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Research

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Identifies immune-related gene pair signature associated with breast cancer prognosis

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Abstract

Background: Breast cancer (BC) is the cancer with the largest number of deaths in women. There is growing evidence that immunity plays an important role in the prognosis of breast cancer.

Methods: In this study, we developed and validated an immune-related gene pair signature (IRGPs) to predict the survival of breast cancer patients. Screening immune-related genes from The Cancer Genome Atlas (TCGA) database and the Gene Expression Omnibus (GEO) database for the construction of IRGPs, and patients with breast cancer in these two cohorts were assigned to low- and high- risk subgroups. Additionally, we used Kaplan-Meier survival analysis, univariate and multivariate Cox analysis to investigate IRGPs and their individualized prognostic characteristics, and analysis of immune cell infiltration in breast cancer.

Results: A 47-IRGP signature was constructed from 2498 immune genes, which could significantly predict the overall survival (OS) of breast cancer patients in the TCGA and GEO cohorts. Immune infiltration analysis showed that a variety of immune cells are significantly related to the prognostic effects of IRGP characteristics in breast cancer patients, especially $CD8^+$ T cells and macrophages.

Conclusions: The IRGP signature constructed in this study can help determine the prognosis of breast cancer and provide new ideas and basis for future research on the role of immune-related genes in breast cancer patients.

Keywords: breast cancer; immune-related gene pair; bioinformation; prognosis

1. Background

BC is the most common malignant tumor in women, with 2.08 million cases and 680,000 deaths in 2018, far exceeding other female cancers(1). Although in the past few decades, early detection, diagnosis, and treatment have led to a gradual decline in mortality, the prognosis of patients with distant metastases would be poor(2). Previous studies have shown that multi-gene signature can better predict the prognosis of BC. Oncotype DX is a 21-gene signature that could predict the risk of distant recurrence and used to weigh the benefits and side effects of chemotherapy(3). The heterogeneity of BC poses a challenge for predicting the prognosis of patients. Some traditional predictors, such as clinical features, pathological features, and tumor markers, cannot accurately predict the prognosis. Therefore, it is very necessary to explore new prognostic predictors and potential therapeutic targets.

A lot of evidence shows that the immune system plays an important role in the development and progression of cancers(4-6). In addition, there is evidence that immune regulation is involved in the development and progression of BC(7, 8). Prognostic signatures constructed based on immune-related gene pairs have been proven to be useful for assessing OS in a variety of cancers, including lung cancer(9), pancreatic cancer(10), melanoma(11), ovarian cancer(12). IRGPs in the progression of BC and the mechanisms underlying the complex interactions between BC and host immune response needs to be further explored.

In this study, RNA-seq data and clinical information were downloaded from TCGA database and the GEO database, we identified prognostic IRGs and constructed an immune-related gene pair index (IRGPI) by univariate Cox regression and LASSO analyses. In addition, we investigated the IRGPI and their clinicopathological prognostic signatures using Kaplan–Meier survival analysis, Cox analysis, and analysis of immune cell infiltration in BC. The potential mechanisms are also investigated by Gene set enrichment analysis (GSEA), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Our IRGPI not only could effectively predict the prognosis of patients with BC, but also provided potential targets for BC diagnosis and treatment.

2. Methods

2.1 Data sources

The RNA-seq data (FPKM) of 1,096 BC samples, and their corresponding clinical data were obtained from the TGGA database (https://cancergenome.nih.gov/). All samples obtained from the TCGA platform were used as the development cohort. To validate the prognostic value of the IRGP signature constructed from the development cohort, the RNA-seq expression dataset GSE20685 from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) as a validation cohort, which includes 327 BC patients. All data were downloaded on 20 June 2021.

2.2 Data preprocessing

The ensemble ID of merged mRNA matrix data was transformed into the corresponding gene symbol through the strength of each set of annotation files. Datasets without complete OS information or follow-up time is less than 90 days were discarded.

