

# TCDD-Inducible Poly-ADP-Ribose Polymerase (TIPARP), A Novel Therapeutic Target Of Breast Cancer

This article was published in the following Dove Press journal:  
*Cancer Management and Research*

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**Background:** TIPARP (TCDD-inducible poly-ADP-ribose polymerase), a mono-ADP-ribosyltransferase and a transcriptional repressor of aryl hydrocarbon receptor (AHR), was one of the potential therapeutic targets for human cancers identified by CRISPR–Cas9 screens recently. Studies about TIPARP on cancers are scarce till now, most of which just focus on expressions, while the functions have not been widely reported yet. Moreover, the TIPARP prognostic significance and therapeutic value of breast cancer is also uncertain.

**Methods:** The present study was performed to comprehensively analyze the expression pattern, prognostic effect, potential therapeutic function of TIPARP in breast cancer by pooling all currently available databases online including Oncomine, UALCAN, bc-GenExMiner, Kaplan–Meier Plotter, COSMIC, UCSC Xena, STRING, DAVID and Comparative Toxicogenomics Database. Further, we also performed several cell biology experiments including RT-qPCR, Western blot and CCK-8 in cellular and clinical sample levels to confirm the conclusions from bioinformatics analysis.

**Results:** TIPARP was expressed lower in tumor tissues comparing with normal tissues. Meanwhile, several clinical parameters of breast cancer patients were correlated with TIPARP expression. Further, higher TIPARP expression was related to preferable survival. Moreover, the mutations and DNA methylation of TIPARP might contribute to TIPARP dysregulation in breast cancer. Interactors with TIPARP were significantly enriched in telomere maintenance, telomere organization and mainly participated in pathways in cancer. Finally, several common drugs including metformin were observed to up-regulate the expression of TIPARP.

**Conclusion:** TIPARP might act as a preferable prognostic marker of breast cancer through multiple biological processes such as DNA methylation, mutation as well as pathway related to telomere and so on. TIPARP could be considered as a potential therapeutic target for breast cancer. However, large-scale and comprehensive research is needed to clarify our results.

**Keywords:** TIPARP, breast cancer, therapeutic target

## Introduction

Breast cancer is the most common malignant tumor and remains a major cause of death in women.<sup>1</sup> With the development of treatment, including surgery, chemotherapy, radiotherapy, endocrine therapy and target therapy, both disease-free survival and overall survival of breast cancer have been significantly improved. However, after systemic therapies, there are still some patients dying of breast cancer, especially for advanced breast cancer. These patients do not

benefit from targeted therapies, partly owing to a limited knowledge of candidate therapeutic targets.<sup>2</sup> Therefore, identifying novel and specific therapeutic targets for breast cancer treatment has attracted much attention from worldwide.

Recently, Behan identified 628 new cancer therapeutic targets by performing genome-scale CRISPR-Cas9 screens in 324 human cancer cell lines from 30 cancer types. The framework informed a data-driven list of prioritized therapeutic targets that would be the strong candidates for the development of cancer drugs. It can also inform the initial stages of drug development by contributing to a new, diverse and more effective portfolio of cancer drug targets.<sup>3</sup> However, most priority targets need more evidence to support their role as drug targets in cancer treatment.

To further evaluate the priority targets above, we chose TIPARP (TCDD-inducible poly-ADP-ribose polymerase) as a promising drug target in breast cancer and performed confirmatory studies for it. TIPARP is a mono-ADP-ribosyltransferase and a transcriptional repressor of aryl hydrocarbon receptor (AHR), revealing a novel negative feedback loop in AHR signaling.<sup>4</sup> TIPARP and AHR have been reported to play an important role in regulating the immune functions, inflammation and gastrointestinal health.<sup>5–8</sup> However, the studies about the role of TIPARP in cancers are scarce till now. Most studies just focused on the expression in tumors. For example, AHR signaling pathway genes including TIPARP have been reported to be upregulated in human meningioma.<sup>9</sup> TIPARP also showed significantly reduced expression in ovarian cancer cells, compared to normal primary human ovarian surface epithelial cells.<sup>10</sup> To our best knowledge, there are extremely few studies about TIPARP on breast cancer. So, the role of TIPARP in breast cancer and the underlying molecular mechanisms responsible for breast cancer development are still unclear. Numerous studies should be performed to investigate the role of TIPARP in breast cancer.

In the present study, we performed a deep bioinformatics analysis of the clinical parameters and survival data as well as biological functions and molecular mechanisms related to TIPARP in breast cancer patients using several online databases. Moreover, we also performed several experiments to confirm the conclusions from bioinformatics analysis in cellular and clinical sample levels.

## Materials And Methods

### ONCOMINE Data-Mining Analysis

ONCOMINE ([www.oncomine.org](http://www.oncomine.org)), an online web-based cancer database for RNA and DNA sequences, is used to facilitate data-mining the transcriptional expressions of genes in 20 types of cancer.<sup>11</sup> Transcriptional expression of TIPARP in cancer samples was compared with that in normal individuals using Student's *t*-test. Statistically significant values and fold change were demarcated as *P*-value  $\leq 1E-4$  and 2, respectively.

### UALCAN

UALCAN (<http://ualcan.path.uab.edu/>) is a user-friendly, interactive web resource for analyzing transcriptome data of cancers from The Cancer Genome Atlas (TCGA).<sup>12</sup> The mRNA expression of TIPARP in breast cancer and normal tissues as well as different stages of breast cancer was detected using the UALCAN web portal. Moreover, the promoter methylation level of TIPARP in breast cancer and normal tissues was obtained from UALCAN.

### bc-GenExMiner v4.2

Breast Cancer Gene-Expression Miner v4.2 (bc-GenExMiner v4.2) is a statistical mining tool of incorporates' 264 independent datasets and three classical mining functions: expression, prognosis and correlation.<sup>13,14</sup> Expression of TIPARP was analyzed with clinical parameters as well as the relevance with metastatic relapse events.

### Kaplan–Meier Plotter

The Kaplan-Meier Plotter (<http://kmplot.com/analysis/>), a platform containing gene expression information and survival data of 5143 clinical breast cancer patients, was applied to verify the prognostic value of TIPARP gene in overall survival (OS), relapse-free survival (RFS), distant metastasis-free survival (DMFS) and post-progression survival (PPS).<sup>15</sup>

### COSMIC Analysis

The Catalog of Somatic Mutations in Cancer (COSMIC) database (<http://www.sanger.ac.uk/cosmic/>), the world's largest and most comprehensive resource for exploring the impact of somatic mutations in human cancer,<sup>16</sup> was performed to analyse the mutations of TIPARP. Pie charts were generated for a distribution overview and substitutions on the coding strand in cancer.

