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Original Article

Identification of an immune cell infiltration-related gene signature for prognosis prediction in triple-negative breast cancer

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Abstract

Triple-negative breast cancer TNBC with higher immunogenicity and tumor-infiltrating lymphocyte (TIL) enrichment can benefit from immunotherapy relative to other breast cancer subtypes. Our work was designed to identify the TIL-related hub genes in TNBC and construct a prognostic signature for TNBC. TNBC gene expression files were obtained from the TCGA database. CIBERSORT algorithm and random forest risk model were used for immune infiltration group division. The TIL-related differentially expressed genes (DEGs) were then selected and subject to GO, KEGG analyses and GSEA. Next, Lasso cox regression analyses were adopted for constructing a prognostic risk model, followed by evaluation using time-dependent ROC curves. The copy number variation between the two risk groups was also analyzed, and major genomic mutation types were identified. Additionally, the nomogram was constructed with calibration curve for clinical prognosis analysis. Our results showed that totally 113 TNBC samples were allocated into the high or low-immune risk groups. We identified 243 DEGs between groups, namely TIL-related DEGs, with 128 upregulated and 115 downregulated genes. Among the TIL-related DEGs, 6 hub genes (SLITRK3, PCDHGB3, NELL2, SRRM4, ASIC2 and B4GALNT2) were screened out and constructed a prognostic risk signature, which had good performance for long-term prognosis prediction. Analysis of genomic mutation showed that the TP53, PIK3CA, TTH, etc. showed high mutation frequency in the two prognostic risk groups. Moreover, the higher risk score of the prognostic risk model predicted poor overall survival in TNBC patients, and nomogram and calibration curve confirmed the potent prediction ability of this model. To sum up, six TIL-related biomarkers (SLITRK3, PCDHGB3, NELL2, SRRM4, ASIC2 and B4GALNT2) were identified and used for the construction of the prognostic risk model, which might provide novel insight for the clinical decisions.

Keywords: Immune cell infiltration, Prognosis, Tumor-infiltrating lymphocyte, Triple-negative breast cancer

1. Introduction

Breast cancer (BC) is a global public-health issue, with nearly 2.3 million new cases, and an incidence rate of 11.7% among all cancers in 2020 [1]. Based on the expression of hormone receptors including ER, PR, and HER2, breast cancer has been classified into different subtypes, among which triple-negative breast cancer (TNBC) refers to the subtype lacking the expression of hormone receptors and with HER2 gene amplification [2]. Clinically, TNBC shows aggressive and heterogeneous behavior and represents 15% to 20% of breast cancer cases [3, 4]. Current treatment for TNBC mainly includes surgery, adjuvant chemotherapy and radiotherapy; additionally, targeted therapy and immunotherapy are promising therapeutic interventions for improving clinical response and survival outcomes [5]. Unfortunately, patients with TNBC have worst prognosis relative to those with other BC subtypes. Over half of TNBC patients undergo recurrence within the first 5 years after diagnosis, and the overall survival of is merely 12-18 months on average [4]. Deepening the understanding of the immune signature in TNBC might provide novel biomarkers for prognosis as well as therapeutic response prediction in TNBC patients.

Cancer has been increasingly recognized as an evolutionary and ecological process with reciprocal contact between cancer cells and the tumor microenvironment (TME) [6]. Various cell types are found in the TME, including fibroblasts, endothelial cells, and immune cells, which secrete factors for chronic inflammation and environment, in which cancer cells can escape from eradication by host immunosurveillance [7]. TNBC is also distinguished from other subtypes in immunogenic characteristics with higher proportion of tumor-infiltrating immune cells, which are found to affect the TNBC progression by regulating cell proliferation, apoptosis and drug resistance via anti-tumor activity or immunosuppression [8, 9]. The evaluation of tumor-infiltrating lymphocytes (TIL) is valuable for the prediction of prognosis in TNBC [10], and provides promising targeting strategy for improving the survival outcome of TNBC patients. Tumor mutation burden

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(TMB), an indicator of tumor antigenicity, is linked with TNBC patient prognosis, and those with high TMB show significantly higher 5-year survival rates relative to those with low TMB [11]. As TNBC is a highly heterogeneous malignancy, the prognosis of patients can be different in patients with similar clinical features, thus identification of the immune signature in TNBC might benefit the screening of patients sensitive to immunotherapy.

