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ORIGINAL ARTICLE

Basement Membrane Related Lncrnas Risk Model for Predicting Prognosis and the Immunotherapy Responsiveness of Breast Cancer

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Abstract:
We developed a risk model for breast cancer (BC) based on basement membrane (BM)-related long non-coding RNAs (lncRNAs) using the Cancer Genome Atlas (TCGA) data. Eight differentially expressed BM-related lncRNAs were identified and used to create the model. The model effectively predicts 1-, 3-, and 5-year survival rates, with high-risk patients showing worse outcomes. Additionally, we assessed tumor mutation load, immune cell infiltration, and responses to treatments. This model offers valuable insights for survival prediction and therapeutic decision-making in BC patients.

Keywords: Breast cancer, basement membrane, Long non-coding RNA, prognostic model, immune microenvironment.

Introduction

Breast cancer ranks among the most prevalent malignancies globally and the prevalence has been steadily increasing at a rate of 0.6 percent per year for the past two decades[1]. Recent meta-analyses reveal a substantial enhancement in the global survival rates of BC patients over recent decades. However, survival rates in developing regions continue to lag behind those in developed areas [2]. Notably, at the initial clinical diagnosis, the incidence of distant metastasis among stage III patients is approximately 45%, nearly threefold higher than in stage II patients [3]. Furthermore, BC manifests as a highly heterogeneous disease, marked by significant differences in gene expression profiles, biological behavior, and clinicopathological features [4].

In clinical settings, the prognosis of BC patients is often evaluated based on tumor stage, molecular subtype, and histological grade. Yet, these clinical indicators fall short in precisely predicting patient prognosis and drug sensitivity, resulting in inaccurate prognostic assessments and inappropriate drug interventions. Thus, investigating prognostic biomarkers holds significant importance in predicting BC patient prognosis accurately and tailoring anti-tumor drug selection and treatment on an individualized basis.

The BM forms a thin, dense layer of extracellular matrix (ECM) [5]. It safeguards tissues against detrimental physical stresses and facilitates signaling between their microenvironment and cells. Within the basement membrane, a plethora of biochemical and mechanical signals are present, crucial for cellular signaling, maintaining

structural integrity, and providing a barrier against abnormal cells and macromolecules in the body[6]. Perturbations in the chemical and mechanical properties of the basement membrane are implicated in various diseases, notably cancer. For instance, the stiffness of BM is modulated by the ratio of netrin-4 (Net-4) to laminin molecules; a higher ratio correlates with a softer BM, consequently reducing the invasion activity of cancer cells[7]. All in all,BM serves as a protective structural barrier against invasion, migration, and exosmosis of cancer cells, playing a crucial role in malignant tumors, especially epithelial cancers[6] .

In epithelial tumors, cancer cells typically infiltrate the surrounding stromal tissue by traversing the BM to metastasize. For individuals diagnosed with breast carcinoma in situ, the 5-year survival rate stands at approximately 99%. However, upon breaching the basement membrane and transitioning to invasive cancer, this rate declines to 85%. Additionally, for BC patients with distant metastasis, the 5-year survival rate decreases to 27%[8]. lncRNAs, which were defined as non-coding transcripts of more than 200 nucleotides long, are known for their importance role in cell differentiation and tumor development[9]. These molecules are pivotal in diversified biological processes, such as cell proliferation, differentiation, apoptosis, and metastasis[10]. Considerable evidence suggests that aberrant expression of lncRNAs significantly contributes to the initiation and progression of breast cancer. For example, He et al. showed that upregulation of lncRNA T376626 enhances proliferation, migration, and invasion of triple-negative breast cancer (TNBC) cells by regulating cell cycle, apoptosis, and epithelial-mesenchymal transition (EMT) pathways [11]. Likewise, Jia et al. discovered circPVT1 facilitates breast cancer cell proliferation by upregulating the expression of ESR1 and downstream ERα target genes, indicating its potential as both a diagnostic marker and therapeutic target for ERα-positive BC [12].Additionally, Li et al. pinpoint laminin subunit α2 (LAMA2) as a differentially expressed gene associated with breast cancer. And revealed that LINC01270 suppresses the MAPK signaling pathway by inhibiting LAMA2 expression, thereby impeding breast cancer progression[13].

Clinical data indicate significant dysregulation of specific lncRNAs in lesions and precancerous tissues of kidney and bladder cancer patients. Hence, these lncRNAs show potential as novel biomarkers for diagnosing tumors, predicting prognosis, and assessing progression[14]. Thus, a thorough grasp of the regulatory mechanisms controlling lncRNAs in breast cancer is crucial for developing innovative therapies. Given the limited literature on the link between basement membrane-related lncRNAs in breast cancer and immunotherapy prognosis, our team formulated a prognostic risk model for these lncRNAs. This model seeks to precisely predict breast cancer outcomes and steer customized clinical interventions.

Methods

2.1 Data Collection and Processing

Transcriptome data and clinical information of breast cancer patients were obtained from the TCGA database (https://portal.gdc.cancer.gov/). A total of 1094 samples from breast cancer patients were collected, including data on mRNA and lncRNA expression, along with somatic mutation data.The transcriptome data underwent processing and standardization utilizing the "TCGAbiolinks" package in the R programming language.

2.2 Detecting Basement Membrane-Related Lncrnas in Breast Cancer Tissues

We used the "limma" package in R to pinpoint genes associated with the basement membrane specific to breast cancer tissues. Subsequently, Pearson correlation analysis was conducted to evaluate the association between these genes and expressed lncRNAs in breast cancer tissues, with a filtering criterion of $|r| > 0.4$ and $P < 0.001$. This process aimed to pinpoint differentially expressed basement membrane-related lncRNAs in breast cancer tissues. Subsequently, the Sankey map was generated using R packages "dplyr," "ggalluvial," and "ggplot2."

2.3 Construction of Prognostic Model

After excluding patients with incomplete clinical information, 1082 eligible patients were randomly assigned to training and validation cohorts in a 7:3 ratio. The training cohort was utilized to develop the risk scoring system, while the validation cohort was utilized to validate its prognostic ability of it. Chi-square test analysis showed no statistical significant difference in age, gender, or

staging between the groups.Within the training cohort, basement membrane-related lncRNAs affecting breast cancer patient prognosis were first identified through one-way Cox regression analysis using the R package "survival," with a screening criterion of $P < 0.05$. To prevent overfitting, we then employed the R packages "survival" and "glmnet" to further screen closely associated basement membrane-related lncRNAs using least absolute shrinkage and selection operator (LASSO) regression. Subsequently, a multifactor Cox regression analysis was performed to ascertain the regression coefficients for the prognostic risk score system[15].

After constructing the breast cancer prognostic prediction model, the most suitable model was selected based on the Akaike Information Criterion (AIC) to determine the optimal fit.The model formula is as follows: risk score = coef (lncRNA1) × expr (lncRNA1) + coef (lncRNA2) × expr (lncRNA2) + ... + coef (lncRNAn) × expr (lncRNAn), where "expr" denotes gene expression level and "coef" indicates regression coefficient.

2.4 Validation of Risk Scoring System

To assess the effectiveness of the prognostic prediction model, risk scores were computed for patients in the training group. Subsequently, patients were categorized into low-risk and high-risk groups based on the median risk score. Survival analyses were carried out using the "survival" and "survminer" R packages across the training, validation, and combined groups. Following univariate and multivariate regression analyses were performed to evaluate if the risk score could serve as a potential independent prognostic indicator for breast cancer patients. The prognostic model's ability to predict 1-, 3-, and 5-year survival in breast cancer patients was assessed by creating receiver operating characteristic (ROC) curves with the "timeROC" package and calculating the area under the curve (AUC). Furthermore,we compare the predictive performance of risk scores and clinical characteristics through ROC curve plotting in the combined group.

2.5 Development and Evaluation of Column-Line Diagrams

We utilized the R package "rms" to generate a column chart by integrating differentially expressed BM-related lncRNAs (DEBMLnc RNAs) and clinical features to predict the survival of BC patients. Calibration curves were generated using the "bootstrap" software package. Additionally, the consistency index (C-index) and ROC curve over time were calculated.

