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ORIGINAL RESEARCH

Telomere Maintenance-Related Genes are Essential for Prognosis in Breast Cancer

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Objective: Telomere maintenance mechanism significantly impacts the metastasis, progression, and survival of breast cancer (BC) patients. This study aimed to investigate the role of telomere maintenance-related genes (TMRGs) in BC prognosis and to construct a related prognostic model.

Methods: Differentially expressed genes were identified from the TCGA-BC cohort, and functional enrichment analysis was conducted. TMRGs were sourced from the literature and intersected with DEGs. Candidate genes were selected using machine learning algorithms, including Lasso Cox, Random Forest, and XGBoost. Multivariate Cox regression analysis was conducted to construct a prognostic model and identify hub genes. Subsequent analyses included survival analysis, gene set enrichment analysis (GSEA), immune infiltration analysis, and drug sensitivity analysis of the hub genes. Finally, in vitro experiments were conducted to validate the expression of the hub genes.

Results: A total of 1329 differentially expressed TMRGs were analyzed, with 128 significantly associated with overall survival. Machine learning identified 7 prognosis-related TMRGs: MECP2, PCMT1, PFKL, PTMA, TAGLN2, TRMT5, and XRCC4. These genes were used to construct a prognostic model, with MECP2, PCMT1, PFKL, TAGLN2, and XRCC4 as harmful factors, while PTMA and TRMT5 were protective. The model demonstrated a significant prognostic value (AUC: 0.81, 0.72, 0.69 for 1-, 3-, and 5-year, respectively). Survival analysis confirmed the prognostic relevance of these genes, and GSEA highlighted their roles in oxidative phosphorylation, glycolysis, and PI3K/AKT/mTOR signaling.

Conclusion: The study identified 7 key TMRGs with significant prognostic value in BC. The constructed model effectively stratifies patient risk, providing a foundation for targeted therapies and personalized treatment strategies.

Keywords: breast cancer, telomere, risk score, machine learning, immune infiltration, seven hub genes

Introduction

Breast cancer (BC) is a malignant tumor originating from mammary epithelial cells and remains the most prevalent and deadly malignancy among women worldwide.[1] From 2010 to 2019, the incidence of BC increased by 0.5% annually.[2] In 2020, approximately 2.3 million cases of BC were diagnosed globally; this number is expected to exceed 3 million cases annually by 2040.[3] In China, BC patients are becoming younger, with most patients being diagnosed between the ages of 45 and 55.[4] Depending on the stage and type of BC, current treatments include surgery, radiotherapy, chemotherapy, hormone therapy, and targeted therapy.[5] Despite advancements in early detection and treatment, the prognosis of BC patients varies significantly. In personalized medicine, traditional prognostic markers, such as tumor size, histological grade, and lymph node status, may not provide sufficient guidance for tailoring treatment strategies in early-diagnosed BC patients.[6,7] This highlights the need for reliable prognostic biomarkers to optimize treatment.

Telomeres are protective caps at the ends of chromosomes, playing a crucial role in maintaining genomic stability. With each cell division, telomeres shorten, and when critically shortened, they ultimately lead to cellular senescence or apoptosis.[8] However, in cancer cells, mechanisms that maintain telomere length are often activated, allowing these cells to evade senescence and continue proliferating. This process is primarily mediated by two key mechanisms: telomerase

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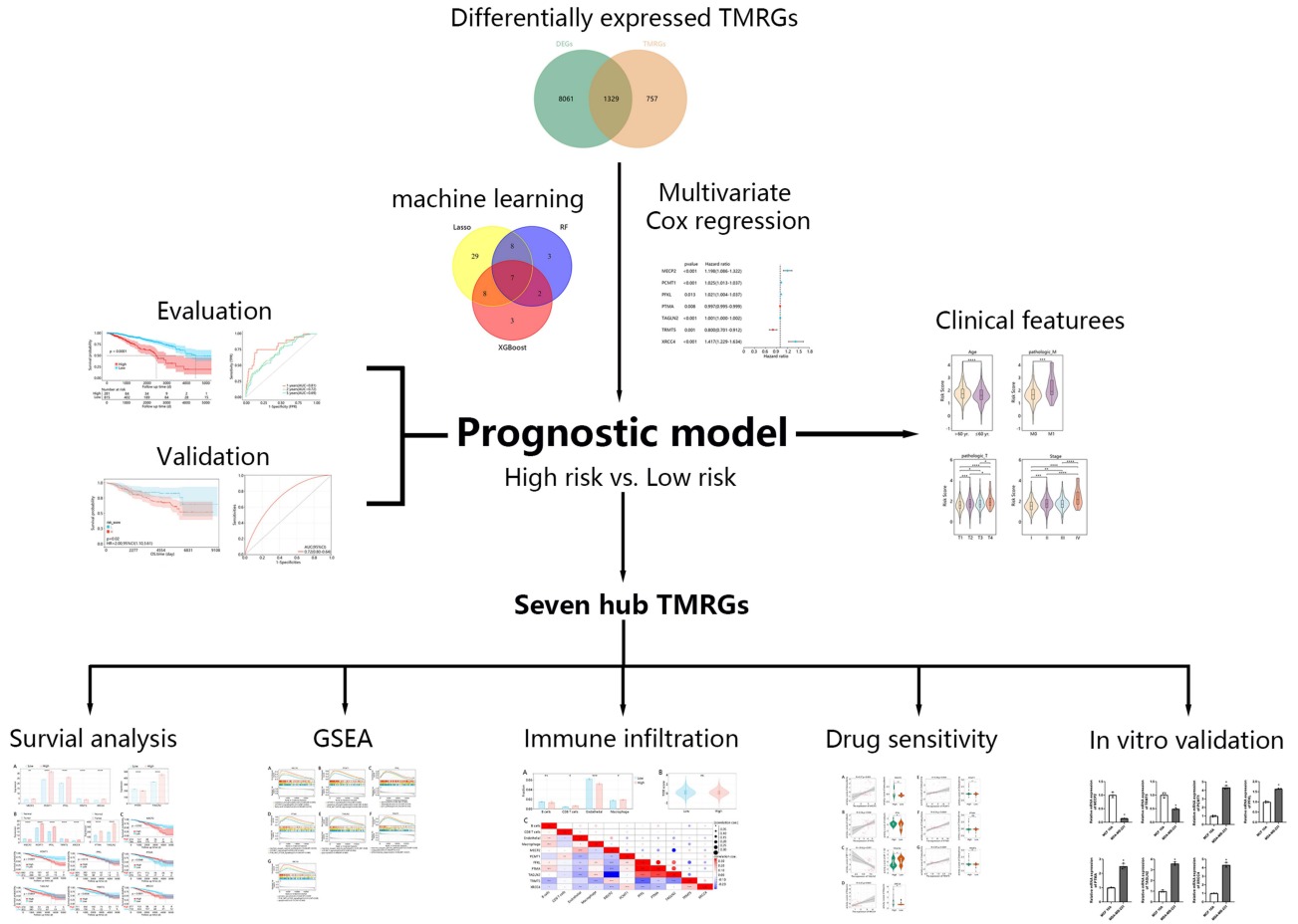
Graphical Abstract



and the alternative lengthening of telomeres (ALT) pathway. Telomerase, a ribonucleoprotein reverse transcriptase, adds telomeric repeats to chromosome ends, counteracting telomere shortening.[9] It is reported that approximately 90% of cancers exhibit telomerase activity.[10] In the 10% of cancers lacking telomerase expression, telomere length is maintained through the ALT pathway. Although the ALT pathway is less common, it remains significant, involving homologous recombination-based telomere elongation mechanisms.[11] A review by Ricardo Leão detailed various genetic and epigenetic mechanisms leading to telomerase reverse transcriptase promoter (hTERT) upregulation in tumors and highlighted its strong potential as a biomarker.[12] Another study found that hypermethylation in specific regions of the hTERT promoter is a significant epigenetic marker in the development of BC.[13] Yang et al further highlighted that therapies targeting hTERT and its regulatory molecules hold promise as viable strategies for BC treatment.[14] Elsharawy et al demonstrated that NOP10, a factor essential for ribosome biogenesis and telomere maintenance, is significantly associated with aggressive BC characteristics, and its high expression is strongly correlated with shorter survival in BC patients.[15]

Telomere maintenance-related genes (TMRGs) are closely associated with tumorigenesis and progression, including in BC.[16] In-depth bioinformatics research into the role of TMRGs in BC holds promise for identifying new prognostic markers, providing clinicians with more references for diagnosis and treatment planning, and ultimately improving patient outcomes. In this study, we utilized bioinformatics techniques and machine learning algorithms to identify key

TMRGs related to BC prognosis and constructed a prognostic model to predict the survival rate of BC patients. Our research offers new insights into personalized treatment for BC patients.