## UCSC Cancer Genomics Browser Analysis

The heatmap of TIPARP expression and DNA methylation status was identified using data in TCGA breast cancer cohort (TCGA-BRCA) by performing the UCSC (University of California at Santa Cruz) Cancer Genomics Browser (<http://xena.ucsc.edu/>).<sup>17</sup>

## String

We analyzed the functional protein association network by STRING database (<https://string-db.org/>).<sup>18</sup> Protein name: TIPARP and organism: homo sapiens were analyzed.

## David

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (<https://david.ncifcrf.gov/home.jsp>) provides a set of complete annotation tools to help us understand the biological significance of a large number of genes and classifies gene function and analyses the functional annotation clustering and functional annotation table for a list of given genes.<sup>19</sup> TIPARP-related genes were submitted to DAVID for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Visualization of the results of GO and KEGG analysis was done using ggplot2 package of R software.

## Comparative Toxicogenomics Database

The Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>) is a robust, publicly available database for toxicogenomic information. It provides manually curated key information about chemical-gene/protein interactions, chemical-disease and gene-disease relationships, from peer-reviewed scientific literatures.<sup>20</sup> Given a target gene, CTD can provide the corresponding chemicals sorted by the interactions between them. Candidate treatments for regulating the expression of TIPARP were predicted using CTD with default parameters.

## Breast Tissue Samples

30 pairs of breast tissue samples used in real-time quantitative PCR (RT-qPCR) were obtained from the First Affiliated Hospital of Nanjing Medical University, China, between 2014 and 2016. The collection and use of the samples was reviewed and approved by the Institutional Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. All patients provided written

informed consent, and that this was conducted in accordance with the Declaration of Helsinki.

## RNA Isolation And RT-qPCR Analysis

Total RNA was isolated using Trizol reagent (TaKaRa, Japan), and 1000 ng RNA was reverse-transcribed into cDNA using Primescript RT Reagent (TaKaRa, Japan). The RT-PCR was performed using FastStart Universal SYBR Green Master (Roche, Switzerland) in a RT-PCR instrument (Applied Biosystems, USA), and  $\beta$ -Actin was used as endogenous control. The following PCR primers were used:

TIPARP forward, 5'-TGCACCCAGTTTCAAGTGAT3'-

TIPARP reverse, 5'- GTTACCAGCTCAAACACGA3'-

$\beta$ -Actin forward, 5'-GCTGTGCT ATCCCTGTACGC3'-

$\beta$ -Actin reverse, 5'-TGCCTCAGGGCAGCGGAACC3'-

## Cell Culture

The human breast cancer cell lines BT474, MDA-MB-231, T47D, MDA-MB-453, SUM1315, MCF-7 and normal mammary epithelial cell line MCF-10A were obtained from American Type Culture Collection (Manassas, USA) and incubated in a humidified atmosphere of 5% CO<sub>2</sub> in air at 37°C and fed with the culture medium of High glucose Dulbecco's Modified Eagle Medium, supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin solution. For routine passages, cultures were split 1:3 when they reached 80–90% confluence generally every 2–3 days. All experiments were performed on exponentially growing cells.

## CCK-8 Assay

We assessed cell viability through cell counting kit-8 assay (CCK-8) according to the manufacturer's protocol (Dojindo, Japan). Briefly,  $5 \times 10^3$  cells were seeded into a 96-well plate in triplicate and 8 hrs later, Metformin (Sigma, USA) was added into the wells at the indicated final concentrations (0, 1, 5, 10, 20 and 40  $\mu$ M). After incubation with metformin for 24 hrs, 48 hrs and 72 hrs, we added the CCK-8 solution (10  $\mu$ L) into each well, and the plates were incubated for 2 hrs at 37°C. The absorbance of cells at 450 nm (OD450) was measured in a microplate reader (Bio-Rad, USA).

## Western Blot

Tissues and cells treated with/without metformin were lysed by RIPA buffer containing protease inhibitors (Sigma, USA). Protein extractions were separated by

10% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA). After incubated with the anti-TIPARP antibody (1:1000, Abcam, USA) and anti- $\beta$ -Actin antibody (1:1000, Cell Signaling Technology, USA), the membranes were then incubated with peroxidase (HRP)-conjugated secondary antibody (1:1000, Cell Signaling Technology, USA). After washes, signals were detected using a chemiluminescence system (Bio-Rad, USA) and analyzed using Image Lab Software.

# Statistical Analysis

Experiments were repeated in triplicate, unless otherwise specified. The data were analyzed using the SPSS 20.0 software (Chicago, USA). We analyzed the statistical significance of the differences between groups using Students *t*-test and oneway ANOVA followed by Bonferroni test, and a statistically significant difference was considered at the level of  $P < 0.05$ .