2.6 Principal component analysis(PCA) analysis

PCA was used to effectively reduce dimensionality, identify patterns, and illustrate groups within the high-dimensional data encompassing full gene expression profiles[16]. This included 224 genes and 563 lncRNAs associated with BM, along with the risk prediction models. The outcomes were visualized utilizing the "scatterplot3D" package within the R software environment.

2.7 Functional Enrichment Analysis 0f Differentiated Expression Genes in High- and Low-Risk Groups

We identified gene disparities between the low-risk and high-risk groups and further scrutinized them using GSEA. This entailed performing Gene Ontology (GO) analysis to explore pertinent biological processes and pinpointing differentially expressed genes linked with KEGG pathways across the two groups. The KEGG gene set (c2.cp.kegg.Hs.symbols.gmt) and the GO gene set (c5.go.Hs.symbols.gmt) were acquired from the website (https://www.gsea-msigdb.org/).

2.8 Analysis of Tumor Immune Microenv-Ironment and Immune-Related Functions

We examined the variances in immune infiltration microenvironment between the high-risk and low-risk groups, considering specific cell types and immune function. This was accomplished using the single-sample gene set enrichment analysis (ssGSEA) algorithm [17]. Additionally, A Wilcoxon test was performed to assess the expression levels of immune checkpoint genes across different risk groups, focusing on 26 immune checkpoint genes identified in previous studies.N6-adenylate methylation (m6a) is a form of internal RNA modification, our study delved deeper into the variations in m6a-modifying gene expression between the low-risk and high-risk groups, utilizing several R packages such as "limma," "GSEABase," "GCVA," "reshape2," "Pheatmap," "ggplot2," and "ggpubr."

2.9 Genetic Mutation Loads Were Analyzed and Quantified.

We obtained mutation data from TCGA, utilizing the somatic mutation data to calculate the TMB score for each group. Next, we identified the optimal threshold based on the TMB score, thus categorizing patients into low and high mutation groups to access the association between prognosis and TMB. The analysis employed R packages such as "limma," "survival," "ggpubr," and "survminer." Furthermore, we utilized the Tumor Immune Dysfunction and Exclusion (TIDE) Algorithm[18]to evaluate potential response to immune checkpoint blocking (ICB) treatment in BC patients.

2.10 Drug Sensitivity Analysis

Given the potential of lncRNAs in modulating antitumor drug sensitivity, we assessed the IC50 of 136 antitumor drugs and compared sensitivity differences between the two risk groups. R packages employed included "oncoPredict R," "ggpubr," "limma," and "ggplot2."

2.11 Statistical Analysis

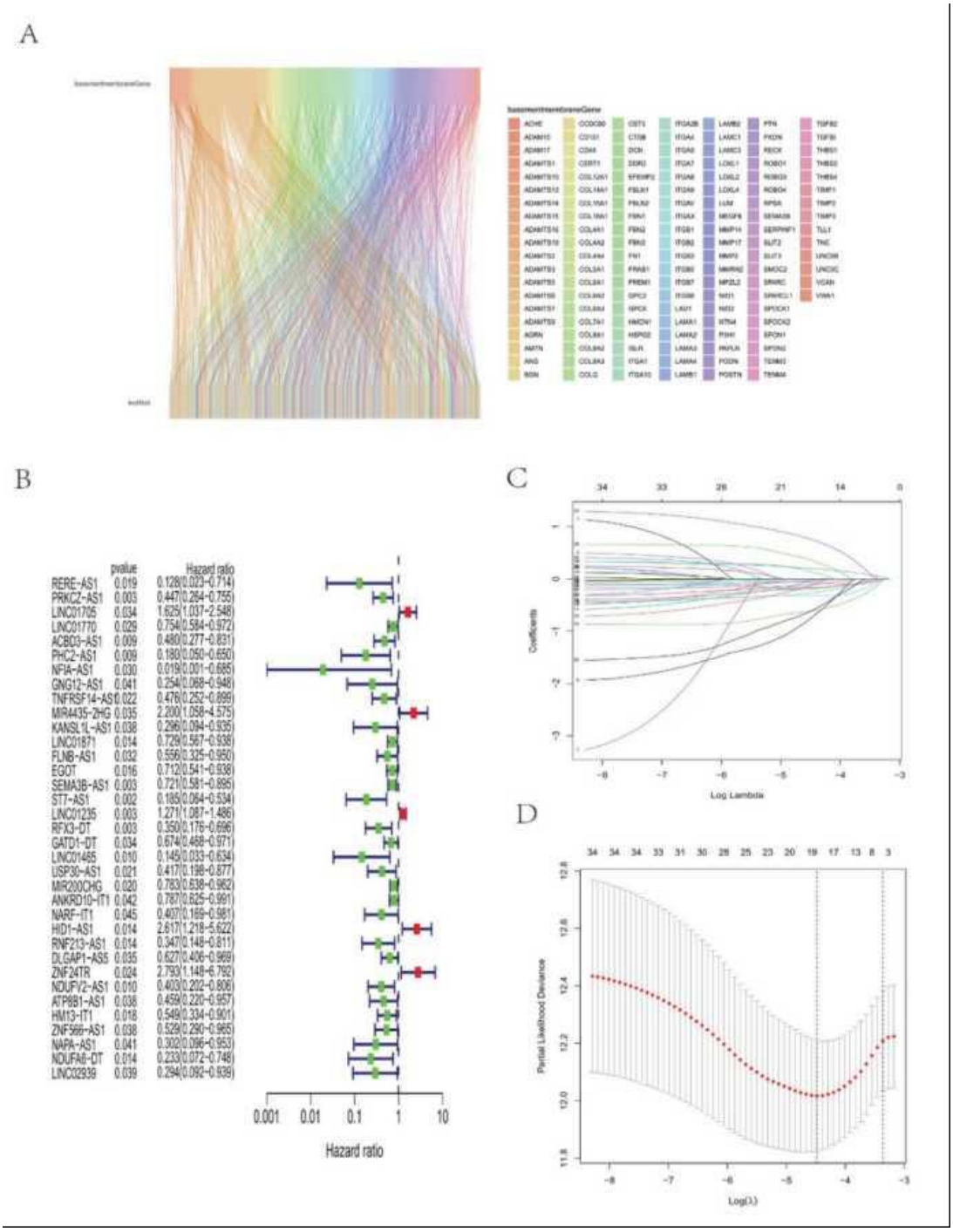
The Chi-square test examined disparities in patients' general characteristics across the two groups. Pearson correlation analysis explored the co-expression connection between lncRNAs and BM-related genes. Kaplan-Meier analysis, coupled with a log-rank test, scrutinized the OS and PFS among patients in distinct groups. Cox regression identified independent prognostic factors, with factors exhibiting $P<0.05$ in univariate regression being incorporated into multivariate regression. Statistical significance was set at $P<0.05$.

Results

3.1 Obtaining BM-related lncRNAs

The genes linked to the basement membrane in the dataset were acquired utilizing the "limma" package in R. Subsequently, 563 BM-related lncRNAs were identified through co-expression analysis. The co-expression relationship graph (Figure1) illustrates the connection between basement membrane-related genes and lncRNAs, with each connection representing a co-expression relationship.

Figure 1: Screening of basement membrane-related characteristic LncRNAs. (A) Sankey diagram of the co-expression relationship between basement membrane-related LncRNAs and related genes; (B) Among the 35 basement membrane-related lncRNAs selected by univariate Cox regression analysis, red lncRNAs were associated with high risk, while green lncrnas were associated with low risk. (C, D) Eight prognostic correlated LncRNAs were identified by cross-validation of LASSO analysis.



3.2Construction of the risk scoring system

The risk scoring system was developed in training cohort and validated in validation cohort. Chi-square test analysis showed no remarkable differences in clinical characteristics between the two cohorts（table1). In the training cohort, Univariate Cox regression analysis identified 35 lncRNAs linked to breast cancer patient prognosis. Further analysis using Lasso regression and multivariate Cox regression revealed 8 DEBMlncRNAs(table2), which were used to create the risk score system. Five of these lncRNAs were identified as protective factors, while the remaining three were considered risk factors. The formula utilized for risk score determination is as follows: Risk score = (-2.24 59 1833224486)*GNG12-AS1+(-1.2789173112972 7)*KANSL1L-AS1+(-0.478944964349569)* LIN C01871+(-0.320947399564139)*SEMA3B-AS1+ (-1.02063899279776)*ST7-AS+ (0.2799 5345162 7031)*LINC01235+ (1.25653857637997)*HID1-AS1+(0.835319830761729)*ZNF24TR.