Methods

Data Collection

The transcriptome expression data and clinical information of BC were downloaded from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov) database. The TMRGs were acquired from the TelNet database (https://malone2.bioquant.uni-heidelberg.de/fmi/webd/TelNet) according to previous literature.[17] As this study involves a secondary analysis of anonymized data from public databases, no ethical approval or consent was required.

Functional Enrichment Analysis of Differentially Expressed Genes (DEGs) in BC

Using the R package "Limma",[18] DEGs between normal and tumor samples were screened out, with $p < 0.05$ and |fold change| $\geq$ 1.5. DEGs were shown in a volcano plot using the "ggVolcano".

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were further performed to reveal the functions of these DEGs. GO terms included biological progress (BP), cellular component (CC), and molecular function (MF). Functional enrichment analysis was conducted using R packages "clusterProfiler"[19] and "org.Hs.eg.db", and results were visualized using "Goplot" package.[20]

Identification of Differentially Expressed TMRGs

The overlapping genes between DEGs and TMRGs were defined as differentially expressed TMRGs, and results were visualized through a Venn diagram drawn using "VennDiagram" package.[21]

Identification of Prognostic TMRGs Using Machine Learning Analysis

Firstly, univariate Cox regression analysis was performed on the differentially expressed TMRGs to identify prognosis-related genes. Then, machine learning analyses (Lasso Cox, Random forest (RF), and XGBoost) were conducted using "glmnet", "randomForestSRC", and "xgboost". Using a Venn diagram, genes in Lasso Cox and the top 20 genes in RF or XGBoost were intersected to find candidate genes. Finally, these candidate genes were subjected to multivariate Cox regression analysis to identify hub prognostic genes. Univariate and multivariate Cox regression analyses were performed using SPSS.

Construction and Evaluation of Prognostic Model

A prognostic model was established using the hub prognostic genes, and the risk score of each TCGA-BC patient was calculated using the expression levels of hub genes and their regression coefficients in the multivariate Cox regression analysis:

$$\sum_{i=1}^{n} gene\ expression_i \times gene\ coefficient_i$$

Then, the TCGA-BARC patients were divided into high-risk or low-risk groups based on their risk scores' optimal truncation value. The predictions of the risk model were assessed through a receiver operating characteristic (ROC) curve using "timeROC" package.

Tumor Microenvironment (TME) Analysis

Immune cell infiltration between low-risk and high-risk groups was determined using the EPIC algorithm in "IOBR" package.[22] Tumor immune dysfunction and exclusion (TIDE) score was measured on the TIDE website (http://tide.dfci.harvard.edu/).

Drug Sensitivity Analysis

The IC50 of potential drugs for BC were downloaded from the CellMiner database (https://discover.nci.gov/cellminer/home.do). The correlation between prognostic gene expression levels and drug sensitivity was analyzed, with |correlation| > 0.3 and $p < 0.05$ as statistically significant.

Cell Lines and Cell Culture

Human mammary epithelial cell line MCF 10A and human breast cancer cell line MDA-MB-231 were purchased from Pricella Biotechnology Co., Ltd (Wuhan, China). Both cell lines were maintained in DMEM/F12 and Leibovitz's L-15 medium (Pricella Biotechnology Co., Ltd) with 10% FBS under standard conditions of 37°C and 5% CO_2.

Quantitative Real-Time (qRT)-PCR

Total RNA was extracted from cells. Under the manufacturer's instruction, cDNA was synthesized through the QuantiText Rev.Transcription Kit (QIAGEN, German). The qRT-PCR reaction was conducted using Hieff UNICON Universal Blue qPCR SYBR Green Master Mix (Yeasen, Shanghai, China). The cycling conditions were 30s at 95°C, followed by 40 cycles of 3s at 95°C and 20s at 60°C. The $2^{-\Delta\Delta Ct}$ method was applied to calculate genes' relative mRNA expression levels normalized to GAPDH.

Statistical Analysis

Statistical analyses were conducted using R software version 4.1.2,[23] SPSS 25, and GraphPad Prism version 10.1.2. The Kaplan Meier (KM) method was employed to compare the overall survival (OS) between different risk groups. For quantitative data, the comparison between the two groups was evaluated via *t*-test. Correlation analysis was performed using the Pearson method. $p < 0.05$ was considered statistically significant.

Results

Functions of DEGs and Identification of Differentially Expressed TMRGs in BC

From the TCGA-BC cohort, 9390 DEGs were acquired, including 5718 up-regulated genes and 3672 down-regulated genes (Figure 1A). Functions of the DEGs were further explored through functional enrichment analysis. These DEGs were mainly associated with BPs, such as cellular protein metabolic process, macromolecule biosynthetic process, establishment of localization, cellular nitrogen compound biosynthetic process, and transport (Figure 1B). The top 5 CCs associated with DEGs were cytosol, nuclear part, protein-containing complex, nuclear lumen, and endomembrane system (Figure 1C). As for MFs, DEGs were mainly enriched in catalytic activity, metal ion binding, nucleic acid binding, anion binding, and enzyme binding (Figure 1D). KEGG enrichment analysis revealed that pathways correlated with DEGs included the PI3K-Akt signaling pathway, MAPK signaling pathway, focal adhesion, cellular senescence, and oxidative phosphorylation (Figure 1E).

In addition, 2086 TMRGs were obtained from previous literature. As shown in Figure 1F, there were 1379 overlapping genes between DEGs and TMRGs. These overlapping genes named differentially expressed TMRGs were used for further analysis.

Selection of Candidate TMRGs Related to BC Prognosis

To identify candidate genes related to the prognosis of BC, univariate Cox regression analysis of 1329 differentially expressed TMRGs first identified 128 genes significantly associated with OS of TCGA-BC patients (Table S1). Then, three machine learning algorithms, including Lasso Cox, RF, and XGBoost, were conducted on these 128 genes. Lasso Cox analysis identified 52 genes with non-coefficients (Figure 2A and B), and these genes are presented in Table S2. According to the importance, the top 20 genes identified by RF or XGBoost were shown in Figure 2C and D, respectively. After intersecting genes identified by three machine learning analyses, 7 prognosis-related candidate TMRGs (MECP2, PCMT1, PFKL, PTMA, TAGLN2, TRMT5, and XRCC4) were finally acquired (Figure 2E).