# Results

## Reduced Expression Of TIPARP In Breast Cancer Tissues

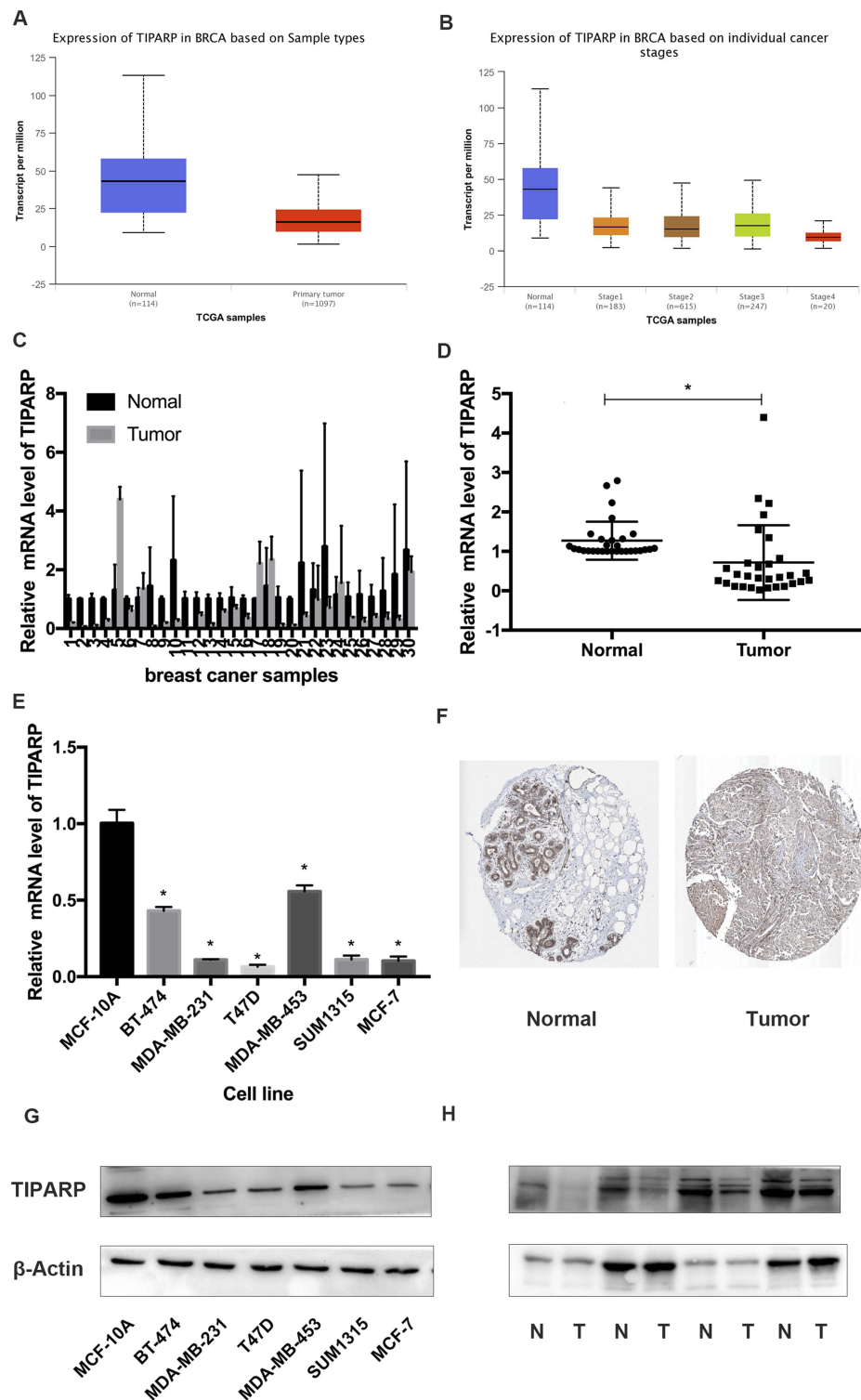
Firstly, the expression of TIPARP in 20 types of cancer was measured and compared to normal tissues using the Oncomine online database (Figure 1). We found that decreased TIPARP (blue) was observed in bladder cancer, cervical cancer, esophageal cancer, leukemia, lung cancer, lymphoma, melanoma and especially breast cancer. Whereas, increased level of TIPARP (red) was observed in colorectal cancer, head and neck cancer, liver cancer and myeloma. Consistently, using UALCAN website, we also found that lower TIPARP was expressed in breast cancer tissues than in normal tissues (Figure 2A,  $P < 0.05$ ). Next, we focused on whether mRNA expression of TIPARP was related to cancer stage in individual patients. As shown in Figure 2B, the results indicated that patients with a more advanced stage of breast cancer tended to express lower levels of TIPARP.

To verify the results above, we further compared the TIPARP expression in breast cancer tissues and adjacent normal tissues of patients in our hospital and found that TIPARP was expressed lower in breast cancer tissues both in mRNA level (Figure 2C, D) and protein level (Figure 2H), consistent with the results from databases (Figure 2C, D,  $P < 0.05$ ). The mRNA expression of TIPARP was also lower

Cancer Type	Cancer vs. Nomal	
Bladder Cancer		1
Brain and CNS Cancer		
Breast Cancer		4
Cervical Cancer		1
Colorectal Cancer	1	
Esophageal Cancer		3
Gastric Cancer		
Head and Neck Cancer	2	
Kidney Cancer		
Leukemia		1
Liver Cancer	1	
Lung Cancer		2
Lymphoma		3
Melanoma		1
Myeloma	1	
Other Cancer		6
Ovarian Cancer		
Pancreatic Cancer		
Prostate Cancer		
Sarcoma		
Significant Unique Analyses	5	22
Total Unique Analyses	402	



**Figure 1** Expression of TIPARP in 20 common cancers versus paired normal tissues using the Oncomine database. Red and blue, respectively, stand for the numbers of datasets with statistically significant ( $P < 0.05$ ) increased and decreased levels of TIPARP gene.



**Figure 2** Decreased expression of TIPARP in breast cancer patients tissues. **(A)** Lower mRNA TIPARP was expressed in breast cancer tissues than in normal tissues ( $P < 0.05$ ) using UALCAN website. **(B)** Patients with a more advanced stage of breast cancer tended to express lower levels of TIPARP. **(C)** TIPARP mRNA expression in 30 pairs of breast cancer and adjacent tissues. **(D)** Average expression level of TIPARP mRNA in 30 pairs of breast cancer tissues and adjacent normal breast tissues. The data are represented as mean  $\pm$  SD of three independent experiments. \* indicates  $P$ -value  $< 0.05$ . **(E)** The mRNA expression of TIPARP was lower in breast cancer cells compared to normal breast cell line MCF-10A. The data are represented as mean  $\pm$  SD of three independent experiments. \* indicates  $P$ -value  $< 0.05$  vs MCF-10A. **(F)** Immunohistochemistry confirmed the lower expression of TIPARP in breast cancer in protein level. **(G)** The protein expression of TIPARP was lower in breast cancer cells compared to normal breast cell line MCF-10A. **(H)** TIPARP protein expression in 4 pairs of breast cancer and adjacent tissues.

in breast cancer cells compared to normal breast cell line MCF-10A both in mRNA level (Figure 2E) and protein level (Figure 2G). Immunohistochemistry from the Human Protein Atlas database confirmed the lower expression of TIPARP in breast cancer in protein level (Figure 2F).

## TIPARP Expression And Clinical Parameters Of Breast Cancer Patients

Using bc-GenExMiner v4.2 software, we implemented Welch's test to compare the abnormal expression of TIPARP among different groups of patients according to clinical pathological features. For age criteria, TIPARP was significantly elevated in  $\leq 51$ -year group with respect to  $> 51$ -year group (Figure 3A). As we know, the Scarff, Bloom & Richardson (SBR) is a histological grade that evaluates tubule formation, nuclear characteristics of pleomorphism and mitotic index.<sup>21</sup> Based on tumor size, lymph node stage and tumor grade, the Nottingham Prognostic Index (NPI) is used to stratify patients into additional prognostic groups. The SBR grade and NPI index are two wonderful prognostic model for breast cancer.<sup>22</sup> More advanced SBR grade and NPI index were associated with lower TIPARP level (Figure 3B and C). ER-positive or PR-positive breast cancer patients tended to express higher TIPARP gene compared with ER-negative or PR-negative patients (Figure 3D and E). Regarding nodal status and HER-2 status, there was no significant difference between positive and negative group (Figure 3F and G). Moreover, TIPARP was significantly increased in non-triple-negative and non-basal-like breast cancer patients compared to triple-negative and basal-like breast cancer patients (Figure 3H and I).