Table I

Clinical characteristics of breast cancer patients

Variables	Total cohort	Training cohort	Validation cohort	P value
	N=1082, (%)	N=759, (%)	N=323,(%)	
age				0.6754
≤65	765 , (70.7%)	225 (69.66%)	540(71.15%)	
> 65	317(29.3%)	317(29.3%)	98(30.34%)	
Gender				0.5604
Female	1070(98.89%)	318(98.45%)	752(99.08%)	
Male	12(1.11%)	5(1.55%)	7(0.92%)	
Stage				0.5871
Stage I	182(16.82%)	55(17.03%)		
Stage II	618(57.12%)	190(58.82%)	428(56.39%)	
Stage III	249(23.01%)	68(21.05%)	181(23.85%)	
Stage IV	20(1.85%)	4(1.24%)	16(2.11%)	
unknown	13(1.2%)	6(1.86%)	7(0.92%)	
T stage				0.9565
T1	277(25.6%)	79(24.46%)	198(26.09%)	
T2	627(57.95%)	190(58.82%)	473(57.58%)	
T3	138(12.75%	42(13%)	96(12.65%)	
T4	37(3.42%)	11(3.41%)	26(3.43%)	
unknown	3(0.28%)	1(0.31%)	2(0.36%)	
N Stage				0.9176
N0	514(47.5%)	157(48.61%)	357(47.04%)	
N1	356(32.9%)	102(31.58%)	254(33.47%)	
N2	117(10.81%)	34(10.53%)	83(10.44%)	
N3	76(7.02%)	24(7.43%)	52(6.85%)	
unknown	20(1.85%)	6(1.86%)	13(1.71%)	
M stage				0.5952
M0	903(83.45%)	275(85.14%)	628(82.74%	
M1	20(1.85%)	4(1.24%)	16(2.11%)	
unknown	159(14.7%)	44(13.62%)	115(15.15%)	

Table II Risk coefcients and hazard ratios for eight lncRNAs

ID	coef	HR	Pvalue
GNG12-AS1	-2.245918332	0.254	0.041
KANS1L-AS1	-1.278917311	0.296	0.038
ST7-AS1	-1.020638993	0.185	0.002
LINC01871	-0.478944964	0.729	0.014
LINC01235	0.279953452	1.271	0.003
HID1-AS1	1.256538576	2.617	0.014
ZNF24TR	0.835319831	2.793	0.024
SEMA3B-AS1	-0.3209474	0.721	0.003

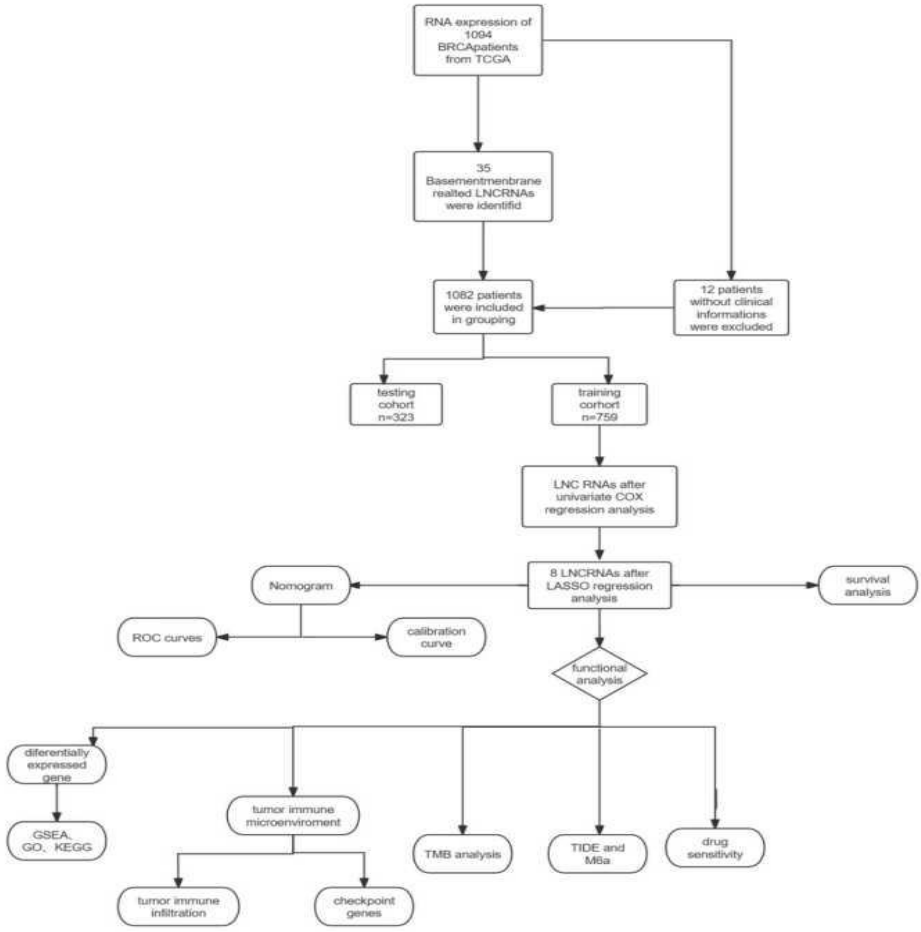


Figure 2: Data collection and analysis flow chart.

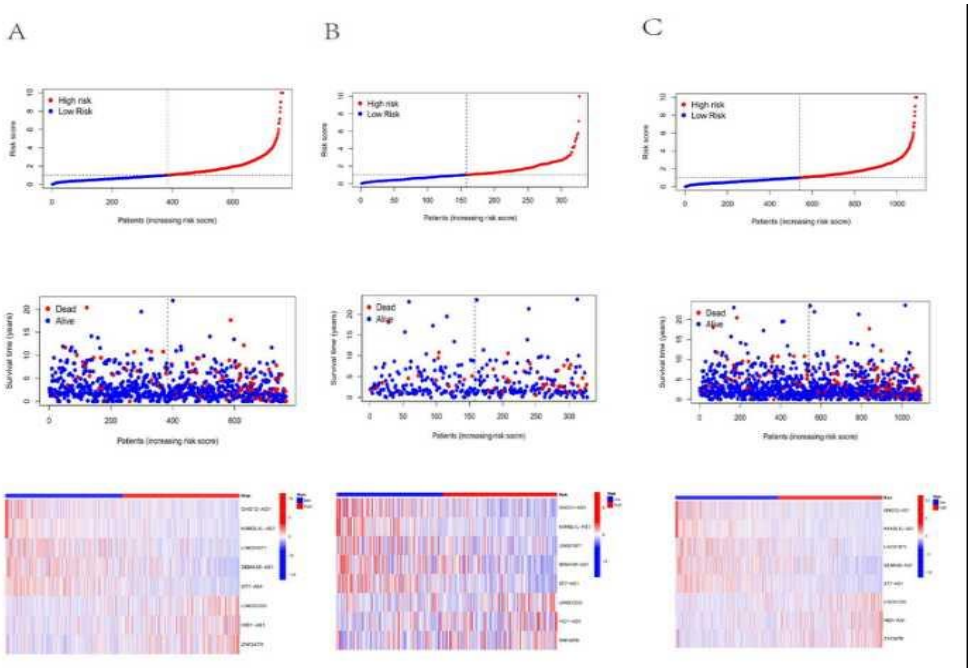


Figure 3. Prognostic value of risk scoring system or models that consisting of eight DEBMLncRNAs in different groups. (A) The figure above, middle and below respectively show the risk score distribution, survival status and the relative expression of 8 DEBMLncRNAs in patients with high risk and low risk of breast cancer in the training group. Validation is also performed in the validation group(B) and the entire group(C).