2.3 Development of a prognostic IRGP signature

IRGs of all datasets were identified through IMMPORT (http://www.immport.org/)(13), which provided a list of 2498 IRGs. The R statistical software package ‘Limma’ of the Bioconductor was applied to analyze the expression levels of the IRGs in two cohorts. IRGs with median absolute deviation (MAD) >0.5 and expressed in both two cohorts were subjected to subsequent pairwise

comparisons. The IRGP was assigned a value of 1 if the expression of the IRG1 was higher than that of the IRG2; else, the index was defined as 0. Performing univariate Cox regression analysis to obtain IRGPs related to OS in the development cohort (p<0.001). The Lasso Cox regression analysis ('glmnet' R package) was performed to build the IRGPI. Finally, the prognostic model constructed based on forty-seven gene pairs. The IRGPI was further used to calculate the risk score for BC patients in two cohorts. According to an analysis of a time-dependent ROC curve ('survivalROC' R package) for survival, the optimal cutoff value for the IRGPI was -1.114 for patients divided into a low- and high-risk groups.

2.4 Validation of the prognostic model

Kaplan–Meier survival analysis was performed to assess the difference in OS between the low- and high-risk subgroups in each cohort.

2.5 Immune cell infiltration in the BC

Many previous studies have confirmed that tumor-infiltrating immune cells are related to the prognosis of tumors(14, 15). CIBERSORT(https://cibersort.stanford.edu/) was used to estimate the infiltration levels of 22 specific immune cell in the low- and high-risk groups. Wilcoxon test was performed to determine the correlation between immune cell infiltration and the different risk groups. Correlations were visualized using boxplots and radar plots.

2.6 Function enrichment analysis of IRGP signature

GSEA was used to analyze the significant functional pathways of our prognostic immune characteristics in the development cohort. The log2 fold change in gene expression was calculated between the different risk groups. The pathways involved in GSEA analysis download from the Molecular Signature Database (version 7.4; C5 databases, http://www.gseamsigdb.org/gsea/downloads.jsp). The 'fgsea' R package was performed to the GSEA analysis and permutated 20,000 times. |Normalized Enrichment Score (NES)| >1.2, normalized p-value <0.05, and false-discovery rate (FDR) q-value < 0.05 were considered statistically significant. GO and KEGG analyses were performed using the 'ClusterProfiler' R package to further explored the underlying mechanisms of immune-related genes signature in BC patients(16). $p < 0.05$ and FDR < 0.05 were considered statistically significant.

3. Results

3.1 IRGP signature construction

This study includes 1096 BC samples downloaded from TCGA and 327 samples collected in GEO (GSE20685). 526 IRGs with fantastically giant mutations were measured in both development and validation sets. Using these 526 genes, 25,750 immune gene pairs were constructed. Then Cox proportional hazards regression analyses was performed for the OS of the development cohort, and obtained 259 IRGP candidates (p<0.001). Next, Lasso Cox regression analysis was used to select the OS-related IRGPs without multicollinearity. Finally, 47 immune-related gene pairs were selected to construct the prognostic IRGPI (Table 1). According to time-dependent ROC curve analysis, the optimal cutoff value for the IRGPI was -1.114 for patients divided into low- and high- risk groups (Fig 1).

Table 1. Construction the prognostic IRGP signature.

IRG 1	IRG 2	Coefficient	IRG 1	IRG 2	Coefficient
HSPA1L	IGLV6-57	0.07	CD79B	IGHD	0.07
PSMB8	PGRMC2	-0.02	RAC2	C3AR1	-0.14
TAPBPL	IL1R1	-0.36	FCGR2B	RASGRP1	0.10

TAPBPL	TGFBR1	-0.08	IGHD	RLN2	-0.18
CXCL14	HMOX1	-0.27	IGHD	NPR3	-0.03
CXCL9	CCL2	-0.11	IGHD	TRAV8-3	-0.05
DEFB1	NPR3	-0.17	IGLV6-57	SEMA6C	0.00
CCL8	ICAM2	0.31	IGLV6-57	CTF1	-0.01
PTGDS	IGLV1-44	0.26	IGLV6-57	IL34	-0.02
S100A16	BST2	0.16	IGLV6-57	TGFA	-0.08
ZC3HAV1L	FLT3	0.02	C5	FLT3	0.10
APOBEC3G	CMKLR1	-0.05	SEMA3B	SEMA6C	-0.06
APOBEC3G	PLXNB3	-0.09	SEMA4D	PLXNB3	-0.02
CTSG	IGHD	0.14	PLXNA3	TNFRSF18	0.51
SOCS3	BRD8	-0.36	PLXNB1	LTBP3	-0.51
CCL5	TNFRSF12A	-0.14	PLXNB3	TNFRSF18	0.12
GNLY	CD79A	0.03	EGF	FLT3	0.05
FURIN	STC2	0.09	SCG2	PTGER3	0.18
DLL4	IL27RA	0.08	STC1	STC2	0.06
IL7R	GHR	-0.14	TOR2A	IL3RA	0.10
ARG2	PLXNB3	-0.44	IGF1R	TNFRSF21	-0.38
GBP2	CSF1R	-0.16	IL27RA	TIE1	-0.10
CCL2	IL2RG	0.12	NGFR	NR6A1	-0.04
PPARG	PLXNB3	-0.10			