Construction of Prognostic Model Using Candidate TMRGs

Furthermore, the seven candidate TMRGs were subjected to the multivariate Cox regression analysis to construct a prognostic model. As shown in Figure 3A, the seven TMRGs were all significantly related to the prognosis of BC patients ($P < 0.05$), thus were all enrolled in the prognostic model, with MECP2, PCMT1, PFKL, TAGLN2, and XRCC4 being identified as harmful features (hazard ratio (HR) > 1), while PTMA and TRMT5 as protective features in BC (HR

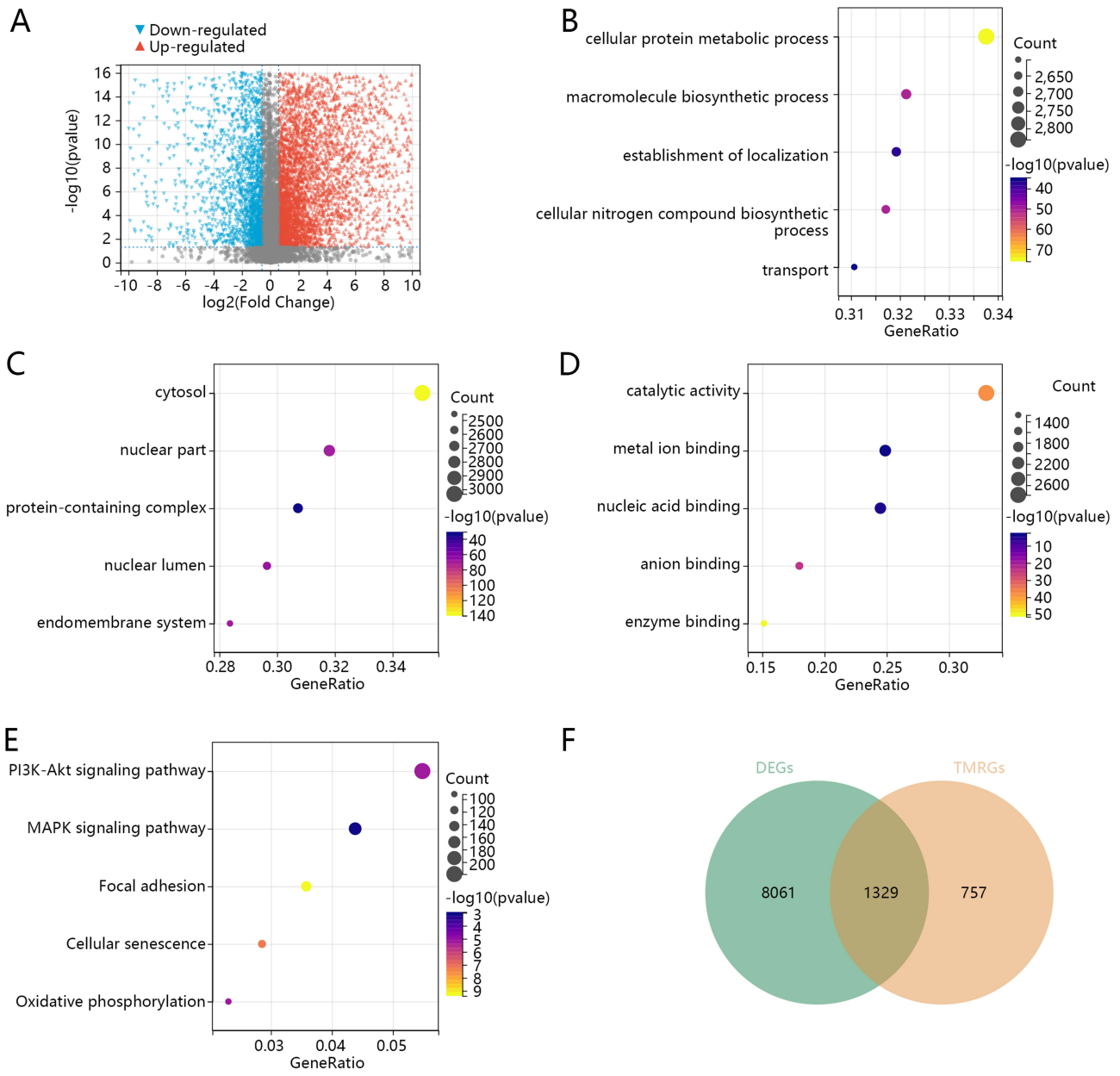


Figure 1 (**A**) Volcano plot of differentially expressed genes (DEGs) in breast cancer (BC). (**B**) Top 5 biological processes associated with DEGs. (**C**) Top 5 cellular components associated with DEGs. (**D**) Top 5 molecular functions associated with DEGs. (**E**) Top 5 KEGG pathways associated with DEGs. (**F**) Differentially expressed telomere maintenance-related genes (TMRGs) in BC were identified using a Venn diagram.

< 1) (all P <0.05). Then, the risk score of each sample in the TCGA-BC cohort was calculated based on these genes' expression levels and correlation coefficients: risk score = 0.181*MECP2 + 0.024*PCMT1 + 0.02*PFKL – 0.003*PTMA + 0.001*TAGLN2 – 0.223*TRMT5 + 0.348*XRCC4. According to the optimal truncation value of risk score, the TCGA-BC samples were divided into low-risk and high-risk groups. The prognostic risk model revealed that with the increase in risk score, the number of dead people increased (Figure 3B), and patients in the high-risk group had poor prognosis compared to those in the low-risk group ($p < 0.0001$, Figure 3C). ROC curves indicated a good prognostic value of this model, with AUCs at 1-, 3-, and 5-year of 0.81, 0.72, and 0.69, respectively (Figure 3D). An external dataset GSE7390 was then used to assess the robustness of this model. As shown in Figure S1A-B, the high-risk group exhibited significantly lower survival probability than the low-risk group ($p < 0.05$), and ROC revealed an AUC value of 0.72. In addition, we compared the differences in risk scores among groups with different clinical characteristics. As shown in

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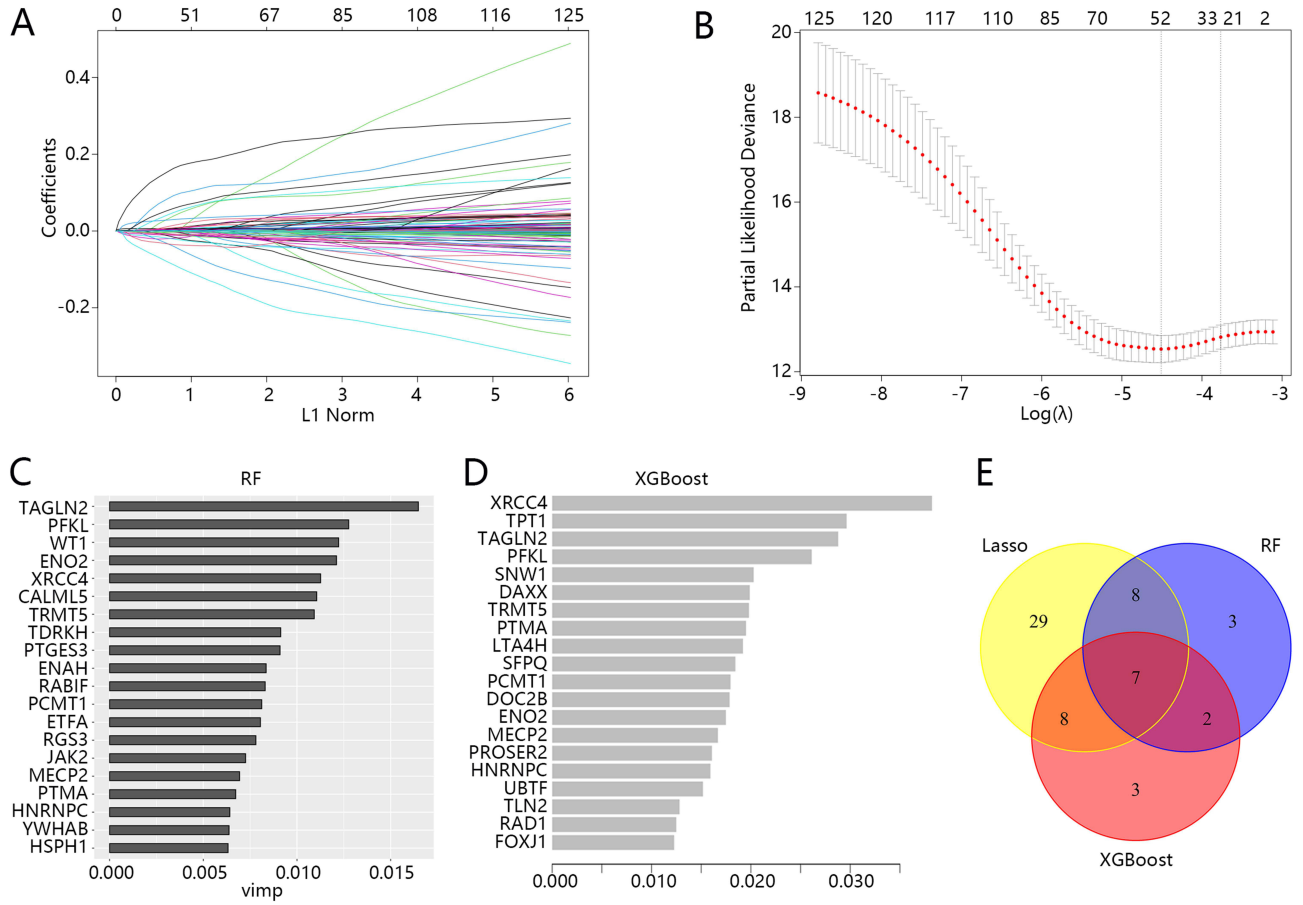