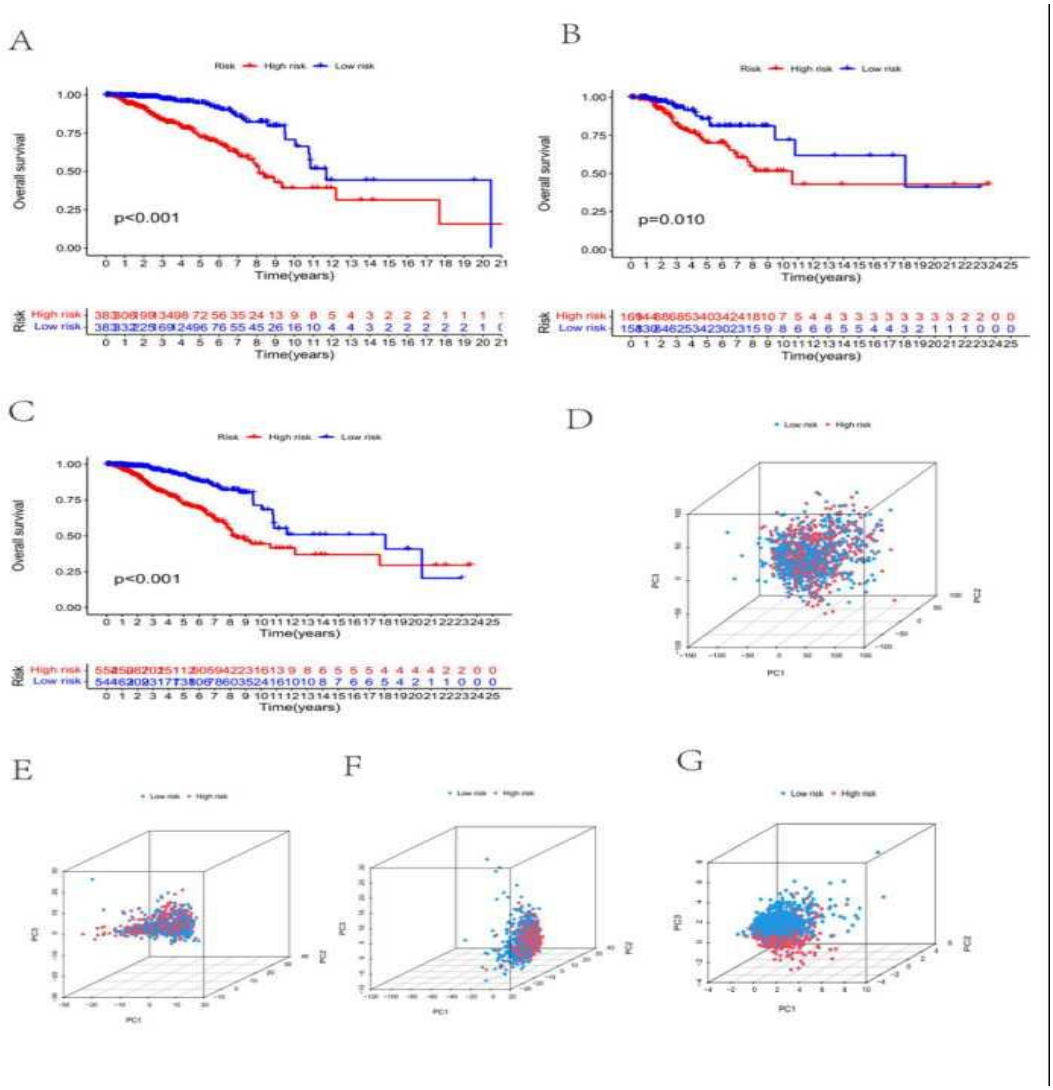


Figure 4: Validation of the risk scoring system. (A) Kaplan-Meier curve based on the overall survival analysis of the training cohort. (B) Kaplan-Meier curve based on the overall survival analysis of the validation cohort. (C) Kaplan-Meier curve for overall survival analysis of the entire cohort.(D) PCA between high-risk and low-risk groups based on all genes; (E) PCA between high-risk and low-risk groups based on basement membrane-related genes; (F) PCA between high-risk and low-risk groups based on basement membrane-related LncRNAs; (G) PCA between high-risk and low-risk groups based on 8 DEBMLncRNAs. PCA, principal component analysis; lncRNA, long non-coding RNA.

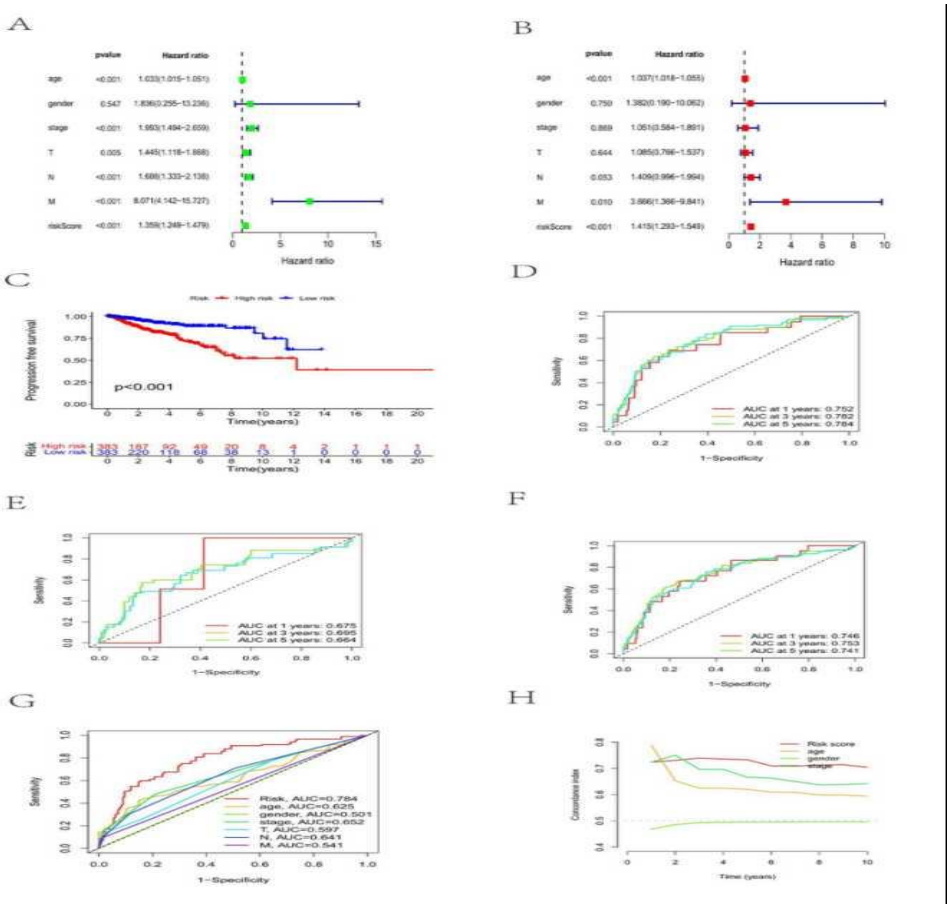
3.3Validation of risk scoring system

Figure 4 demonstrates that the high-risk group of patients had a more unfavorable prognosis compared to the low-risk group. In order to assess the prognostic significance of DEBMlncRNAs for BC patient survival, a thorough analysis was conducted, incorporating heat maps, scatterplots, and Kaplan-Meier curves to identify survival disparities among distinct cohorts. Kaplan-Meier analysis results indicated a poorer OS rate in the high-risk group in contrast to the low one (Figure 4A), a consistent finding across validation and overall cohorts (Figure 4B, 4C). Risk scoring systems are valuable for predicting prognosis in patients at different clinical stages. Figures 6A and 6B demonstrate that patients in the high-risk group have a worse prognosis compared to those in the low-risk group, regardless of their clinical stage. PCA showcased differentiation effects of different gene sets across four graphs (Figure 4). Comparison of PCA analyses based on the 8 DEBMlncRNAs distinctly categorized patients into two groups, (Figure 4D-G). These findings emphasize the effectiveness of prognostic characteristics in identifying different risk patients.

The study utilized Cox regression analysis to determine if predictive features act as independent prognostic factors in BC patients. Both univariate

and multivariate Cox regressions were used to assess the prognostic value of the variables (Figure 5A, 5B). Kaplan-Meier analysis further confirmed the predictive value of the risk score in forecasting PFS of two group (Figure 5C), the predictive ability of independent prognostic factors was assessed using the ROC curve, resulting in AUC values of 0.752, 0.782, and 0.784 for 1-year, 3-year, and 5-year outcomes respectively (Figure 5D). The predictive ability of the risk scoring system were subsequently validated in the validation and overall groups. (Figure 5E and 5F). Additionally, the C-index indicated that the risk scoring system had better predictive accuracy compared to other prognostic indicators (Figure 5H).