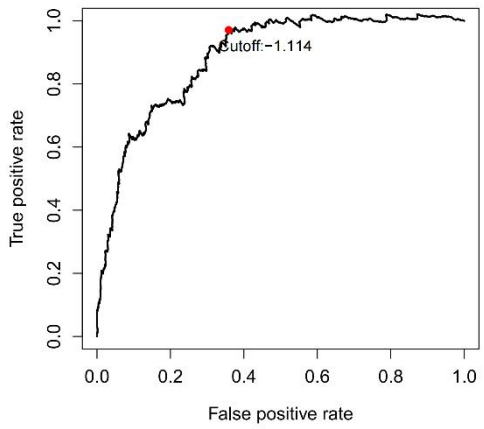
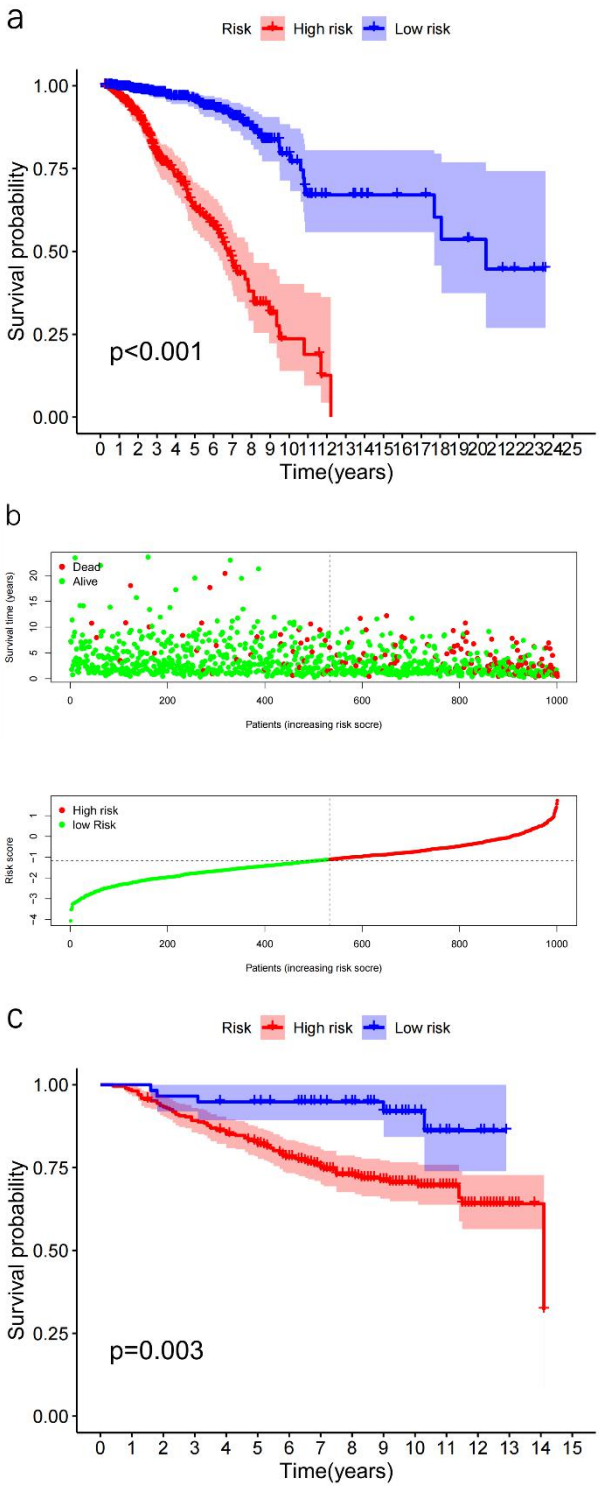