The current study explored the immune infiltration pattern as well as related biomarkers in TNBC using bioinformatics analysis. A random forest risk model was established, dividing patients into high or low-immune-related risk groups. The TIL-related differentially expressed genes were further screened and their biological functions and enriched pathways were analyzed. Furthermore, a prognostic risk model was established based on the TIL-related differentially expressed genes. Moreover, the mutation characteristics of TNBC patients were explored and the predictive accuracy of the model was analyzed. The findings of our study are expected to provide novel insight into the TIL-related gene signature in TNBC and benefit clinical decisions.

2. Materials and Methods

2.1. Data collection

Gene expression files (read counts) with clinical data were downloaded from The Cancer Genome Atlas (TCGA)-Breast Invasive Carcinoma (BRCA) (n=1174) dataset using TCGAbiolinks R package. Protein-coding genes and TNBC samples (n=113) were filtered for further analysis.

2.2. Immune score calculation and immune-related risk model establishment

CIBERSORT identifies the proportion of 22 immune cell subtypes in human by analyzing the expression matrix of biomarkers with a deconvolution algorithm according to the principle of linear support vector regression [12] and was adopted to calculate the immune cell infiltration levels in TNBC.

RandomForest R package was adopted for constructing a random forest risk model. The TNBC samples were randomized into a training and a validation dataset at a ratio of 7:3. TNBC patients were separated to the high- (n=56) or low-risk (n=57) group according to the median immune-related risk score. The baseline characteristics of patients in the high or low-risk groups are shown in Table 1. Additionally, the ROC curve was applied to evaluate the prediction ability of this model.

2.3. Identification of TIL-related DEGs

To find the crucial genes linked with the immune cell infiltration in TNBC, DEseq2 R package was used for screening out the DEGs between groups, under the condition of $|\log2FoldChange| > 1$, $Pvalue < 0.05$. The results were visualized as a heatmap and volcano plot using the pheatmap and ggplot2 R packages, respectively.

2.4. Enrichment analysis of the TIL-related DEGs

Gene Ontology (GO) enrichment analysis is a well-known method for ascribing functions to genes, in terms of cellular components (CC), molecular functions (MF), and biological pathways (BP) [13]. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a knowledge base for analyzing gene functions as well as biochemical pathways [14]. The GO functions as well as KEGG pathways of candidate TIL-related DEGs were analyzed using clusterprofiler R package [15].

Gene Set Enrichment Analysis (GSEA) refers to a method for gene distribution trend assessment from *a*

Table 1. Baseline characteristics of TNBC patients between high or low-risk groups.

Characteristic	High Risk	Low Risk	p
n	56	57	
T stage, n (%)			< 0.001
T1	19 (16.8%)	6 (5.3%)	
T2	27 (23.9%)	46 (40.7%)	
T3	9 (8%)	2 (1.8%)	
T4	1 (0.9%)	3 (2.7%)	
N stage, n (%)			0.912
N0	34 (30.1%)	38 (33.6%)	
N1	14 (12.4%)	11 (9.7%)	
N2	6 (5.3%)	6 (5.3%)	
N3	2 (1.8%)	2 (1.8%)	
M stage, n (%)			0.148
M0	45 (39.8%)	52 (46%)	
M1	1 (0.9%)	1 (0.9%)	
MX	10 (8.8%)	4 (3.5%)	
Satge, n (%)			0.028
Stage I	14 (12.7%)	4 (3.6%)	
Stage II	29 (26.4%)	42 (38.2%)	
Stage III	10 (9.1%)	9 (8.2%)	
Stage IV	1 (0.9%)	1 (0.9%)	
Age, mean ± SD	54.14 ± 11.13	55.6 ± 12.44	0.514

priori set of genes for phenotype correlation to justify contribution of phenotypes [16]. We acquired the "c2.cp.v7.2.symbols.gmt[Curated]" dataset from MSigDB (https://www.gsea-msigdb.org/gsea/msigdb/), and the clusterProfiler R package was used for GSEA under the condition of false discovery rate< 0.25, Pvalue < 0.05.

2.5. Prognostic risk signature construction with TIL-related DEGs

The least absolute shrinkage and selection operator (LASSO) prediction model was established using the glmnet R package [17]. The predictive accuracy of this model was determined by area under the ROC curves at 1, 3, and 5 years with a timeROC R package. The survival curves of the TNBC patients in the high-risk or low-risk groups were drawn with survival R package.