Figure5: Development of a nomogram based on 8 DEBMLncRNAs used to predict the survival of BC patients(A) and (B) Cox regression analysis of risk model and clinicopathological features based on training cohort. Green (A) represents univariate Cox regression analysis; Red (B) indicates multivariate Cox regression analysis.;(C) Kaplan-Meier analysis for predicting the PFS of different risking patients with BC; (D) ROC of risk models predicting 1 -, 3 -, and 5-year survival of breast cancer patients in the training group; (E) AUC values for BC 1-year, 3-year, and 5-year survival predicted by the risk model in the validation cohort; (F) AUC values for 1-year, 3-year, and 5-year survival rates predicted by the risk model in the overall group; ROC and C index curves (5G and 5H) showed that the AUC value and consistency index of risk model were higher than other clinical features.

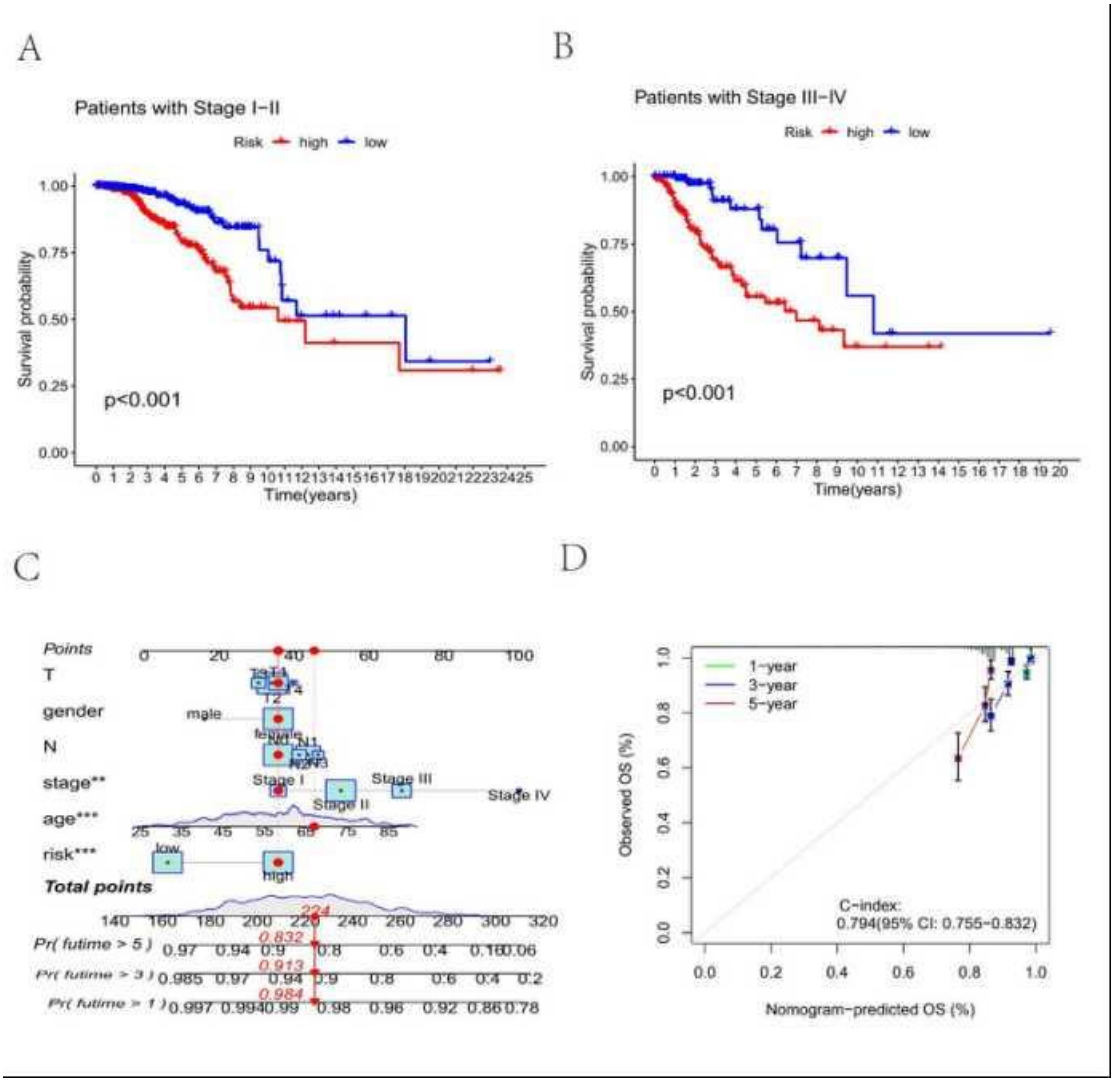


3.4Development and assessment of the Nomogram

Based on these findings, a prognostic nomogram was devised, integrating various independent prognostic factors including age, gender, clinical stage, T-stage, N-stage, and risk score (Figure 6C). The predictive performance and clinical utility of the nomogram were evaluated through calibration curves subsequently (Figure 6D).

Figure6: Survival analysis and nomogram predicted the survival rate of BC(A) Kaplan-Meier curves of patients in stages I–II. (B)

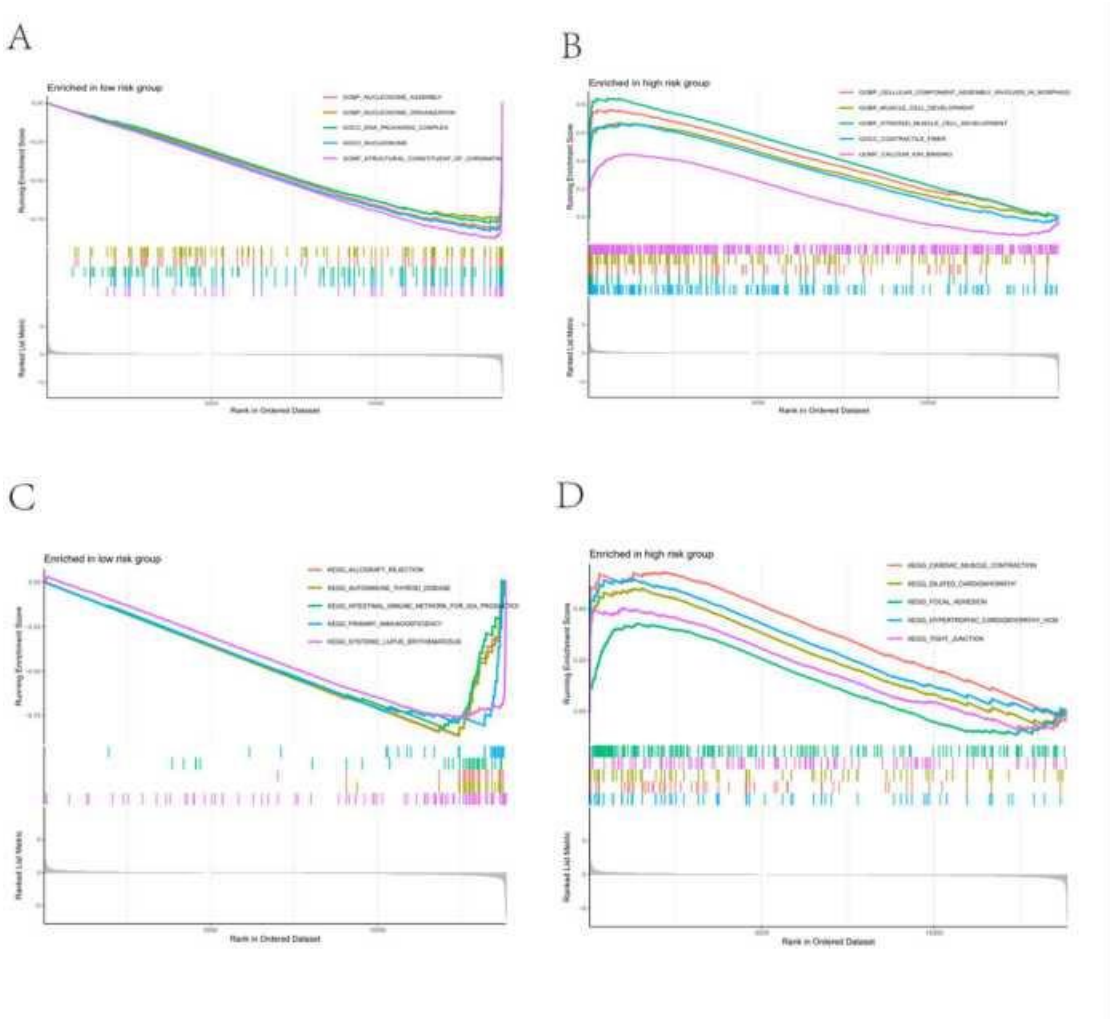
Kaplan-Meier curves of patients in stage III–IV . (C) A nomogram is used to predict prognosis. (D) A calibration plot to assess the prediction power of the nomogram.. T , tumor; N, node; OS, overall survival.