Figure 1. ROC curve for IRGPI at 5 years in the TCGA cohort

3.2 Validation of the Prognostic Value of IRGP signature

According to the optimal cutoff of the risk scores, development cohort was divided into low- and high-risk groups. The Kaplan–Meier survival analysis showed that the IRGPI had a good prognosis for the development cohort and the OS of the high-risk group was extensively shorter than that of the low-risk group ($p< 0.001$) (Fig 2a). IRGPI also has good predictive performance in the validation cohort (GSE20685), a higher risk scores associated with a shorter OS time ($p=0.003$) (Fig 2c). Survival status and risk score distributions are shown in (Fig 2b) and (Fig 2d), indicating that higher mortality and shorter OS times will be seen among patients with higher risk scores Univariate Cox

analysis showed that age, tumor stage (T), lymph node metastasis (N), distant metastasis (M), and risk score all had prognostic effects in the TCGA cohort. However, multivariate Cox analysis shows that only IRGPI can be used as an independent prognostic factor ($p<0.001$) (Fig 2e). Similar to that observed in the development cohort, IRGPs showed significant correlation with OS of patients with BC ($p<0.001$) (Fig 2f).



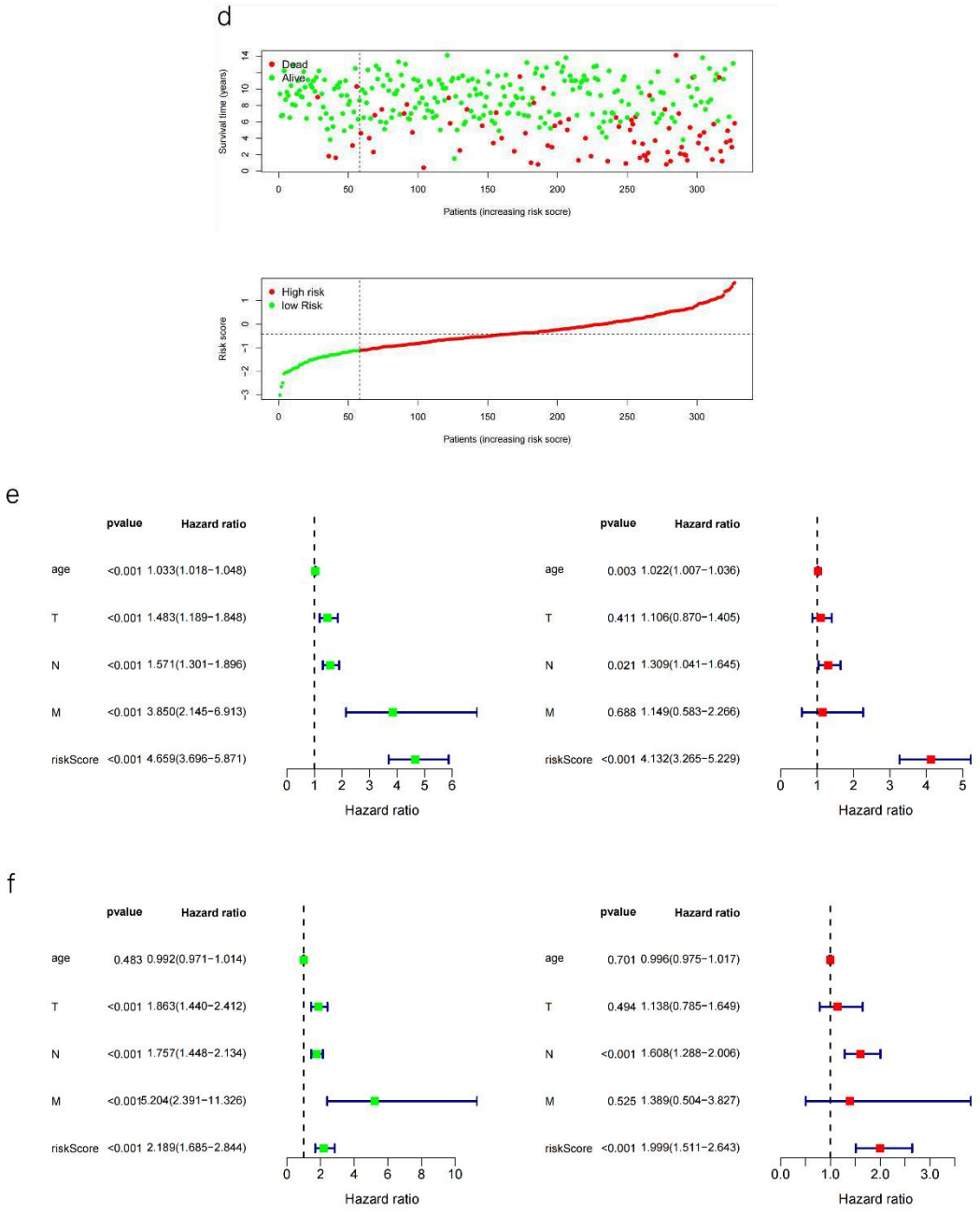