Figure 2 Selection of candidate genes related to BC prognosis using machine learning algorithms (**A-B**) Lasso Cox analysis. (**C**) Random forest (RF) analysis. (**D**) XGBoost analysis. (**E**) Venn diagram showed the overlapping genes identified by Lasso Cox, RF, and XGBoost.

Figure 3E, patients >60 years old had significantly higher risk scores compared to those aged ≤60 years ($p < 0.0001$). Patients with distant metastasis (M1) had significantly higher risk scores than those without distant metastasis (M0, $p < 0.001$). The primary tumor stage (pathologic_T) showed a gradual increase in risk scores from T1 to T4, with T4 patients having significantly higher risk scores compared to other stages ($p < 0.05$). Regarding clinical stages, the risk scores increased progressively from stage I to stage IV, with late-stage patients (III–IV) showing significantly higher risk scores than early-stage patients (I–II, $p < 0.01$). These results indicate that the risk score distinguishes patients with different ages, pathological features, and clinical stages.

The heatmap illustrated the results of the KEGG pathway enrichment analysis comparing high-risk and low-risk groups. Key pathways, including KEGG_RIBOSOME, KEGG_PROTEASOME, and KEGG_STEROID_BIOSYNTHESIS, show distinct expression patterns between the two groups (Figure S2). High-risk patients exhibited upregulation in pathways such as KEGG_DNA_REPLICATION, KEGG_MISMATCH_REPAIR, and KEGG_CELL_CYCLE, indicating enhanced DNA replication and repair processes, as well as cell cycle activity in high-risk patients.

Expression and Survival Analysis of Seven Hub Prognostic TMRGs

The expression levels of seven hub prognostic TMRGs were compared between the high-risk and low-risk groups. As shown in Figure 4A, except for TRMT5 and PTMA, the other 5 TRMGs' expression levels were increased in the high-risk group ($p < 0.01$). Survival analysis was performed to further analyze the prognostic values of the identified seven hub TMRGs in BC. Based on the TCGA-BC cohort, our data showed that expression levels of PCMT1, PFKL, XRCC4, PTMA, and TAGLN2 were up-regulated while MECP2 and TRMT5 were down-regulated in the tumor samples

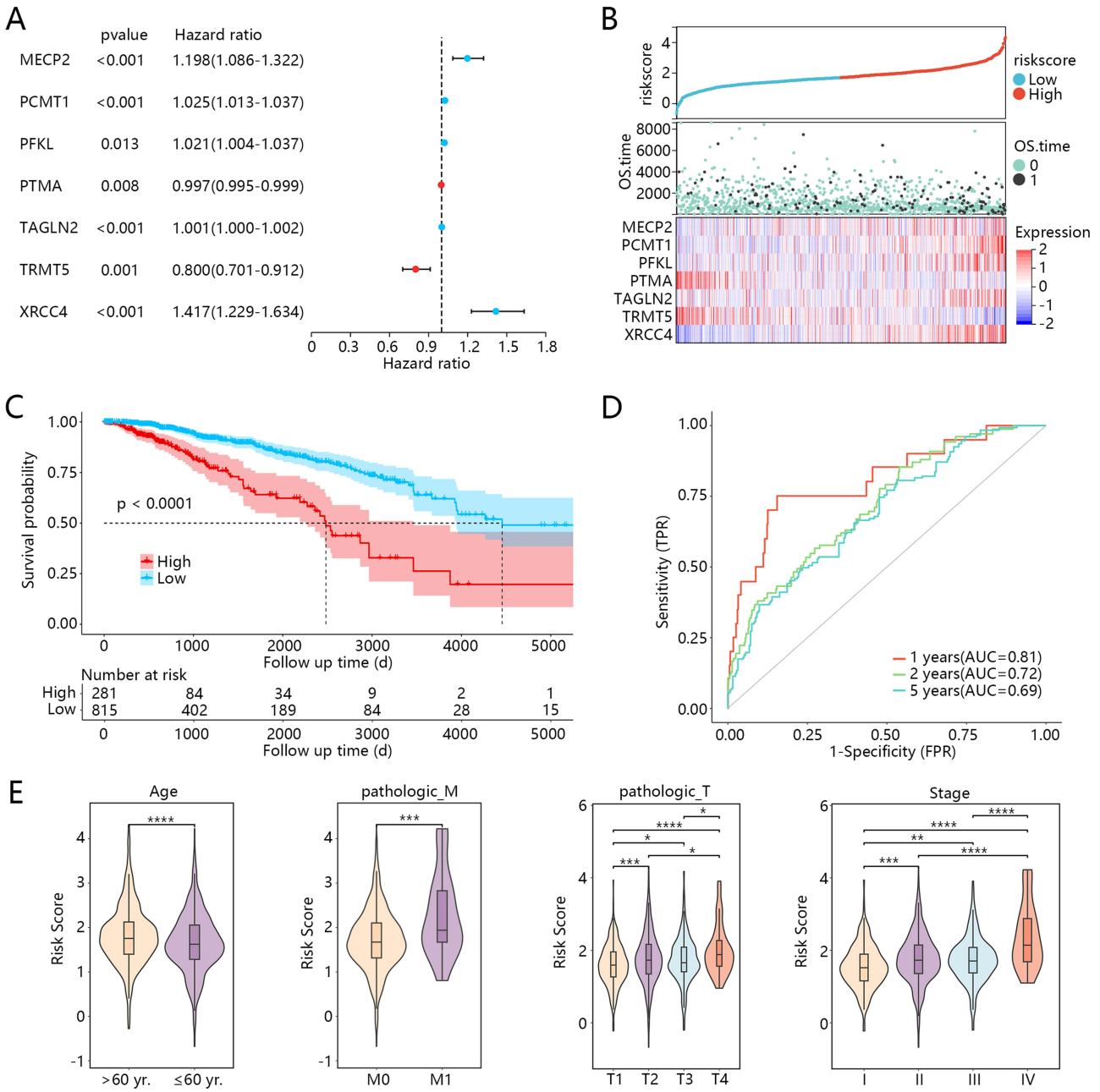

Figure 3 Construction of prognostic model using candidate TMRGs. (**A**) Multivariate Cox regression analysis. (**B**) Risk score distribution, overall survival (OS) time of each sample in the TCGA-BC cohort, and correlation heatmap of 7 prognostic TMRGs expression in each BC sample with high-risk or low-risk groups. (**C**) Kaplan Meier curve for patients in the high-risk and low-risk groups. (**D**) Receiver operator characteristic curve at 1-, 3-, and 5-year. (**E**) Risk score differences between different clinical groups. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

compared to the normal samples ($p < 0.0001$, Figure 4B). KM curves further revealed that high expression levels of MECP2, PCMT1, PFKL, TAGLN2, and XRCC4 while low expression levels of PTMA and TRMT5 indicated lower survival probability (Figure 4C).