3.5Analysis of differences in signaling pathways and functional enrichment between high-risk and low-risk groups.

Firstly, we initiated functional enrichment analysis to investigate signaling pathways and cellular functions linked to risk scores. As the prognosis differed in the two groups, we conducted a differential expression gene analysis to explore potential variances in molecular function (MF), cellular components (CC), and biological processes (BP) between the groups, as depicted in Figure 7. GO analysis indicated significant enrichment of calcium binding in the high-risk subgroup, whereas the low-risk group showed prominence in chromatin composition. Regarding biological processes, the high-risk group primarily engages in "cell morphology formation," while the low-risk group shows enrichment in nucleosome assembly. KEGG analysis revealed differentially expressed genes were significantly enriched in in "cardiac muscle contraction," "focal adhesion," and "tight junctions" in the high-risk group. In contrast, pathways enriched in the low-risk group primarily relate to immune-related dysfunctions, including " allograft rejection," "primary immunod efficiency ," and "autoimmune disease."

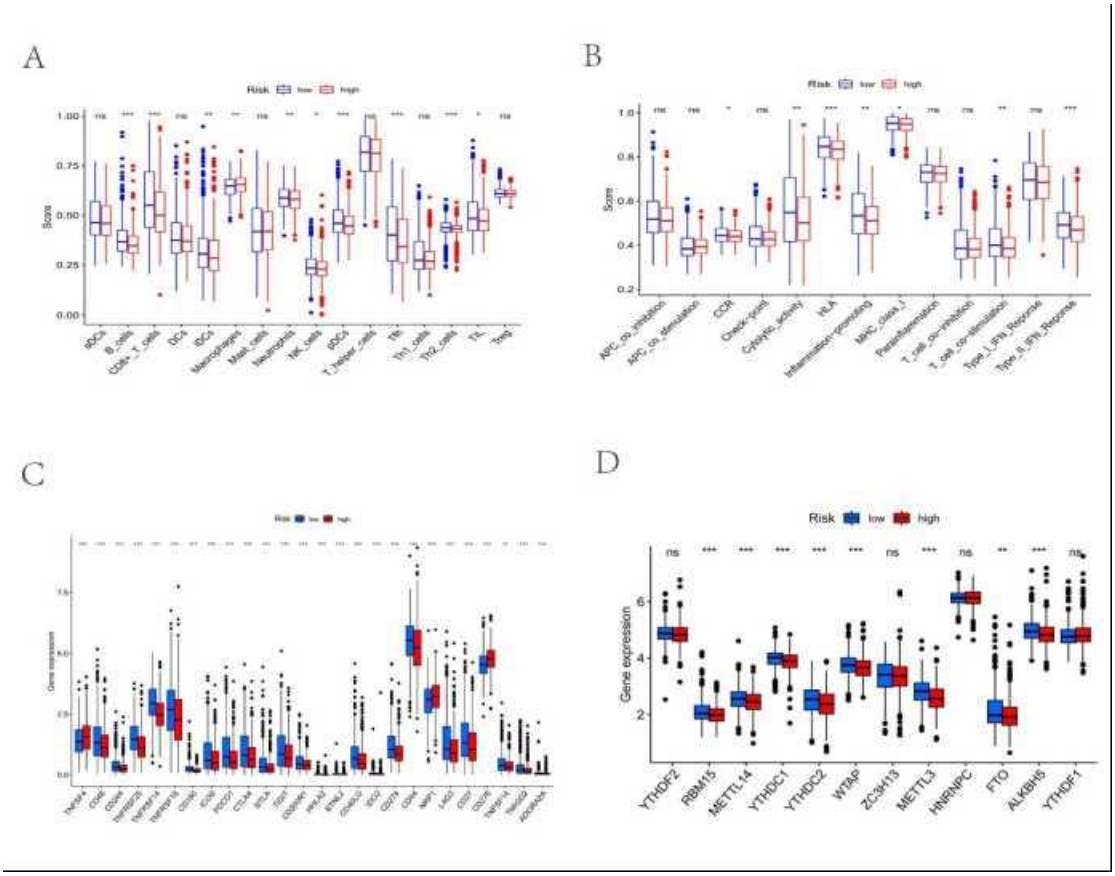
Figure7: GSEA for different risk groups. Gene set enrichment analysis based on Gene Ontology （ 7A、7B）and Kyoto Encyclopedia of Genes and Genomes (7C、7D)of two groups.



3.6Tumor immune characteristics and M6a analysis

To delve deeper into the correlation between risk scores and immune cells and functions, we utilized ssGSEA to compute enrichment scores of every immune cell subset and their related functions. Notable variations were observed in the expression of plasmacytoid dendritic cells (pDCs), B cells, CD8+ T cells, T follicular helper cells (Tfh), and helper T cells type 2 (Th1) between the low-risk and high-risk groups, as depicted in Figure 8A. Moreover, scores indicating "Type II IFN response," "cytolytic activity," "inflammatory promotion," "T cell co-stimulation" and "CCR" were higher in the low-risk group compared to the high-risk group ($P<0.05$), suggesting additional suppression of relevant immune functions in high-risk populations (Figure 8B). We additionally assessed the correlation between risk scores and immune checkpoint genes, revealing statistically significant disparities in the expression of 26 immune checkpoint genes across the two groups (Figure 8C).In view of the abnormal expression of genes regulating m6a modification enzymes being closely linked to tumor onset and progression, Our team further explored the variance in the expression of m6a-modified genes between low-risk and high-risk groups. Statistical variances were noted in the expression of all m6a-modified genes except for "ZC3H13," "YTHDF1," and "HNRNPC" (FIG. 8D).

Figure 8:Difference analysis of tumor immune microenvironment and m6a gene modification in different risk populations.(A) Infiltration of 16 types of immune cells in high-risk and low-risk groups.(B) Enrichment of 13 kinds of immune functions in high-risk and low-risk groups. (C) The expression of immune checkpoint genes was different between high-risk and low-risk groups.(D) There were certain differences in the expression of m6a modified genes between high-risk and low-risk groups.