## TIPARP Expression And Survival Data Of Breast Cancer Patients

We then investigated the prognostic value of TIPARP. The Kaplan–Meier curves indicated that higher level of TIPARP correlated with preferable RFS, OS, DMFS and PPS (Figure 4A–D). To confirm the prognostic value, we also obtained data about metastatic relapse-free survival of TIPARP from bc-GenExMiner and found that patients with decreased TIPARP expression presented worse survival (Figure 4E). The pooled results revealed that low TIPARP expression was correlated with longer survival.

## TIPARP Mutation In Human Cancer

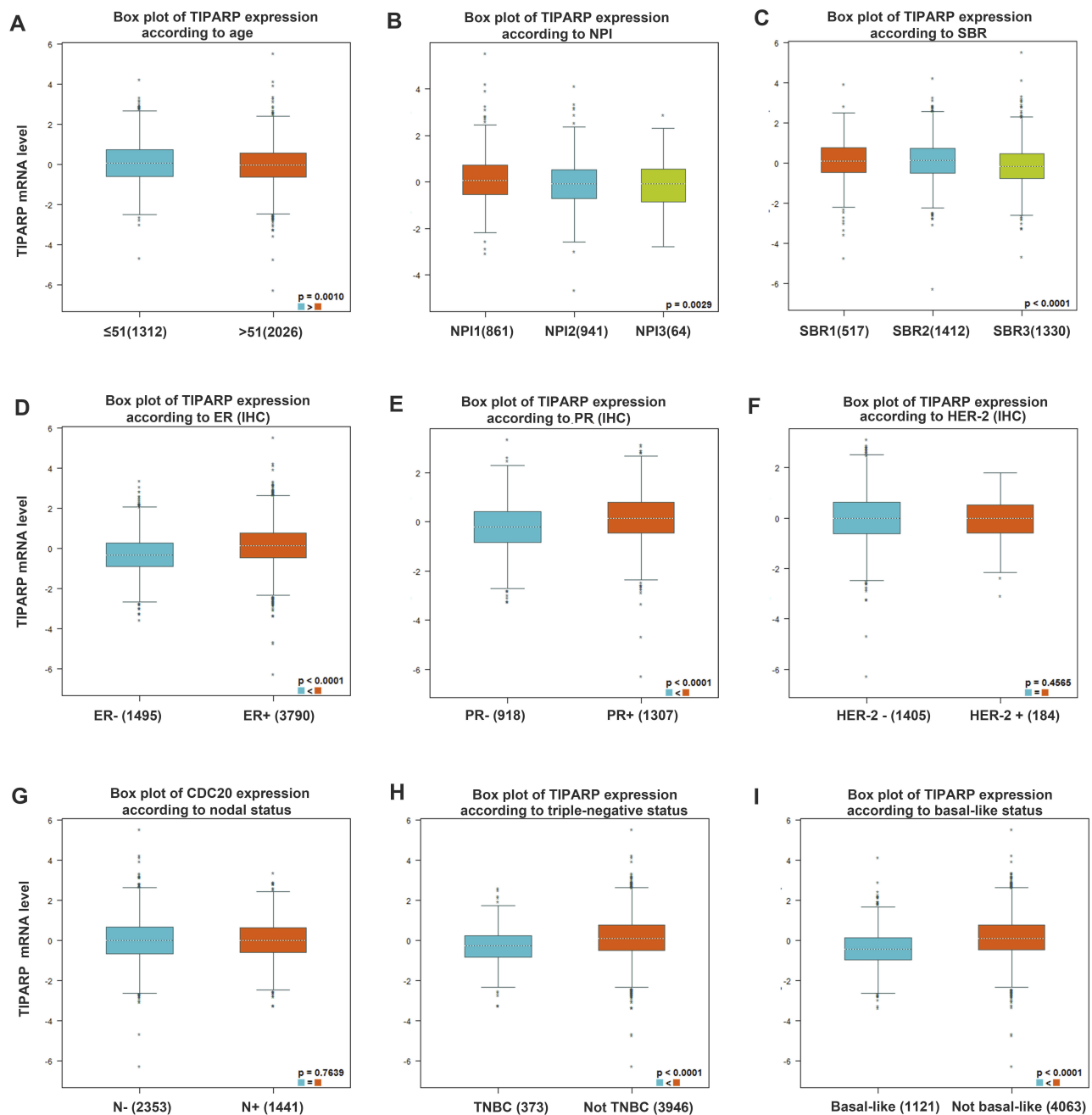
The pie chart including the information of mutations of nonsense substitution, missense substitution, inframe insertion, synonymous substitution, frameshift insertion, frameshift deletion and inframe deletion was generated by COSMIC. Missense substitution rate was 73% while synonymous substitution rate was 18% of mutant samples of human cancer (Figure 5A). In the study, the profile of mutations was displayed using the six substitution subtypes: C>A, C>G, C>T, T>A, T>C and T>G. Respectively, human cancer had 44% C > T, 22% T > C and 15% C > A mutation in TIPARP coding strand (Figure 5B). Moreover, the pie charts of mutation types for mutational signatures of human cancers were displayed according to the data from COSMIC. We also drew the pie charts of mutation types identified from 53 treatments according to data from an excellent research published in Cell recently.<sup>23</sup> Interestingly, comparing with other treatments, semustine resulted in 52% C > T, 13% T > C and 13% C > A mutation, which was also similar to the mutation of TIPARP (Figure 5C, the data for other treatments are not shown).

## TIPARP DNA Methylation Status In Human Breast Cancer

Heatmap of TIPARP expression and DNA methylation status revealed that its expression might be negatively related with DNA methylation in breast cancer (Figure 6A). Consistently, using UALCAN website, we also found that higher promoter methylation level of TIPARP was expressed in breast cancer tissues than in normal tissues (Figure 6B,  $P < 0.05$ ).