Figure 2. Validation of the Prognostic Value of IRGP signature. (a) Kaplan-Meier survival curve of TCGA cohort. (b) Survival status and risk score distribution scatter plots in TCGA cohort. (c) Kaplan-Meier survival curve of GSE20685 cohort. (d) Survival status and risk score distribution scatter plots in GSE20685 cohort. Univariate and multivariate Cox regression analysis of the clinicopathological features in TCGA (e) and GSE20685 (f) cohorts

3.3 Infiltration of immune cells in different risk groups

In previous microenvironment-related researches, CIBERSORT has been used to estimate immune cell subset frequencies(17, 18). In this study, CIBERSORT was used to estimate the relative proportions of 22 tumor-infiltrating immune cells in the development cohort in different risk groups (Fig 3a). Our results showed that resting NK cells (p=4.99e-06), Macrophages M0 (p= 1.86e-12), Macrophages M2 (p= 7.13e-13), and Neutrophils cells (p= 0.001) were significantly enriched in the

high-risk group. Naive B cells (p= 1.57e-14), Plasma cells (p= 1.20e-12), CD8 T cells (p= 9.10e-17), resting memory CD4 T cells (p=2.9e-4), delta gamma T cells (p=3.77e-05), activated NK cells (p=7.8e-3), resting Dendritic cells (p=6.7e-4), were clustered in the low-risk subgroup (Fig 3b).

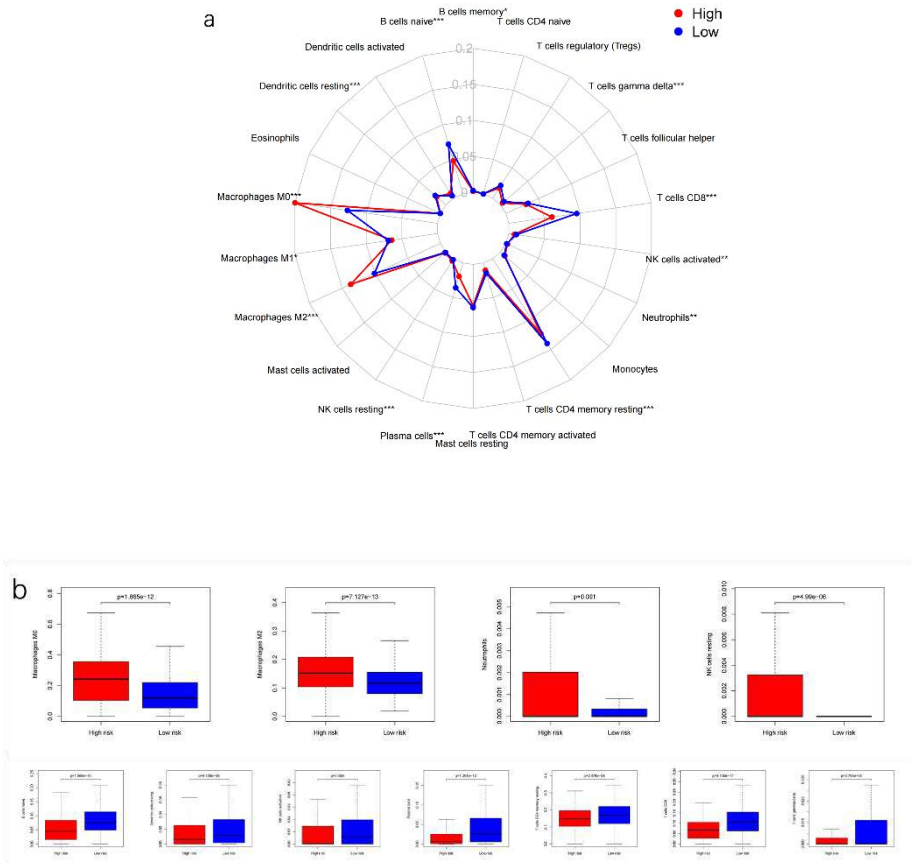


Figure 3. The infiltration level of immune cells in the constructed IRGPI risk model. (a) A comparative summary of the CIBERSORT outputs for the high-risk group and low-risk group. p-values are based on t-test (*p< 0.05, ** p< 0.01, ***p< 0.001). (b) The boxplot shows the distribution of specific immune cells in different risk subgroups from the TCGA cohort(p<0.01)