Functions of Seven Hub Prognostic TMRGs

Additionally, the biological functions of seven prognostic hub TMRGs were explored using GSEA. These genes were mainly enriched in metabolic signaling pathways, such as oxidative phosphorylation (OXPHOS), glycolysis, mTORC1 signaling, and PI3K/AKT/mTOR signaling (Figure 5A–G).

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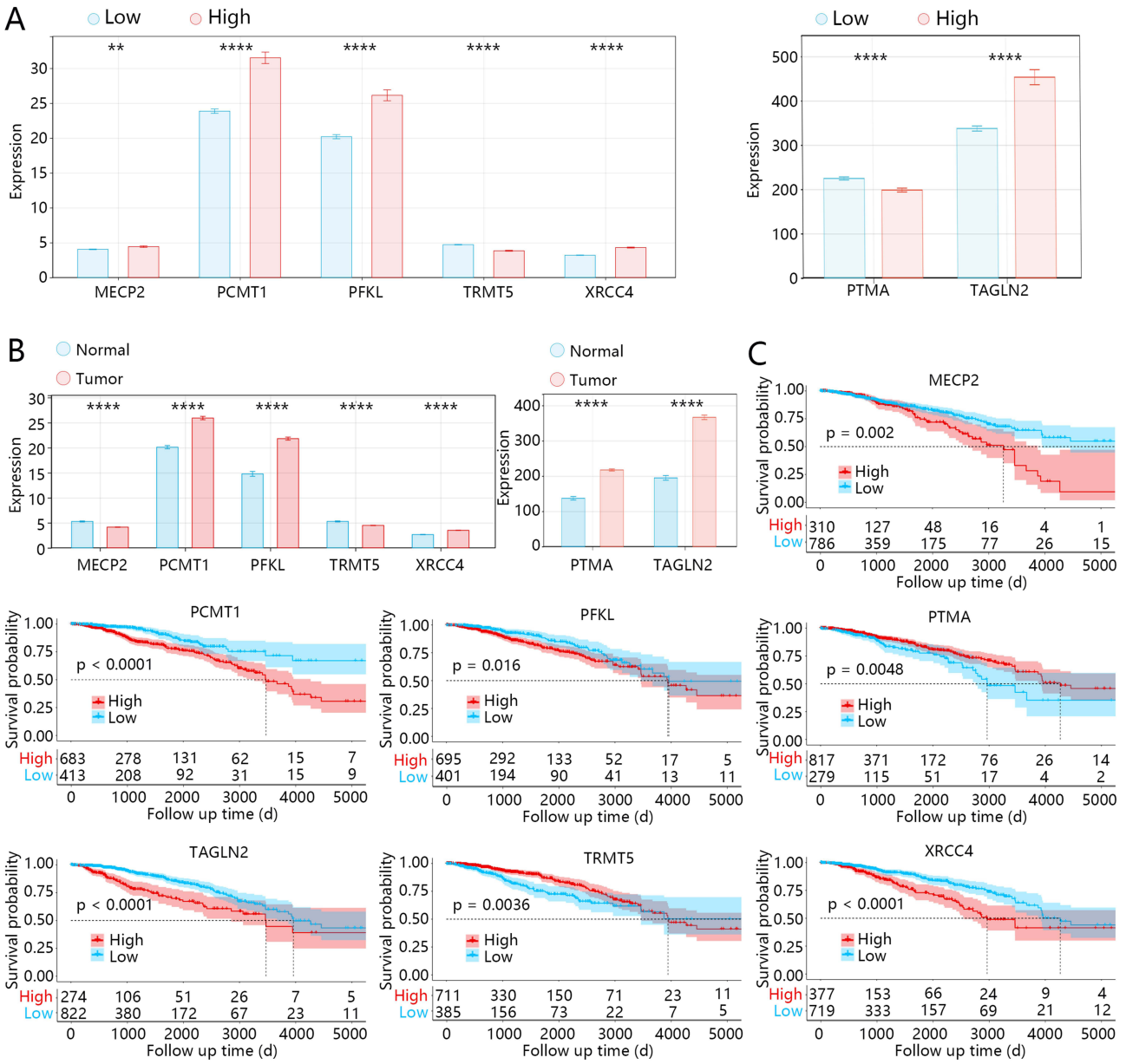

Figure 4 Survival analysis of seven hub prognostic TMRGs. (**A**) Expression levels of MECP2, PCTM1, PFKL, PTMA, TAGL2, TRMT5, and XRCC4 in the high-risk and low-risk groups. (**B**) Expression of seven prognostic TMRGs in normal and tumor samples. (**C**) Kaplan Meier curve revealed the association of seven prognostic TMRGs' expression levels with TCGA-BC patients' survival probability; d: day. **P < 0.01, ****P < 0.0001.

Role of Seven Hub Prognostic TMRGs in Tumor Microenvironment

To further characterize the performance of the prognostic model and the role of hub TMRGs in TME, immune infiltration analysis was performed. Four types of cells (B cells, CD8 T cells, endothelial, and macrophages) showing significantly different fractions in high and low-risk groups were identified ($p < 0.05$, Figure 6A). T cells are strongly associated with anti-tumor effects. Given the significantly different CD8 T cells, we further compared TIDE scores between high and low-risk groups. However, no significant difference in the TIDE score was observed (Figure 6B). Figure 6C shows the correlation between these 4 cell types and 7 prognostic TMRGs. Specifically, PFKL, PTMA, and TRMT5 were significantly associated with B cells; PFKL, TAGLN2, and TRMT5 were associated with macrophages; and only PCMT1 was associated with CD8 T cells (Figure 6C).