## TIPARP Related Protein–Protein Interaction Analysis

STRING is a database known to be used to predict protein–protein interactions. The co-expression analysis revealed that TIPARP was directly co-expressed with 10 interactors including poly(ADP-ribose) polymerase family member 16 (PARP 16), PIN2/TERF1-interacting telomerase inhibitor 1 (PINX1), intracellular hyaluronan-binding protein 4 (HABP4), cytochrome P450 1A1 (CYP1A1), poly [ADP-ribose] polymerase 3 (PARP3), cytochrome P450 1B1 (CYP1B1), immunoglobulin superfamily DCC subclass member 3 (IGDCC3), aryl hydrocarbon receptor (AHR), poly(ADP-ribose) polymerase family member 6 (PARP 6) and indolethylamine N-methyltransferase (INMT). Among the directly co-expressed 10 interactors, some have been reported to play an



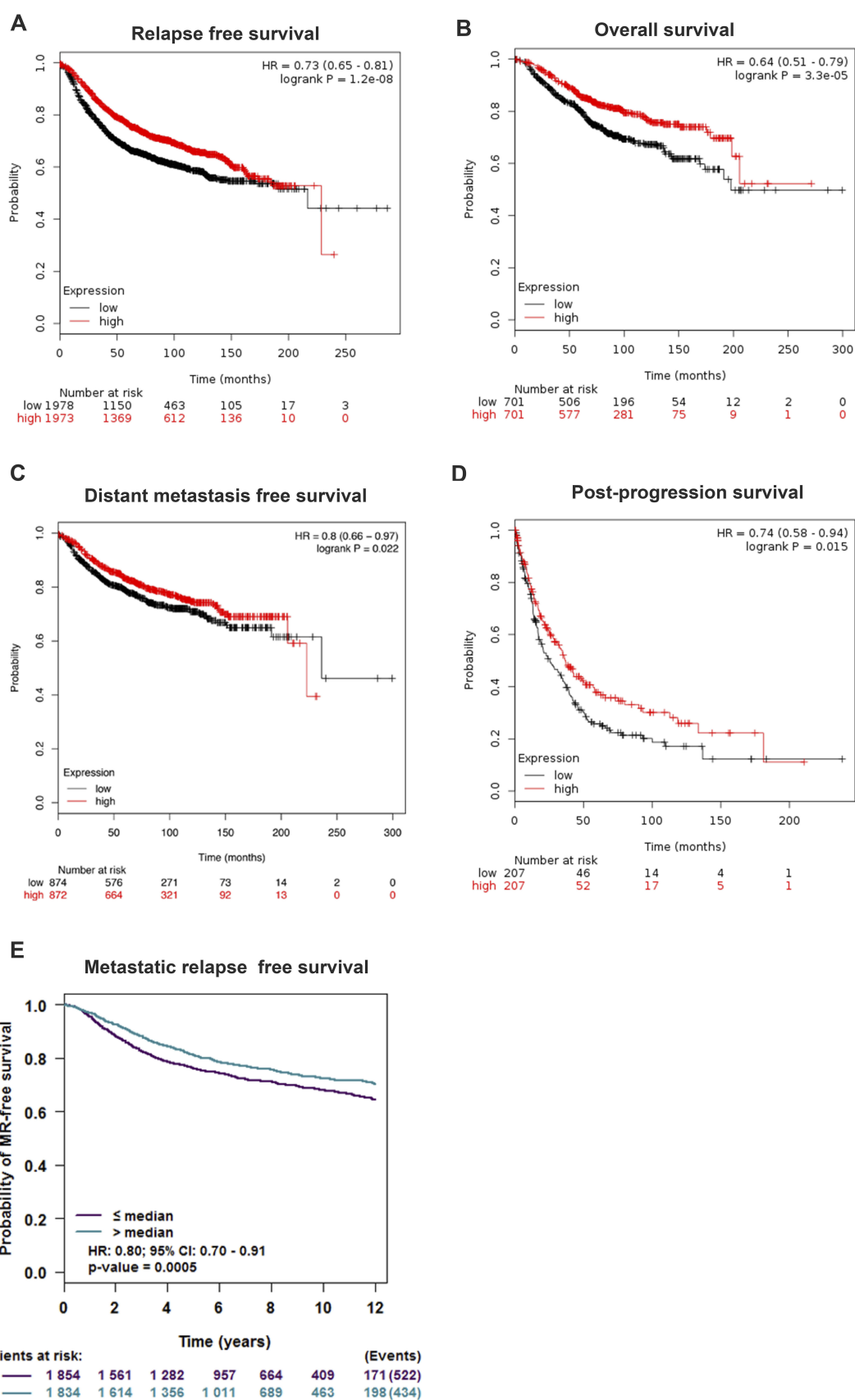
**Figure 3** Box plot evaluating TIPARP expression among different groups of patients based on clinical parameters using the bc-GenExMiner 4.2. Analysis is shown for (A) age, (B) NPI index, (C) SBR grade, (D) ER, (E) PR, (F) HER-2, (G) nodal status, (H) triple-negative status and (I) basal-like status.

important role in breast cancer, such as PINX1<sup>24</sup> and PARP3.<sup>25</sup> Meanwhile, TIPARP interacted indirectly with other 11 proteins (Figure 7A).

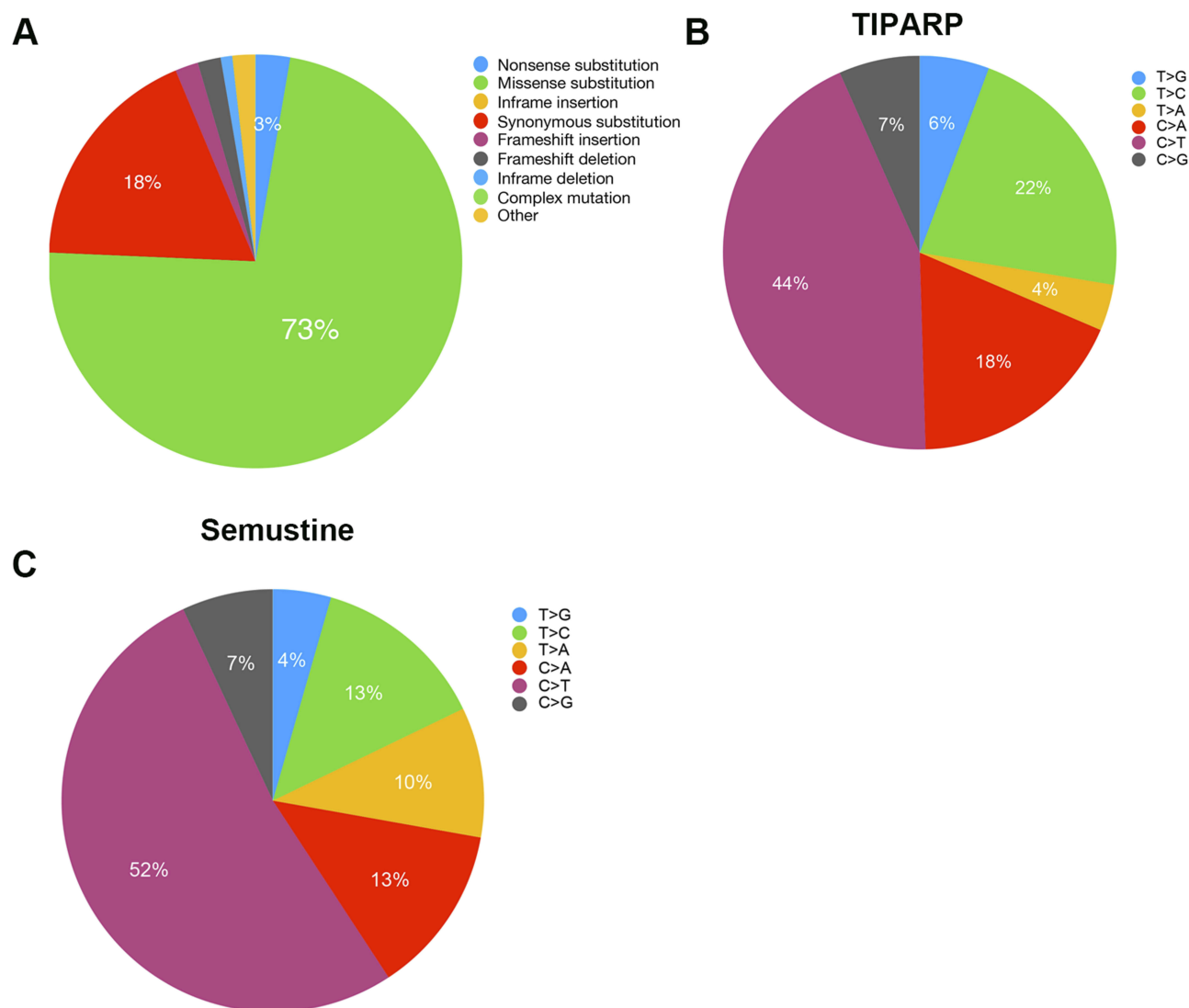
## GO Functional And Pathway Enrichment Analysis