3.7Genetic changes of BM-related Genes

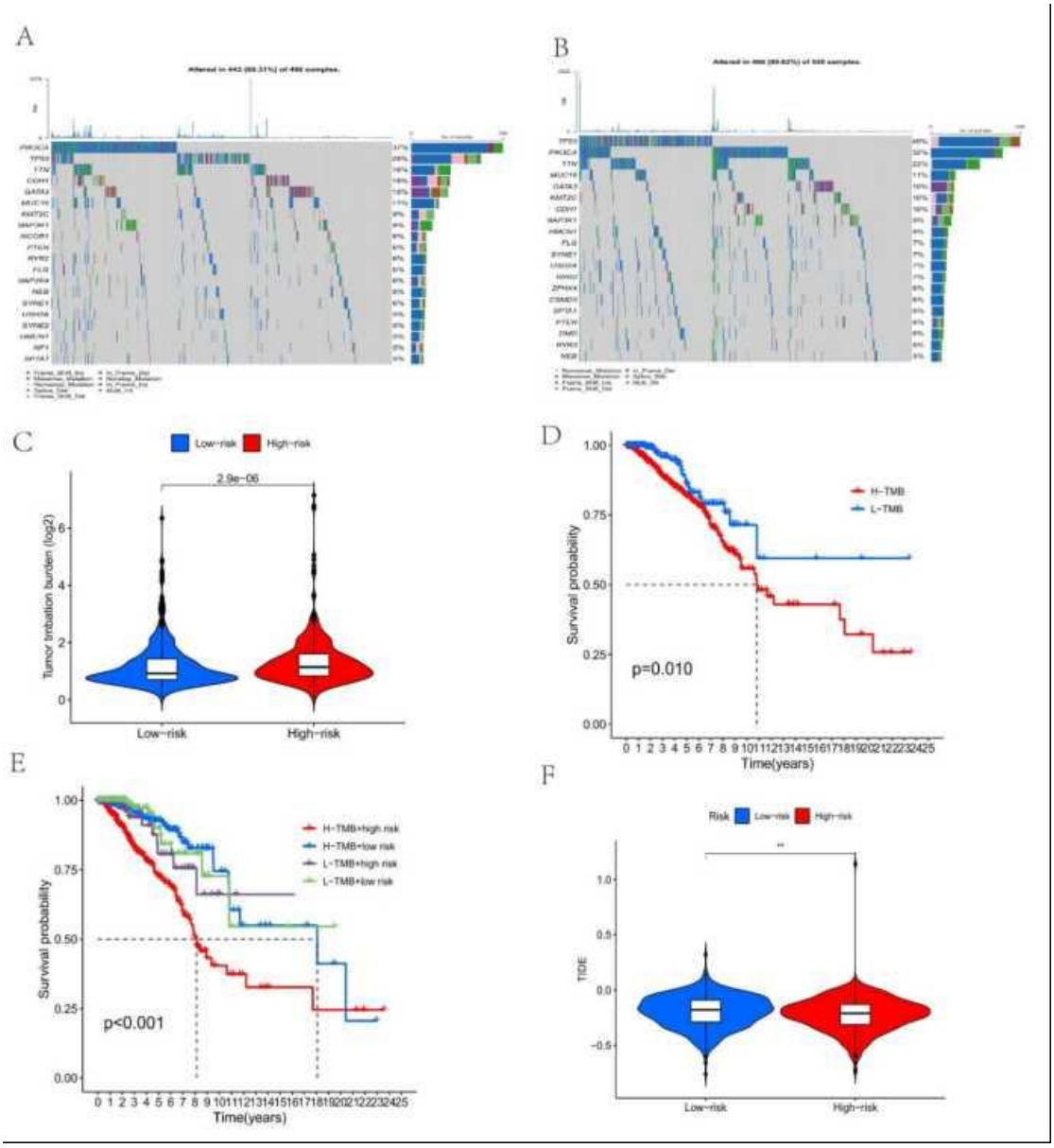
Changes in cancer-related genes are pivotal in cancer development. This study investigated disparities in mutation patterns of tumor-related genes between high-risk and low-risk groups. Figure 9 illustrates the top 20 driver genes exhibiting the most frequent alterations between these groups. The findings reveal a higher tumor mutation burden in the high-risk group (Figure 9C). Missense mutations predominated among all mutation types (Figure 9A, 9B). Patients were categorized into high and low TMB groups based on their tumor mutation burden. Kaplan-Meier analysis revealed that patients in the high TMB group exhibited a poorer prognosis than those in the low TMB group (Figure 9D) ($P<0.05$). Survival analysis, integrating the risk model with TMB, unveiled superior survival probabilities in the low-risk group compared to the high-risk groups, irrespective of high or low mutation burden (Figure 9E) ($p < 0.001$).

3.8immunotherapy Efficacy Prediction

Research on drug therapy for breast cancer holds significant promise and currently commands considerable attention. Immune checkpoint inhibitors (ICIs) have garnered approval for treating PD-L1+ metastatic triple-negative BC[19] .We compared the expression of immune molecules associated with checkpoints between high-risk and low-risk groups. The results indicated that in the low-risk group, the expression of most immune checkpoint related molecules was upregulated (Figure A), suggesting an underlying target for immunotherapy in BC patients with different risk scores. To further prognosticate the efficacy of immunotherapy in high-risk and low-risk groups, TIDE scores were employed to assess the effectiveness of immune checkpoint inhibitors, particularly PD-1 inhibitors, in two groups. Interestingly, TIDE scores were observably lower in high-risk than in low-risk ($p<0.001$, Figure 9F), indicating a potential superior response to immunotherapy of high-risk group.

Figure 9: Tumor mutation load in high and low risk groups and survival analysis of corresponding subgroups, TIDE score. (A) The top 20 driver genes with the highest mutation frequency in the low-risk group. (B) The top 20 driver genes with the highest mutation frequency in the high-risk group. (C) Total TMB between the high-risk and low-risk groups. (D, E) K-M survival curves of low TMB group and high TMB group. (F)

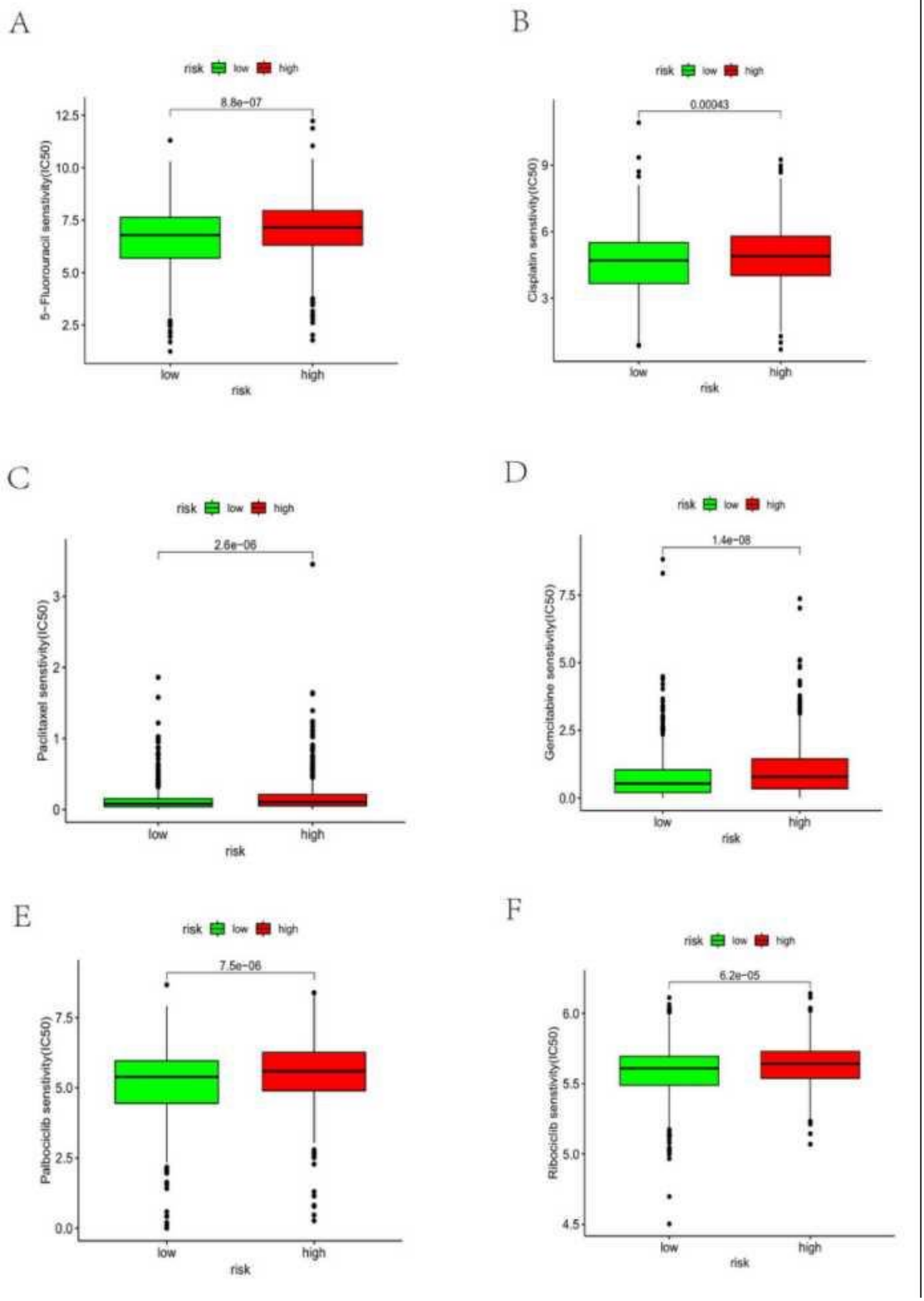
Differences in TIDE scores between high-risk and low-risk groups.