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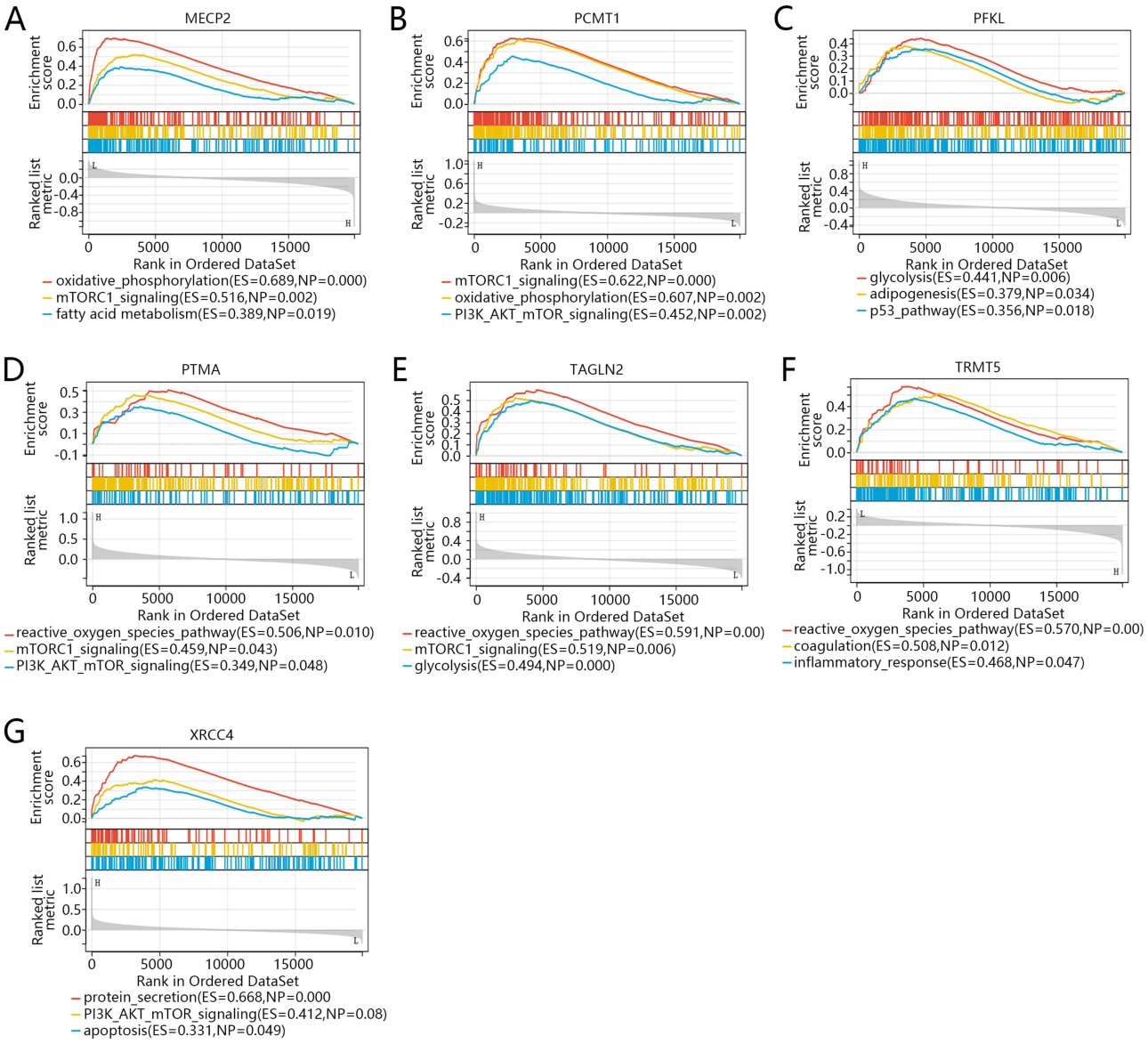

Figure 5 Functions of seven hub prognostic TMRGs. Gene set enrichment analysis revealed the functions of MECP2 (**A**), PCTM1 (**B**), PFKL (**C**), PTMA (**D**), TAGLN2 (**E**), TRMT5 (**F**), and XRCC4 (**G**).

Drug Sensitivity Analysis of Seven Hub Prognostic TMRGs

The potential relationship between the expression of seven hub TMRGs and drug sensitivity was further explored using the CellMiner database. Our data showed that MECP2, PCMT1, PFKL, PTMA4, TAGLN2, TRMT5, and XRCC4 expression levels were correlated with sensitivity of 6, 5, 3, 32, 12, 8, and 5 drugs, respectively (Table S3). The results with the highest correlation were visualized in Figure 7. MECP2, PFKL, and XRCC4 levels were positively correlated with the sensitivity of Vemurafenib, Indibulin, and Ribavirin, respectively (Figure 7A–C), and expression levels of PCMT1, PTMA, and TRMTS were positively related to Chelerythrine sensitivity (Figure 7E–G). In contrast, TAGLN2 expression was negatively related to BP-1-102 sensitivity (Figure 7D).

Expression of Seven Prognostic TMRGs in Cells

Finally, the expression of seven prognostic TMRGs was validated in vitro. As shown in Figure 8, expression levels of MECP2 and TRMT5 were reduced in the MDA-MB-231 cells compared with MCF-10A cells, while expression levels of the other five TMRGs were elevated in the MDA-MB-231 cells compared with MCF-10A cells ($p < 0.05$).

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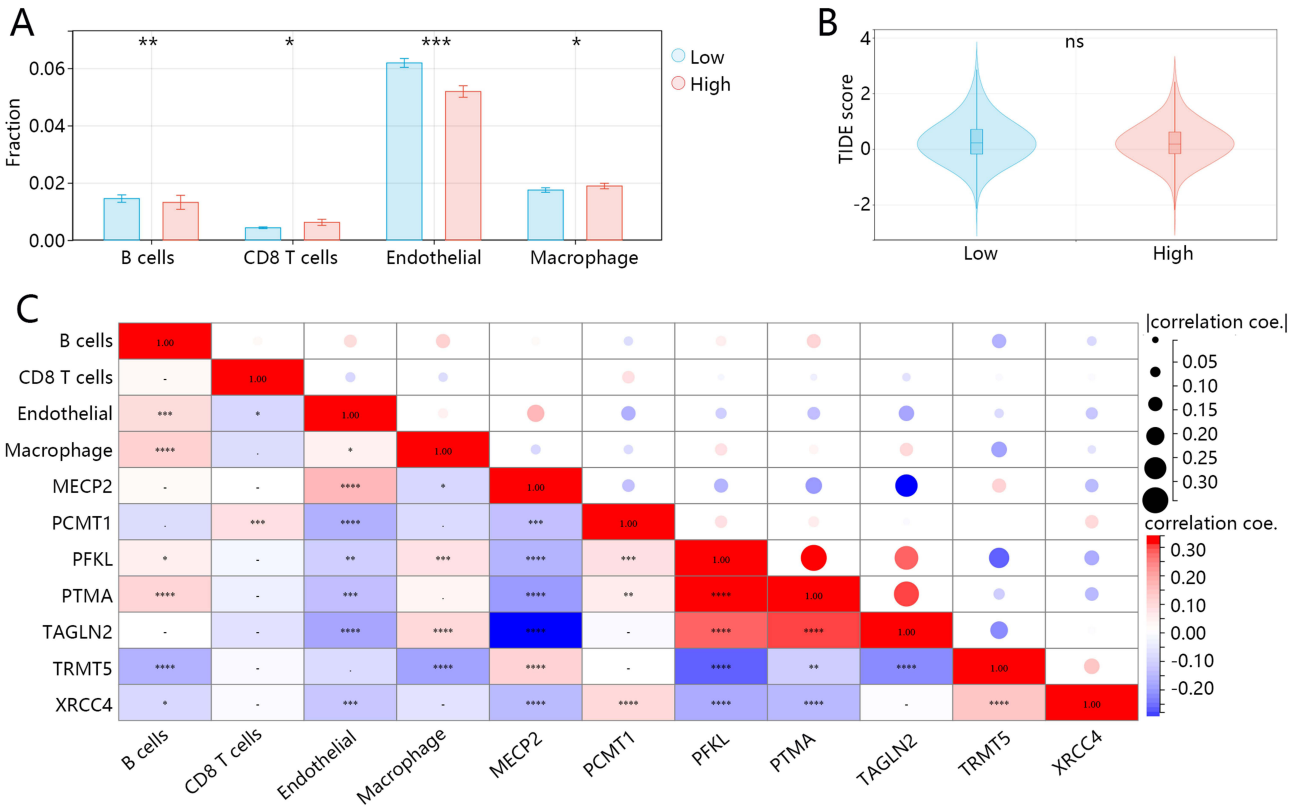

Figure 6 Role of seven hub prognostic TMRGs in tumor microenvironment. (**A**) Immune cell infiltration analysis. (**B**) TIDE score in normal and tumor samples; ns: no significant. (**C**) Correlation heatmap between immune cells and prognostic genes; coe: coefficient. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns: not significant.