To determine the function and mechanisms of these 21 interactors mentioned above, all of them were submitted to

the DAVID for GO analysis and KEGG pathway analysis. We found that the interactors were significantly enriched in response to xenobiotic stimulus, telomere maintenance, telomere organization and response to organic substance and so on (Figure 7B). According to the KEGG pathway enrichment analysis, the 21 related genes mainly participated in pathways in cancer, tryptophan metabolism and steroid hormone biosynthesis and metabolism of xenobiotics by cytochrome P450 (Figure 7C).



**Figure 4** Survival curve evaluating the prognostic value of TIPARP using Kaplan–Meier Plotter and bc-GenExMiner. Analysis is shown for (A) relapse-free survival, (B) overall survival, (C) distant metastasis-free survival, (D) post-progression survival and (E) metastatic relapse-free survival.



**Figure 5** TIPARP mutation in human cancer. **(A)** Pie chart including the information of mutations generated by COSMIC. **(B)** Pie chart of human cancer mutation types in TIPARP coding strand. **(C)** Pie chart of mutation types identified from semustine.

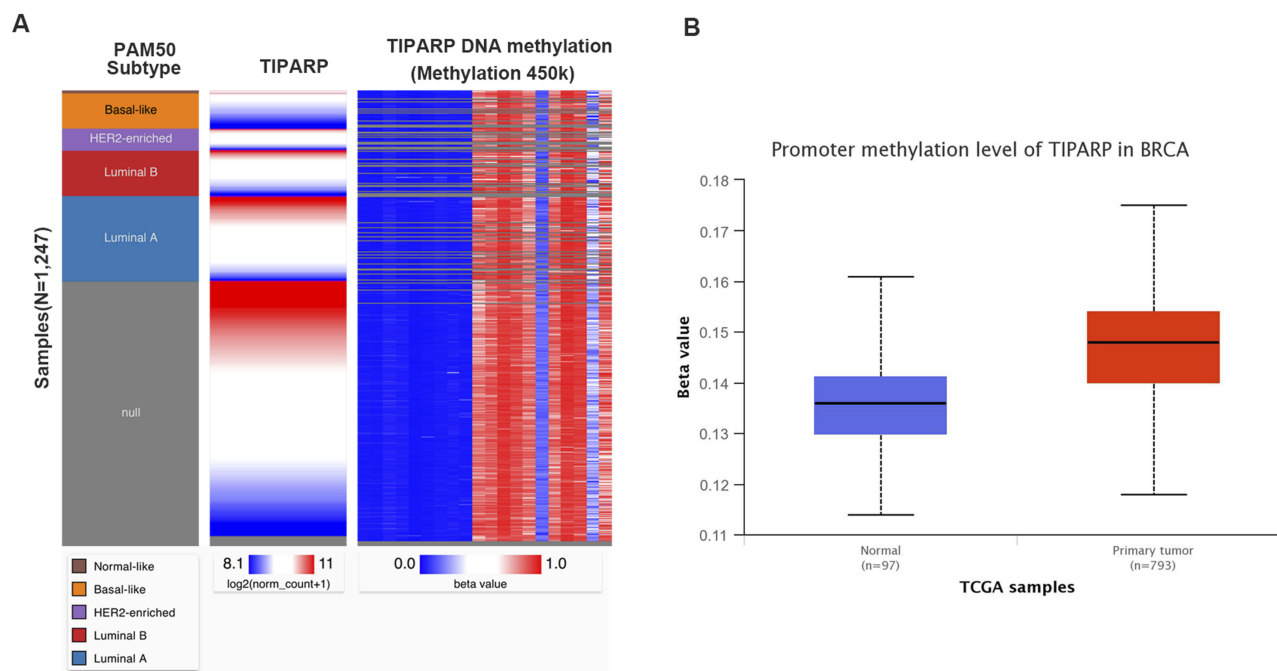
## TIPARP Acting As A Target Of Several Drugs

Using CTD, we found a list of chemicals that interacted with TIPARP. From the 151 chemicals, we picked 10 “old” drugs used commonly for treatment of several diseases, including breast cancer, diabetes, bacterial infection and so on. As shown in [Figure 8A](#), the 10 drugs including tamoxifen, raloxifene, estradiol, ethanol, gentamicins, metformin, acetaminophen, zoledronic acid, rosiglitazone and testosterone have been demonstrated to improve the mRNA expression of TIPARP. To substantiate the results, we investigated the effect of metformin on TIPARP. Metformin showed growth inhibition in BT-474 and MDA-MB-231 breast cancer cell lines in both dose-dependent and time-

dependent manners ([Figure 8B and C](#)). After exposure to 10  $\mu$ M metformin for 48 hrs, metformin increased the mRNA and protein expression of TIPARP ([Figure 8D and E](#)), consist with the result from CTD database.

## Discussion

Despite the development in early clinical diagnosis and treatment strategies, breast cancer remains a significant global burden.<sup>26</sup> Therefore, the development of more effective drugs for breast cancer patients is urgently needed. As is well known, identifying novel drug targets according to the molecular features of tumors could expand the range of drugs and then improve the success rates of new cancer therapies for breast cancer.<sup>27</sup> In the



**Figure 6** TIPARP DNA methylation status in human breast cancer. **(A)** The heatmap of TIPARP expression and its DNA methylation status. **(B)** Higher promoter methylation level of TIPARP was expressed in breast cancer tissues than in normal tissues.