3.9Drug sensitivity Analysis

We finally studied the clinical utility of the risk score system by extrapolating IC50 values for 136 drugs to explore its correlation with anti-cancer drug sensitivity. Our findings unveiled noteworthy disparities in drug sensitivity between the high-risk and low-risk groups (P<0.05).Figure 10 illustrates six drugs that exhibited substantial disparities. The high-risk group exhibited higher IC50 values for chemotherapy and targeted drugs, including 5-fluorouracil, cisplatin, paclitaxel, gemcitabine, Palbociclib, and Ribociclib, indicating that the low-risk group was more responsive to these drugs (all P<0.001).

Figure 10: There were significant differences in the treatment sensitivity of 6 antitumor drugs between high-risk and low-risk groups.



Discussion

Breast cancer ranks among the most common malignant tumors globally and remains the leading cause of death among female cancer patients. Cancer cells in epithelial tumors must penetrate the surrounding stromal tissue via the basement membranes to achieve metastasis. A growing body of research suggests that basement membrane-related lncRNAs play vital roles in affecting various aspects of tumorigenesis and progression. Certain characteristic lncRNAs hold promise as novel biomarkers for tumor diagnosis, prognosis assessment, and disease progression prediction [20]. Therefore, identifying new

molecular prognostic markers is essential to supplement clinical parameters for improved prognosis prediction in breast cancer patients. Our study established a prognostic model of 8 DEBMlncRNAs using genetic information from the TCGA database. LINC01235 emerges as a critical prognostic factor in breast cancer, playing a pivotal role in promoting proliferation and migration. This suggests its potential as a biomarker for diagnosing and treating breast cancer [21]. The decreased expression of the lncRNA ST7-AS1 in breast cancer correlates with more advanced clinicopathologic features, shorter survival time, and a poorer prognosis. Moreover, ST7-AS1 expression correlates with immune cell infiltration extent in the breast cancer tumor microenvironment [22]. Investigators have found that GNG12-AS1 regulates MET signaling and the level of its downstream target MAP2K4 expression, suggesting that GNG12-AS1 may exert its tumor-suppressor function through the regulation of oncogenes such as MET [23]. LncRNA SEMA3B-AS1 is downregulated in TNBC, associated with poor prognosis, and its knockdown significantly increases proliferation and invasiveness of TNBC cell lines, while its overexpression reverses these properties [24]. Elevated expression of LINC01871 correlates with the activation of "interferon gamma", "interferon alpha", and "inflammation", indicating its potential as a prognostic marker for patients with the basal-like subtype of TNBC [25]. A study has shown that HID1-AS1, an lncRNA linked to the stemness index [26], could predict the prognosis of BC patients and serve as a potential diagnostic marker for BC.As for ZNF24TR,further exploration is needed as it has not been reported so far.

Breast cancer drug therapy-related research holds significant promise, being a current research hotspot and attracting widespread attention. Immune checkpoint suppression therapy has been widely approved for some tumors [27],such as breast cancer、melanoma and non-small cell lung cancer , and therapeutic blocking of the PD-1 pathway is arguably one of the most important breakthroughs in the history of cancer treatment . However, not all breast cancer patients benefit from this kind of treatment [28]. The group with low risk exhibited elevated expression of the majority immune checkpoint-related genes. However, the high-risk group exhibits elevated expression of immune checkpoint genes such as TNFSF4, NRP1, and CD276. CD276, commonly overexpressed in various human solid cancers, is frequently linked with adverse clinical outcomes in patients[29].It influences not only innate and adaptive immunity but also regulates cancer cell invasiveness through various non-immune pathways. Tumor immunogenicity is a key determinant of ICIs efficacy .The absence of pre-existing T cell infiltration in the tumor is often a sign of low tumor immunogenicity [30]Tumors that showing stronger immunogenicity generally responding better to ICIs [28].During the early stages of tumor development, immune cells breaching the basement membrane zone to interact with cancer cells and impede cancer invasion [31],This may reinforce the findings of the network meta-analysis led by Cortes, J and fellow researchers [32], which indicating that utilizing pembrolizumab anti-tumor immunotherapy during neoadjuvant and adjuvant chemotherapy stages can enhance survival rates among early-stage TNBC patients. In addition, studies have shown that ICI combined chemotherapy has a higher clinical response rates in advanced TNBC. In a series of KEYNOTE trials [33,34], the clinical efficacy of pembrolizumab alone and its combination with chemotherapy drugs such as paclitaxel and albumin-bound paclitaxel in the treatment of metastatic TNBC patients was compared. The findings prompted the FDA to approve the drug for patients with PD-L1-positive in mTNBC in 2020 [33].

Our investigation uncovered significant differences in immune cell infiltration profiles between high and low-risk BC groups.The low-risk group demonstrated increased infiltration of CD8+ T cells, T follicular helper cells (Tfh), and helper T cell type 2 (Th2). As is widely recognized, CD8+ T cell infiltration has emerged as a significant prognostic marker in TNBC, highlighting its clinical importance[35]. While the high-risk group had more macrophage infiltration, which are another important component of tumor-infiltrating immune cells in breast cancer, and extensive clinical and experimental evidence shows that Tumor-associated macrophages (TAMs) always promote the progression of

malignant tumors[36].

BC patients categorized as high-risk may harbor a greater frequency of genetic mutations, potentially rendering them more responsive to ICI therapy compared to those in the low-risk category. Our study revealed higher mutation rates in TP53 and TTN genes within the high-risk group, whereas the low-risk group predominantly exhibited PI3KCA mutations. Zhang et al. linking TP53 mutations to reduced OS in their study [37]. TP53 mutations could function as prognostic biomarkers for breast cancer, closely linked to heightened infiltration of immune cells within the tumor microenvironment and promote BC immunogenicity [38]. HR+/Her2-mBC patients harboring PIK3CA mutations displayed diminished chemotherapy sensitivity and poorer overall survival[39], while those with PIK3CA mutations exhibited improved overall survival compared to PIK3CA-negative TNBC cases. The influence of PI3K mutations on diverse BC subtypes necessitates further exploration. TMB, reflecting tumor immunogenicity to some extent, emerges as a promising biomarker for predicting the response to immunotherapy in cancer patients[40]. However, accurately predicting the response to immune checkpoint inhibitor treatment in breast cancer patients may entail integrating TMB metrics with other potential biomarkers, as mentioned above, PD-L1 expression and tumor-infiltrating lymphocytes (TILs)[41].

However, the study has limitations, primarily being retrospective and relying on bioinformatics analysis from an online database, without prospective external validation. Additionally, further exploration is needed to understand the mechanisms of DEBMLncRNAs in breast cancer tumorigenesis, progression, and prognosis. To validate our bioinformatics predictions, additional studies involving 8 DEBMLncRNAs are imperative, encompassing functional experiments and elucidation of molecular mechanisms.

In conclusion, we developed an innovative prognostic model using 8 DEBMLncRNAs. This model accurately predicts patient survival and prognosis. Furthermore, Our analysis underscores disparities in the immune microenvironment, response to immunotherapy, and drug sensitivity between high and low-risk subgroups. These insights deepen our comprehension of basement membrane-related lncRNAs' involvement in breast cancer development and progression, offering novel perspectives for treatment decisions.

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Data Availability Statement

The datasets generated and/or analysed during the current study are available in the TCGA repository, http://cancergenome.nih.gov/abouttcga The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship contribution statement

Feng Lin: Conception and design, Visualization, Validation, Methodology, Investigation, Writing – review & editing. Qiongyao Tao: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Hang Li: Funding acquisition, Project administration, Resources, Supervision. Jianchan Fang and Wenhua Huang: Data curation, Supervision, Data curation. Yu Chen: Project administration, Resources, Supervision, Validation. Feng Lin、Qiongyao Tao and Hang Li contributed equally to this work. Final approval of manuscript: All authors.

Ethics approval and consent to participate

Not applicable

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Declaration of competing interest

The authors declared no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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