Discussion

Given the limitations of existing BC prognosis models, which rely heavily on clinical parameters and the anatomically focused AJCC TNM staging system that fails to accurately predict recurrence,[24] more reliable models are needed to predict BC patient prognosis and enhance personalized treatment. A thorough analysis of clinical and biological characteristics is crucial for determining appropriate treatment options during clinical decision-making.[25] Studies have shown that a composite measure combining clinicopathologic data with biomarkers can more accurately predict the benefit of different treatment regimens in BC patients.[26] Telomere maintenance is crucial for tumorigenesis and cancer progression. In this study, a telomere maintenance-related seven-gene risk prognostic model was established using three machine learning algorithms. This model may assist clinicians in more accurately stratifying patient management and optimizing treatment strategies to improve long-term survival rates. Additionally, survival analysis revealed that MECP2, PCMT1, PFKL, PTMA, TAGLN2, TRMT5, and XRCC4 were identified as independent prognostic factors, and some drugs related to these seven TMRGs were acquired.

Telomere maintenance plays a crucial role in predicting cancer prognosis. Prognostic models based on telomere-related genes have been developed for various cancers, including head and neck squamous cell carcinoma,[27] renal cancer,[28] and colorectal cancer.[29] Previous studies have highlighted that telomere maintenance mechanisms influence metastasis and treatment response in BC.[30] In this study, we constructed a seven-gene prognostic model associated with telomere maintenance. The model effectively stratified BC patients into high- and low-risk groups and demonstrated excellent prognostic value. The risk score calculated by the model successfully differentiated patients across various ages, pathological features, and clinical stages.

Among the seven identified hub TMRGs, MECP2, PCMT1, PFKL, TAGLN2, and XRCC4 have been previously reported to be associated with BC. MECP2 is an important epigenetic regulator that has been linked to prognosis in various cancers. A pan-cancer analysis found that low expression of MECP2 is associated with better OS in BC.[31] This study also

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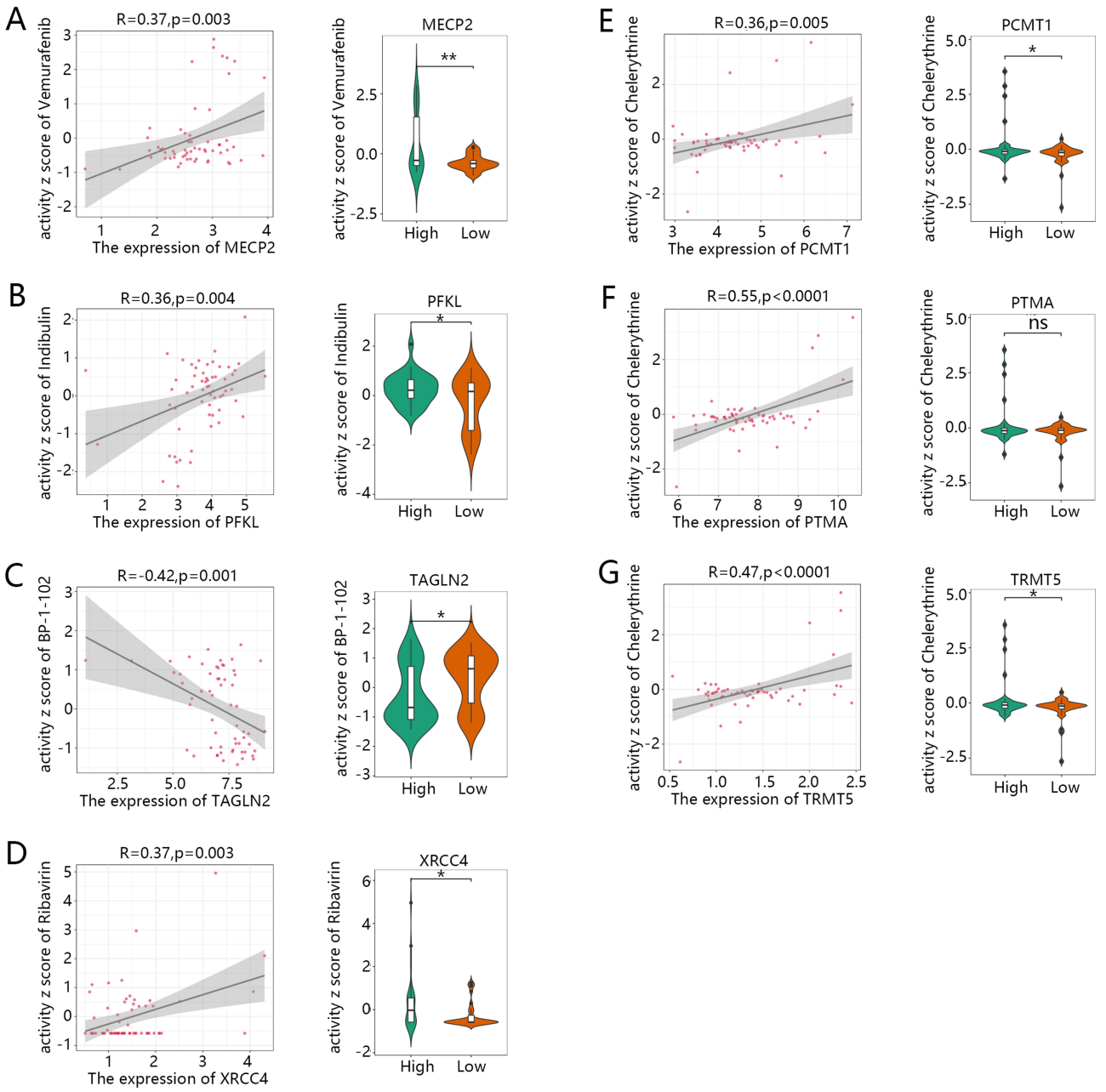

Figure 7 Drug sensitivity analysis of seven prognostic TMRGs. Correlation of drug sensitivity with expression levels of MECP2 (**A**), PFKL (**B**), XRCC4 (**C**), TAGLN2 (**D**), PCMT1 (**E**), PTMA (**F**), and TRMT5 (**G**). *P < 0.05, **P < 0.01, ns: not significant.

found that high expression of MECP2 is associated with a worse prognosis in BC patients. However, when comparing tumor tissues with normal tissues, we observed a significant decrease in MECP2 expression in tumor tissues. Similarly, compared to the normal breast cell line MCF-10A, MECP2 expression was significantly downregulated in the triple-negative breast cancer (TNBC) cell line MDA-MB-231. This result is consistent with the study by Jiang et al, which found that compared to Luminal BC, MECP2 protein expression was almost undetectable in TNBC, and overexpression of MECP2 reduced the migratory ability of MDA-MB-231 cells.[32] We speculate that MECP2 expression may differ among different BC subtypes. Specifically, in TNBC, the low expression of MECP2 may reflect the high heterogeneity and invasive characteristics. The expression pattern of MECP2 may depend on the stage of tumor progression and microenvironmental factors, such as tumor malignancy, local immune response, and intercellular interactions in TNBC. Therefore, although high expression of MECP2 is associated with poor prognosis in some BC patients, its biological role may differ significantly across subtypes. Further