3.4 Functional enrichment of the IRGPI signature

GSEA was conducted to investigate the enrichment of the functional pathways between the high-risk and low-risk groups. This analysis confirmed that 9 hallmark gene sets were associated with the IRGPI signature (FDR < 0.05), including those responsible for 'detection of chemical stimulus', 'spliceosomal snRNP assembly', 'SM like protein family complex', 'small nuclear ribonucleoprotein complex', etc. These pathways provided a basis for studying the molecular mechanisms of IRGPI signaling predicting the prognosis of BC (Fig 4). The GO analysis results (Fig 5a) showed that IRGs of IRGP signature were primarily involved in critical biological process (BP), such as regulation of leukocyte migration, regulation of chemotaxis, leukocyte proliferation. These IRGs were also found to be enriched in response to the external side of plasma membrane, semaphorin receptor complex, immunoglobulin complex in the cellular component (CC) category and receptor ligand activity, signaling receptor activator activity, immune receptor activity in the

molecular function (MF) category. Furthermore, KEGG enrichment analysis indicated that the IRGs was significantly associated with the pathways of cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, MAPK signaling pathway (Fig 5b).

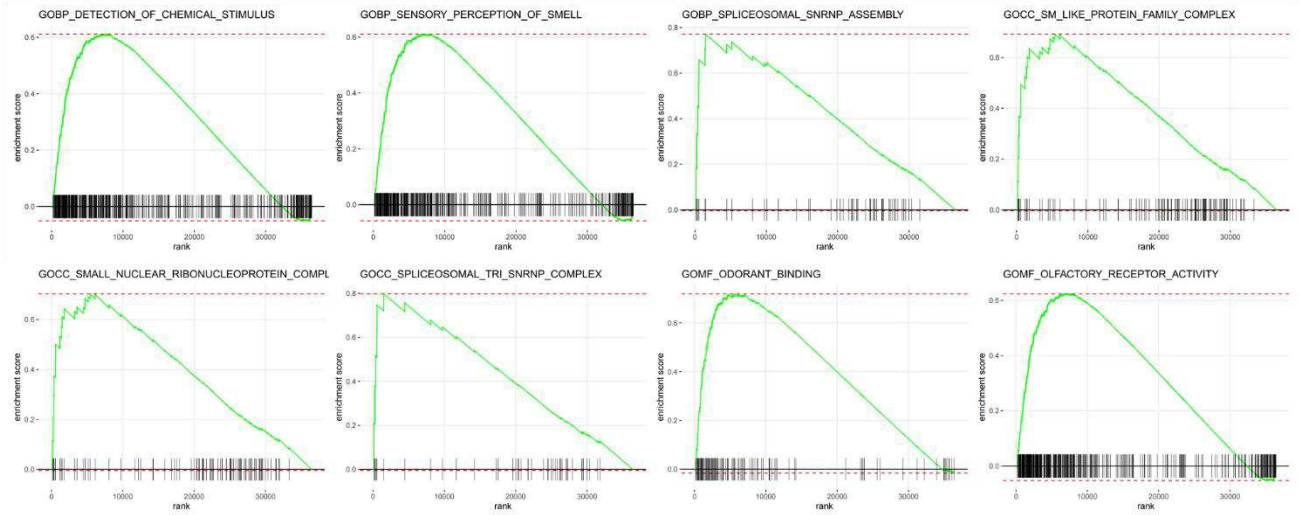
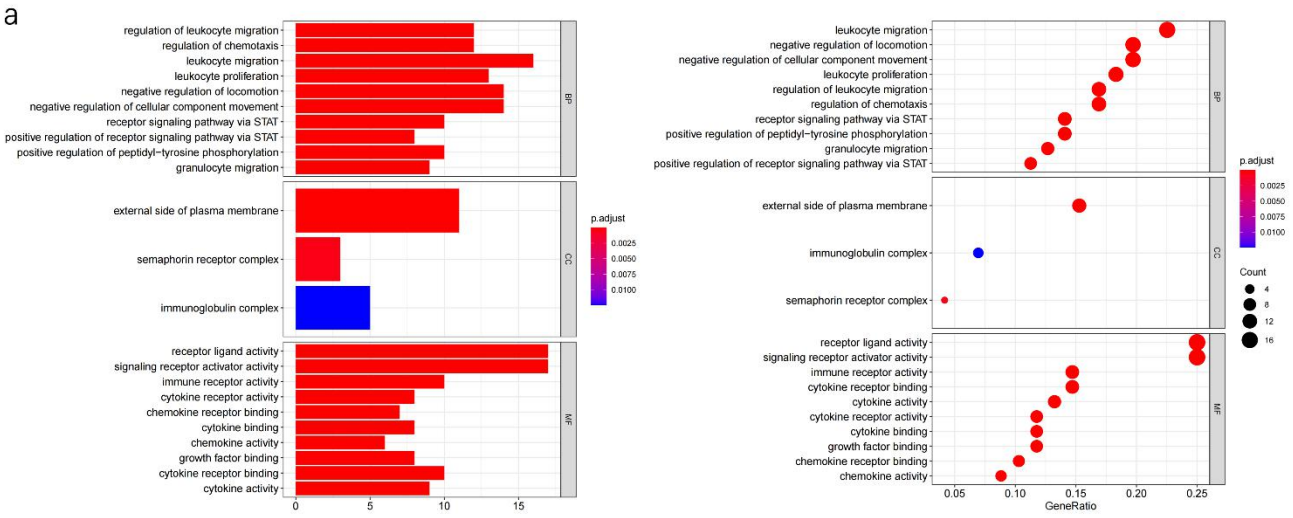