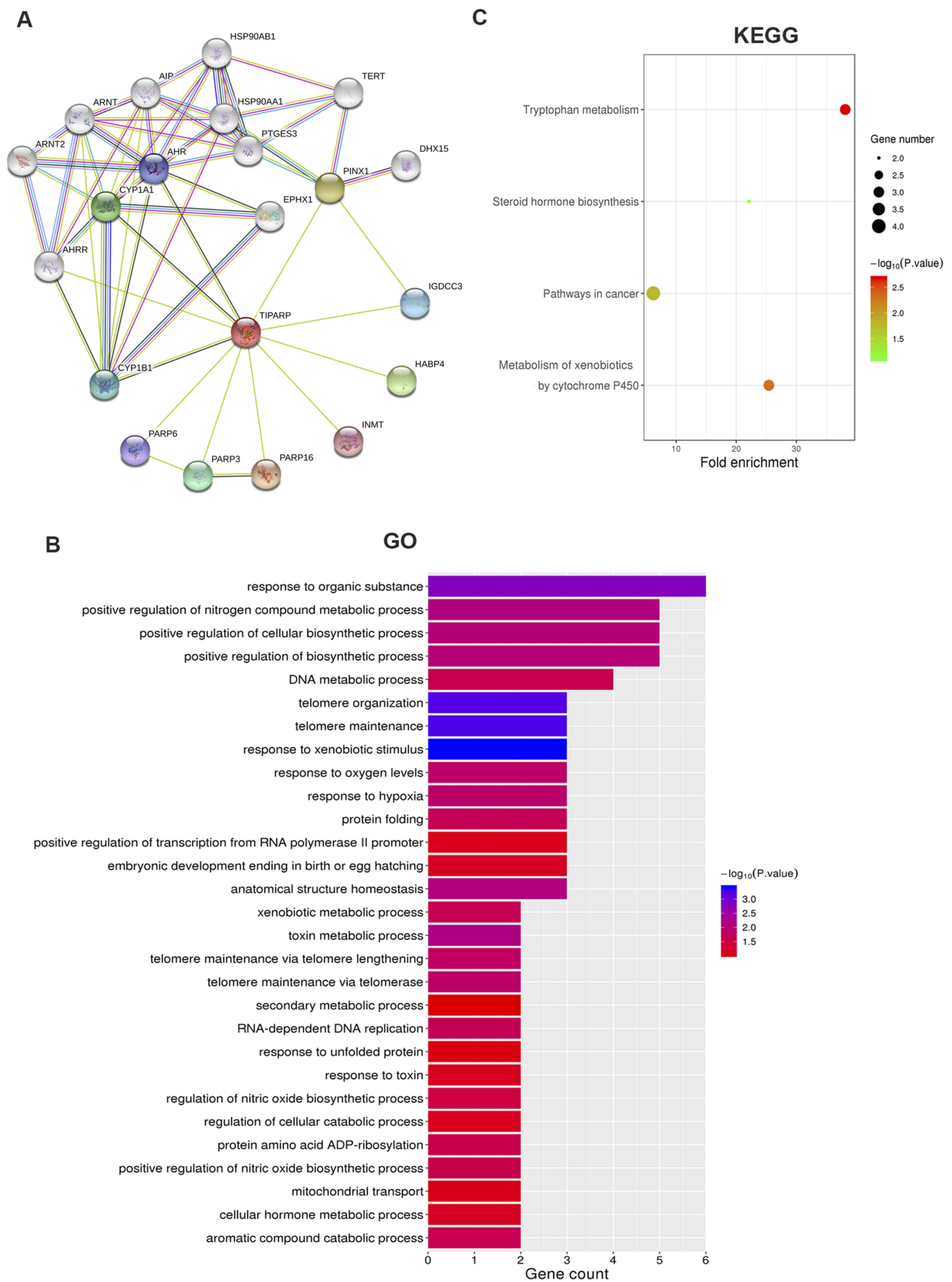
present study, we performed a comprehensive bioinformatics analysis as well as several experiments to investigate the potential therapeutic role of TIPARP in breast cancer.

TIPARP expression decreased in breast cancer significantly and a more advanced stage of breast cancer tended to express lower levels of TIPARP. Consistently, TIPARP also expressed lower in breast cancer tissues, compared to adjacent normal tissues of patients from our hospital, confirming the results from databases online. Meanwhile, ER and PR status were found to be positively correlated with TIPARP expression. Conversely, basal-like status, TNBC status, SBR grade and NPI index were negatively correlated with TIPARP expression. It is generally known that breast cancer patients with ER- or PR-positive, non-basal-like or non-triple-negative status have a preferable outcome.<sup>28</sup> We further investigated the prognostic value of TIPARP in breast cancer using Kaplan–Meier plotter and bc-GenExMiner tool, and the pooled results revealed that low TIPARP expression was correlated with longer OS, RFS, DMFS and PPS. Together, as there is no research about the role of TIPARP in breast cancer, it is the first time to report that TIPARP might act as a preferable prognostic marker of breast cancer.