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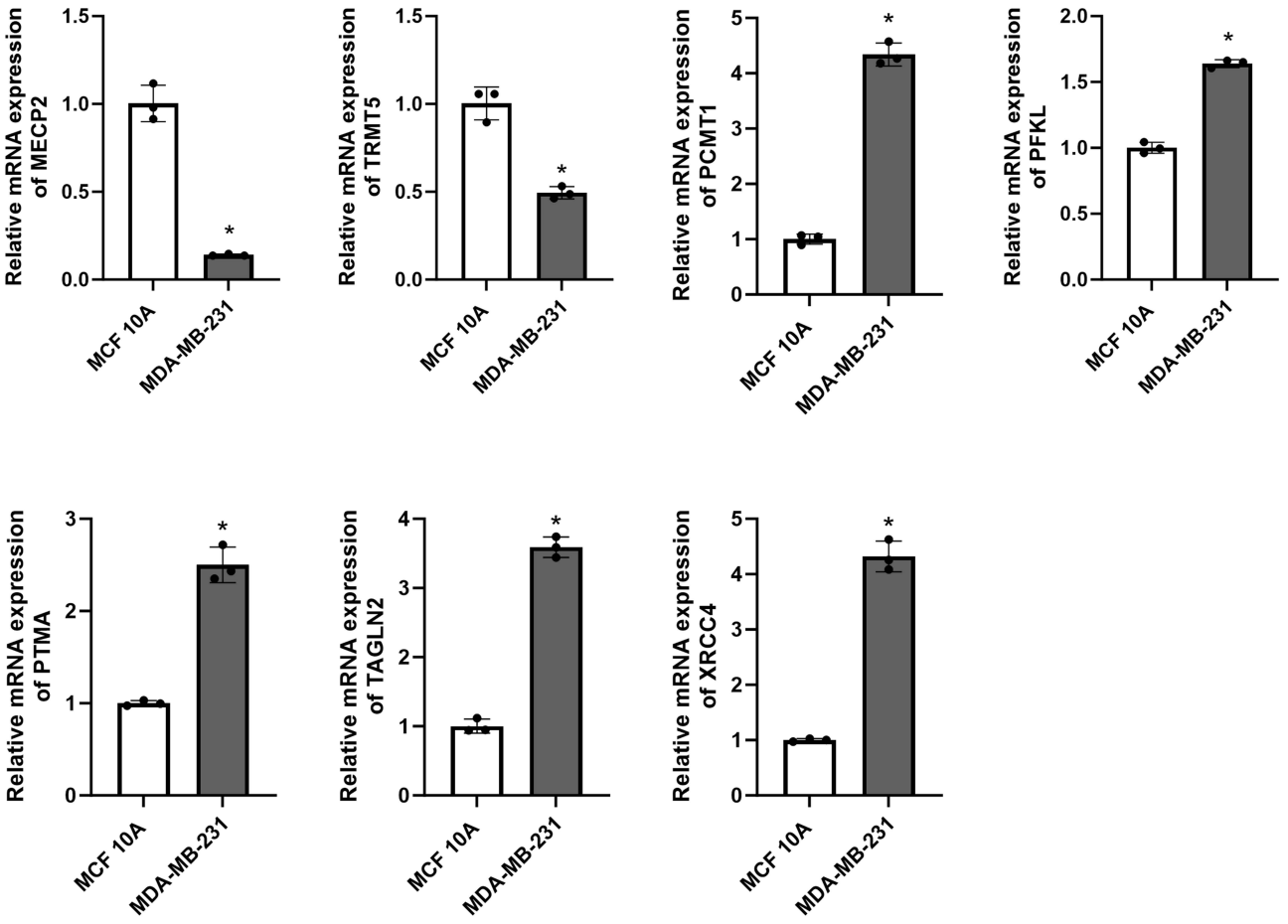

Figure 8 Expression of seven prognostic TMRGs in cells. *P < 0.05.

experiments and data analysis are needed to uncover the complex regulatory mechanisms of MECP2. PCMT1 is a methyltransferase that regulates cancer-related processes such as apoptosis by modulating proteins.[33] High expression of PCMT1 in BC has been noted, and Guo et al identified it as a prognostic biomarker related to BC immune infiltration.[34] We also observed high expression of PCMT1 in BC cells and tissues. PFKL, a subtype of PFK, plays a crucial role in glycolysis. Previous studies have shown that PFKL can predict the prognosis of BC patients.[35,36] TAGLN2 is an actin-binding protein significantly overexpressed in BC tissues.[37] Additionally, Liu et al found that overexpression of TAGLN2 enhances migration and invasion of human BC cells by activating the PI3K/AKT signaling pathway.[38] XRCC4 is a DNA repair gene, and its high expression is significantly associated with poor progression-free survival in BC patients' post-radiotherapy.[39] It can also effectively predict the risk of BC metastasis.[40] We observed high expression of PFKL, TAGLN2, and XRCC4 in BC tissues and cell lines.

Currently, there is limited research on PTMA and TRMT5 in BC, although their roles in other cancers have been studied. PTMA is a nuclear oncogene involved in cell cycle regulation, with overexpression associated with tumor aggressiveness and poor prognosis in glioma,[41] colorectal cancer,[42] and esophageal cancer.[43] TRMT5 has been reported to be upregulated in liver cancer tissues, and knocking down TRMT5 inhibits liver cancer progression by enhancing cellular oxygen levels and inactivating the HIF-1 signaling pathway.[44] In our study, PTMA was significantly overexpressed while TRMT5 was downregulated in BC tissues and cells. The specific roles of PTMA and TRMT5 in BC remain to be further investigated.

Telomere dysfunction in cells has been reported to result in enhanced glucose metabolism both in glycolysis and in the tricarboxylic acid cycle at the organismal level.[45] We investigated the potential mechanisms of these hub TMRGs in BC, metabolic pathways such as OXPHOS, glycolysis, and PI3K/AKT/mTOR signaling were enriched. Aerobic glycolysis is the

most common form of abnormal metabolism in cancers, but cancer cells also utilize OXPHOS to produce ATP. BC exhibits extensive metabolic heterogeneity.[46] Previous studies have observed upregulation of OXPHOS in BC tumor tissues, with proteins related to mitochondrial OXPHOS being overexpressed in BC cells.[47,48] The PI3K/AKT/mTOR signaling pathway is important in cancer progression by regulating metabolism.[49] The production of ROS during OXPHOS enhances the PI3K/AKT pathway, thereby promoting tumor progression.[50] Targeting PI3K/AKT/mTOR is a potential pathway to treat BC.[51] A previous study reported that the telomere maintenance-associated protein dyskerin triggers functional autophagy through inhibition of the PI3K/AKT/mTOR pathway.[52] Another study demonstrated that PI3K/AKT signaling participated in telomere maintenance.[53] In our study, GSEA showed that MECP2, PCMT1, PTMA, TAGLN2, and XRCC4 are associated with OXPHOS, glycolysis, or the PI3K/AKT/mTOR signaling pathway. These findings revealed that hub TMRGs may influence the development of BC by regulating metabolic pathways.

In this study, we preliminarily explored the prognostic value of TMRGs in BC patients. However, there were some limitations in the study. Our results were primarily based on public databases, with potential biases inherent, and future studies need to further verify the role of TMRGs through cell and animal experiments. Additionally, more research is needed to validate the potential molecular mechanisms of hub TMRGs in BC. Addressing these aspects was expected to enhance the understanding of telomere maintenance in BC, with the ultimate goal of improving personalized prognosis and developing targeted therapies.

Conclusion

Overall, this study emphasized the importance of TMRGs in BC prognosis and therapy. By identifying seven hub TMRGs and establishing a robust prognostic model, we demonstrated their utility as potential biomarkers for stratifying BC patients and optimizing treatment strategies. The findings not only deepen our understanding of the molecular mechanisms underlying BC, particularly the role of telomere maintenance but also pave the way for exploring targeted therapeutic approaches. Our results underscore the potential of TMRG-based interventions to improve personalized treatment, enhance prognostic accuracy, and ultimately contribute to better clinical outcomes for BC patients. This study provides a valuable framework for future research to further validate and expand the clinical applications of TMRGs in oncology.

Data Sharing Statement

Any additional data, not shared with this article, is available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The Ethics Committee of The First People's Hospital of Jiande deemed that this research is based on open-source data, so the need for ethics approval was waived.

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Disclosure

The authors declare no competing interests in this work.

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