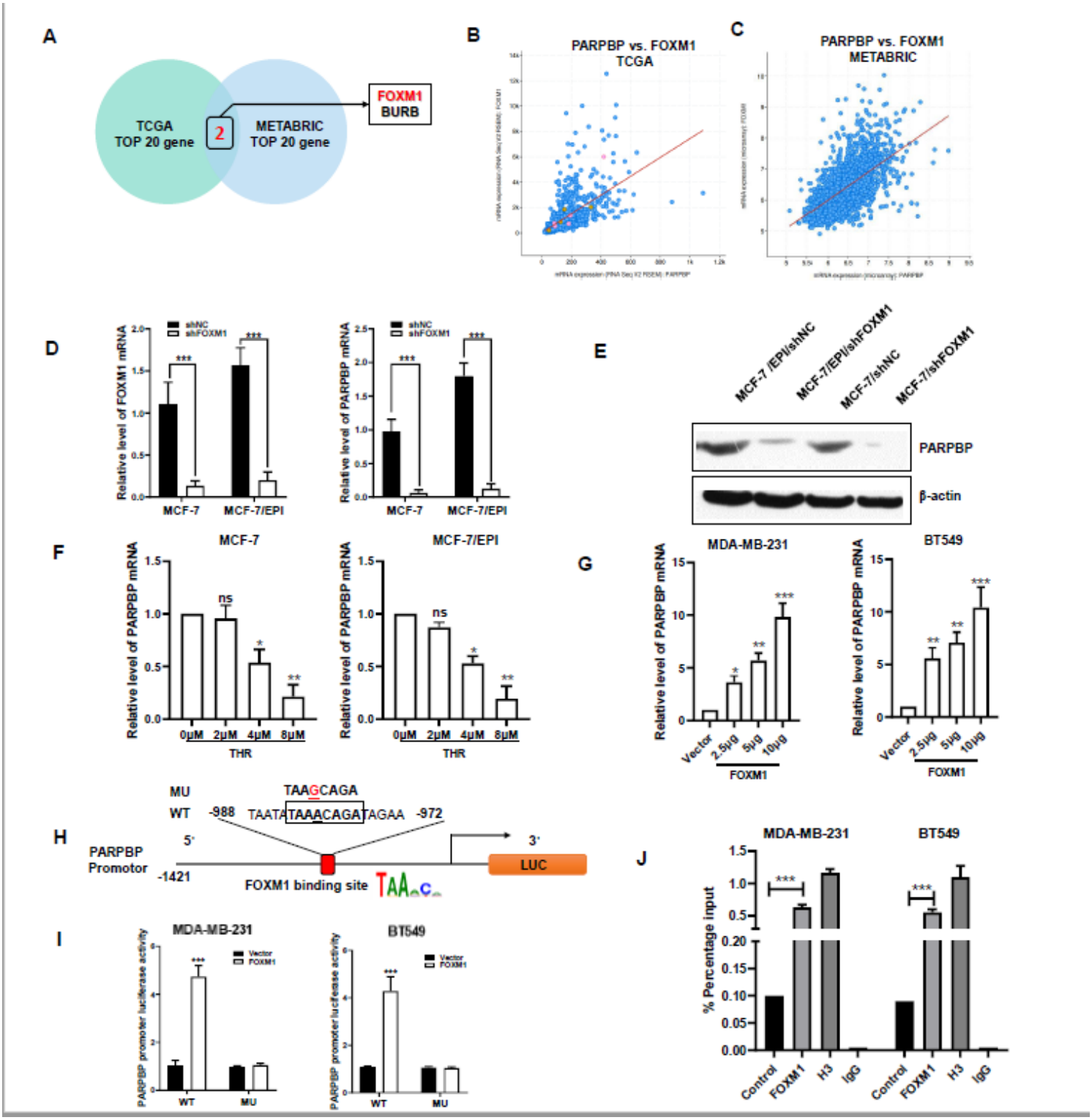

Figure 12

FOXM1 directly binds to the PARPBP promoter and regulates its activity (A) Venn diagram show that FOXM1 and BURB are the intersection of the top 20 co-expression genes in TCGA and d METABRAC

datasets. Correlation between FOXM1 and PARPBP in (B)TCGA and (C)METABRAC. (D) Downregulation of FOXM1 by transfecting shFOXM1 reduced the PARPBP by qRT-PCR and (E) western blotting. (F) MCF-7 and MCF-7/EPI cells treated with FoxM1 inhibitor thiostrepton (THR) at concentrations of 0,2, 4 and 8 μM. (G)Upregulation of FOXM1 by transfecting FOXM1 expression plasmid in MDA-MB-231 and BT549 cells increased the mRNA level of PARPBP. (H) Schematic of the PARPBP promoter reporter and its putative FOXM1-binding site. (I)Luciferase reporter assay in MDA-MB-231 and BT549 cells (with FOXM1 expression plasmid or empty vector) transfected with luciferase reporter constructs containing wild-type (WT) or mutant (MU). Data represent means ± SD of at least three independent experiments. **P < 0.01. (J) ChIP assay. Chromatins were isolated from MDA-MB-231 and BT549 cells. The enrichment percentage = 2% X 2(CT [Input sample] – CT [IP sample]). Normal IgG and Histone H3 were used as negative and positive controls, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable.pdf
- SupplementaryTable.pdf