Figure 4. Gene set enrichment analysis (GSEA) between high-risk and low-risk subgroups. The results show that 8 cancer hallmark gene sets are enriched in the high-risk subgroup (FDR < 0.05)

a



b

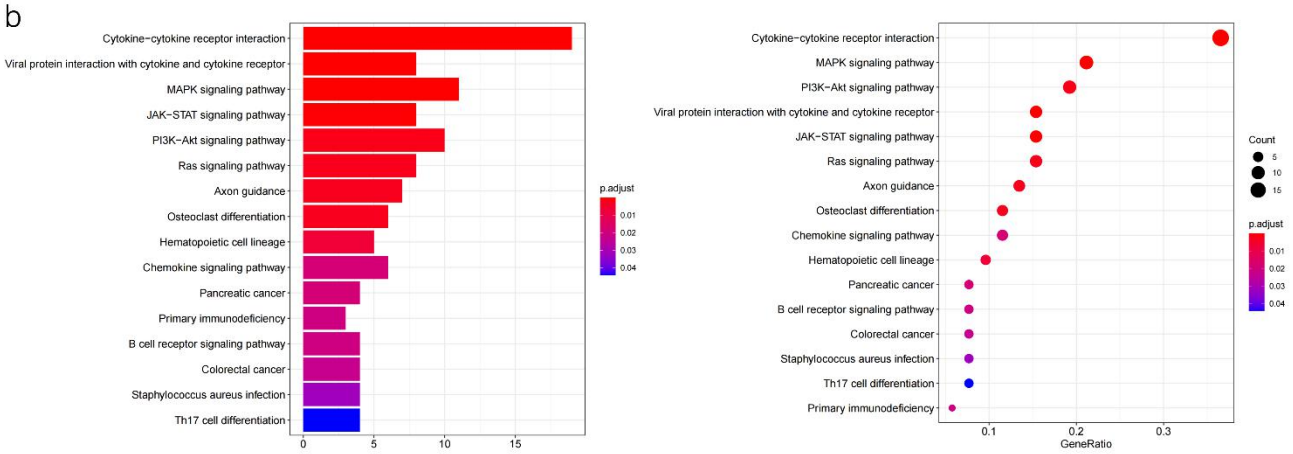


Figure 5. Functional enrichment of the IRGPI signature. (a) Top 10 of gene ontology enrichment terms in biological process (BP), cellular component (CC), and molecular function (MF). (b) Top 10 classes of KEGG enrichment terms. In each bubble plot, the size of the dot represents the number of enriched genes

4. Discussion

BC is the most common cancer among women worldwide. Like other types of cancer, the prognosis of BC also depends on early detection, diagnosis and treatment. BC is highly heterogeneous, and each subgroup has its own biological and clinical characteristics(19). Accurately identifying the risk of long-term recurrence and metastasis for each patient is very important for the choice of treatment options. Previous research has shown that immune regulation is involved in the development and progression of BC(7, 8). Studies have shown that infiltrating immune cells is related to the immune response of tumors and can affect the development and progression of cancer(20, 21). These findings have made us interested in using IRGPs to predict the prognosis of BC and determine the mechanism of the complex interaction between cancer and the host immune response.