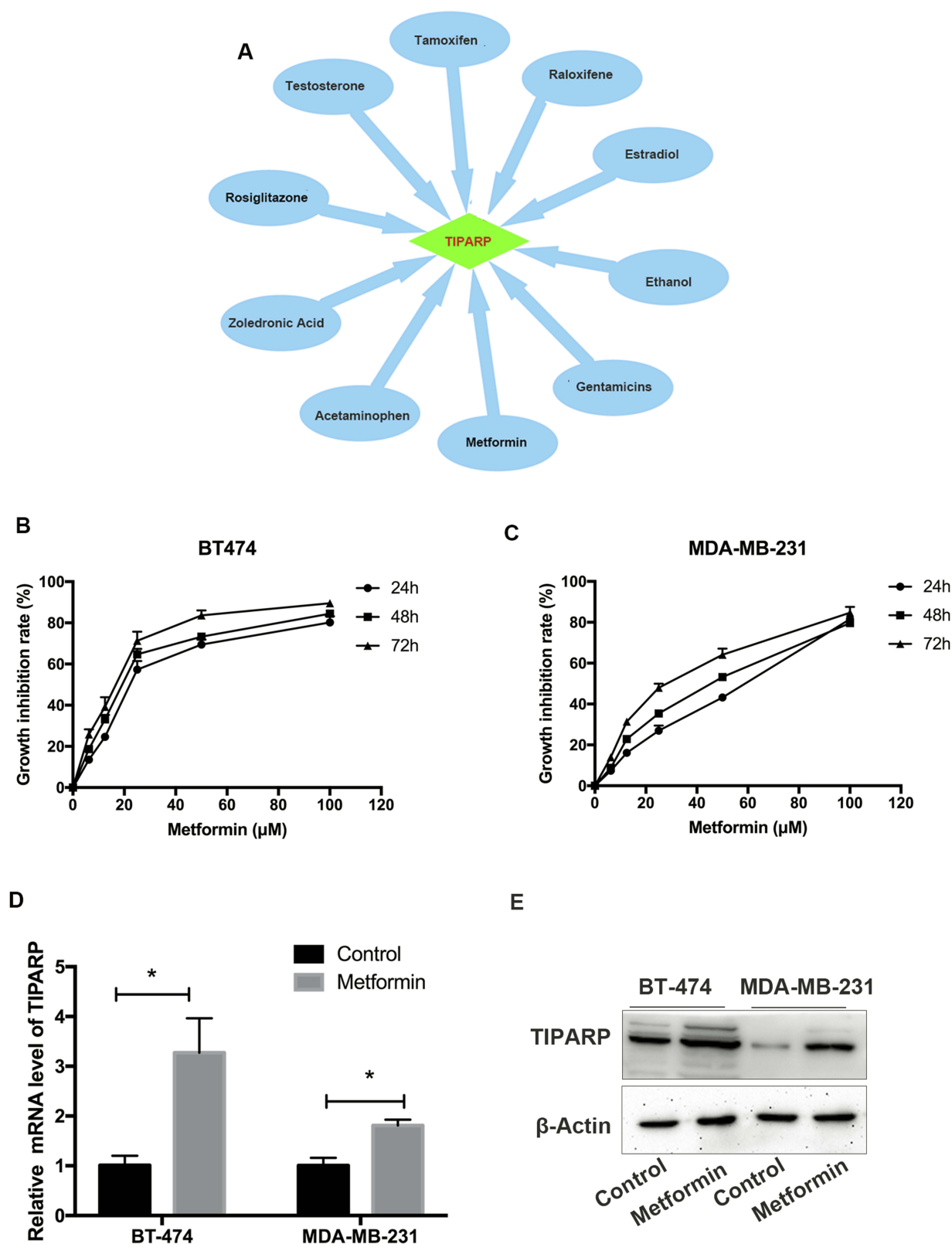
Then, we tried to investigate the mechanisms of TIPARP dysregulation in breast cancer. Through

examining its DNA methylation status in TCGA, we observed a negative correlation between methylation status and TIPARP expression. These findings suggested that DNA methylation might be an important mechanism of TIPARP dysregulation in breast cancer, as the same as many other biomarkers, for instance, MEG3 expression gradually decreased with increasing DNA methylation in breast cancer.<sup>29</sup>

To explore the mechanisms of TIPARP acting as a preferable prognostic marker in breast cancer, we further analyzed the mutation of TIPARP using COSMIC and performed PPI analysis. In TIPARP coding strand, human cancer had 44% C > T, 22% T > C and 15% C > A mutation, respectively. This shared a similar pattern with mutation types identified from semustine according to Kucab.<sup>23</sup> Notably, the mutation types of TIPARP were quite different from those identified from common environmental agents such as radiation, aldehydes and so on, suggesting that the mutation of TIPARP was not caused by single environmental agents. Semustine was known as a drug for treatment of brain, gastrointestinal cancers and melanomas.<sup>30</sup> The results above suggested that exposure to semustine for a long time might induce the mutation of TIPARP, therefore promote the development of breast cancer. Next, PPI as well as GO and KEGG analysis demonstrated that interactors with TIPARP significantly enriched in telomere maintenance, telomere



**Figure 7** TIPARP-related protein–protein interaction analysis. **(A)** PPI network of TIPARP. **(B)** GO analysis of interactors of TIPARP. **(C)** KEGG analysis of interactors of TIPARP.



**Figure 8** TIPARP acting as a target of several drugs. **(A)** 10 common drugs improved the mRNA expression of TIPARP according to CTD. **(B and C)** Metformin showed growth inhibition in BT-474 and MDA-MB-231 breast cancer cell lines in both dose-dependent and time-dependent manners. **(D)** Metformin increased the mRNA expression of TIPARP in both two breast cancer cell lines. The data are represented as mean  $\pm$  SD of three independent experiments. \* Indicates  $P$ -value  $< 0.05$ . **(E)** Metformin increased the protein expression of TIPARP in both two breast cancer cell lines.

organization and mainly participated in pathways in cancer. As we know, telomere plays an important role in regulating cell growth and division. And telomerase is overexpressed in 80–95% of cancers and likely to participate in cell malignant transformation.<sup>31</sup> TIPARP dysregulation may play an important role in breast cancer through the pathway related to telomere.

Finally, we investigated the role of TIPARP acting as a potential therapeutic target of breast cancer. Several common drugs could up-regulate the expression of TIPARP, one of which was metformin. Metformin is the first-line therapy for type 2 diabetes mellitus and one of the most widely prescribed oral antidiabetic medications, having been widely used for over 40 years. In recent years, several studies suggest a potential role for metformin in anti-cancer therapy including breast cancer.<sup>32,33</sup> Our observations might provide a new potential mechanism for metformin treating breast cancer.

In conclusion, over the past several decades, much research has focused on identifying new prognostic markers and therapeutic targets in order to make better clinical decisions and improve therapy and outcomes of breast cancer. The present study was performed to comprehensively analyze the expression pattern, prognostic effect and potential therapeutic function of TIPARP in breast cancer by pooling all currently available data online as well as performing experiments. TIPARP might act as a preferable prognostic marker of breast cancer through multiple biological processes such as DNA methylation, mutation as well as pathway related to telomere and so on. More in-depth experiments are needed to validate the value of TIPARP acting as a therapeutic target of breast cancer.

## Abbreviations

TIPARP, TCDD-Inducible Poly-ADP-Ribose Polymerase; AHR, Aryl hydrocarbon receptor; ER, Estrogen receptor; PR, Progesterone receptor; AR, Androgen receptor; HER-2, Epidermal growth factor receptor-2; SBR, Scarff-Bloom-Richardson; NPI, Nottingham prognostic index; RFS, Relapse-free survival; OS, Overall survival; DMFS, Distant metastasis-free survival; PPS, Post-progression survival; TCGA, The Cancer Genome Atlas; PPI, Protein-Protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

## Acknowledgments

This work was financially supported by Natural Science Foundation of China (81502294, 81572595) and Changzhou Science and Technology Program (CJ20159044).

## Disclosure

The authors declare no conflicts of interest in this work.

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