Considering the differences in sequencing between different platforms, and the prognostic risk model needs to accurately reflect the expression of genes, this is a difficult point in data analysis. In order to predict the calculation results of the model more robustly, we use a new data analysis method to avoid technical differences between different platforms.

In our study, a 47-IRGP signature was developed to predict the OS of patients with BC. Patients were divided into low-risk and high-risk groups according to the IRGPI. Through survival analysis, we can observe that both in the development cohort and validation cohort, the OS of patients in the high-risk group were significantly shorter than that in the low-risk group. Results of the multivariate Cox analysis suggest that the IRGPI was an independent prognostic factor in patients with BC.

Various studies have confirmed that the immune response is significantly associated with tumor initiation and progression and that it plays a critical role in malignant cancer occurrence(22, 23). In this study, IRGP signature were confirmed to be related to multiple functional pathways in immune response, such as primary immunodeficiency. Next, we used CIBERSORT analyze the relationship between immune cell infiltration and IRGPs. The results demonstrated that poor prognosis related to increase in the numbers of NK cells, Macrophages M0, Macrophages M2, Neutrophils cells and lack of Naive B cells, Plasma cells, CD8 T cells, resting memory CD4 T cells, delta gamma T cells, activated NK cells, resting Dendritic cells. These results are similar to previous studies on immune cells. For example, M2 macrophages have been shown to be associated with poor prognosis in several cancers(24, 25). These results explain to a certain extent the mechanism of how IRGPs predicts the prognosis of BC patients accurately. We have also identified some pathways, including cytokine-cytokine receptor interaction pathway, MAPK signaling pathway, B cell receptor signaling pathway, and chemokine signaling pathway, were significantly related to the IRGPs. Among them, the cytokine-cytokine receptor pathway, as the top-ranked pathway, stimulated our interest. Previous researches have shown that cytokine-cytokine receptor interaction is significantly associated with many biological processes of BC, such as chemoresistance and metastasis of BC(26, 27), which often leads to poor prognosis of patients with BC. In addition, MAPK signaling pathway(27), B cell receptor signaling pathway(28), and chemokine signaling pathway(29) are also important roles that

affect the OS of patients with BC. These results may provide new ideas and basis for future research on the role of immune-related genes in BC patients.

However, our research has certain limitations. First of all, because of the high cost, prognostic characteristics based on gene expression characteristics generated by RNA-seq or microarray platforms are difficult to promote clinically. Secondly, the IRGP signature was constructed using RNA-seq and microarray expression data. It needs to be validated experimentally with BC patients in the future.

5. Conclusions

In conclusion, we constructed and systematically analyzed an IRGPs-based risk model. Our IRGP signature can effectively predict the prognosis of patients with BC, and provide references for BC immunotherapy.

Full name	Abbreviation
Breast cancer	BC
Immune-related gene pair signature	IRGPs
The Cancer Genome Atlas	TCGA
Gene Expression Omnibus	GEO
Overall survival	OS
Immune-related gene pair index	IRGPI
Gene set enrichment analysis	GSEA
Gene Ontology	GO
Kyoto Encyclopedia of Genes and Genomes	KEGG

6. Declarations

6.1Ethics approval and consent to participate

Our study was reviewed and approved by the Ethics Review Committee of Guangxi Medical University Affiliated Cancer Hospital.

6.2Consent for publication

Not applicable.

6.3Availability of data and materials

The RNA-seq data (FPKM) of 1,096 BC samples, and their corresponding clinical data were obtained from the TGGA database (https://cancergenome.nih.gov/). The RNA-seq expression dataset GSE20685 was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/).

6.4Competing interests

The authors declare that they have no competing interests.

6.5 Funding

This study was supported by the Natural Science Foundation of Guangxi (No. 2020GXNSFAA297152).

6.6Authors' contributions

CW contributed to the conception of the study; TS collected and analyzed data and wrote the manuscript; QT helped perform the analysis with constructive discussions.

6.7Acknowledgements

Not applicable.

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