2.6. Copy number variation and mutation burden analysis

Copy number variation (CNV) is the genomic structural variation causing abnormal gene copy numbers, such as gene amplification, gain, loss and deletion. The CNV data were downloaded from TCGA database using TCGAbiolinks package, and GISTIC2.0 analysis was then conducted on the GenePattern database. GISTIC2.0 calculated the information on deletions and duplications by analyzing the CNV data of each sample. The visualization of the genomic mutation types and tumor mutation burden (TMB) in TNBC was conducted using maftools package in R.

2.7. Prognostic analysis and nomogram construction

Univariate cox regression was adopted for assessing the predictive ability of 6 hub genes, risk score of the model as well as the clinicopathologic features for OS in TNBC patients with survival package in R. Next, those statistically significant covariates were subject to multivariate cox regression analysis. Furthermore, a rms R package was adopted for the nomogram construction. Calibration plot was adopted to evaluate the degree of fit between estimation of nomogram and actual OS probabilities. The statistical significance was assessed using Wruskal-Wallis test.

2.8. Statistical analysis

The calculation and analyses of all data were conducted with the R 4.0.2 software. Student t and Wilcoxon rank-sum tests were employed for calculating the difference between normally or non-normally distributed variables, respectively. P value less than 0.05 was set as the threshold value.

3. Results

3.1. Establishment of immune risk model in TNBC

The immune cell infiltration level is closely associated with the cancer patient prognosis. CIBERSORT algorithm was used for calculating the immune infiltration in TNBC. The results indicated that T cells CD4 memory activated, T cells CD8, Monocytes, Mast cells resting, NK cells resting, and Macrophages M2 showed high infiltration levels in TNBC (Fig. 1A-B). Then data were sampled and subject to cross-validation using a training dataset and a validation dataset. The random forest algorithm was applied for constructing an immune infiltration-related risk model (Fig. 1C). The risk score was elevated in the death group

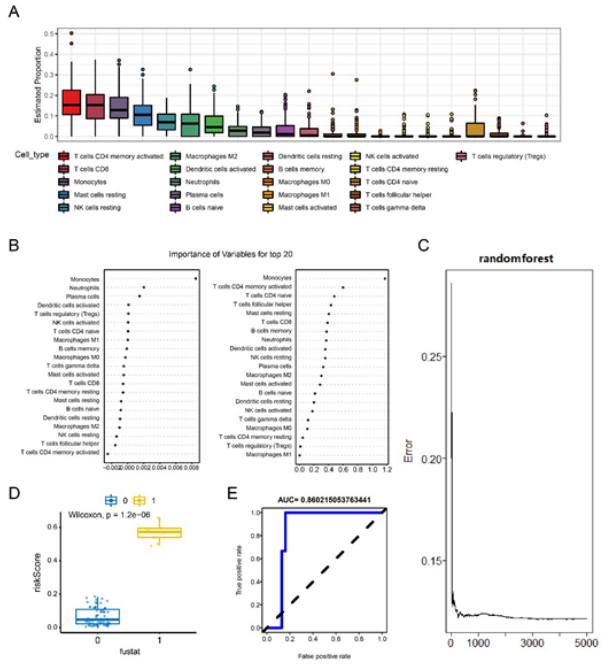

Fig. 1. Immune cell infiltration analysis in TNBC. (A) Box plot of the immune cell infiltration in TNBC. (B) The top 20 key immune cell types in the random forest model. (C) The error rate confidence intervals for random forest model. The whole dataset was divided into the training dataset and validation dataset in a ratio of 7:3. (D) The immune-related risk score in the survival (0) and death groups (1) in the prediction of intragroup data using random forest model. (E) ROC curve of the training dataset.

(1) relative to the survival group (0) (Fig. 1D), and the ROC curve revealed an AUC of 0.86 of this model, indicating the favorable prediction power of this model (Fig. 1E).

3.2. Identification of TIL-related DEGs and potentially relate pathways in TNBC

With a median risk score=0.069 in this immune risk model, we separated 113 TNBC samples into the high or low-immune risk groups. A total of 243 DEGs were screened out between groups, including 128 upregulated genes and 115 downregulated genes (Fig. 2A-B). Moreover, as shown in Fig. 2A, the DEGs showed distinct expression patterns to distinguish the high or low immune infiltration risk groups.

Furthermore, enrichment analyses were conducted to explore the functions and related signalings in TNBC. According to GO enrichment analysis, DEGs were primarily related to biological processes including antimicrobial humoral response, skeletal system development, chemokine-mediated signaling pathway, presynapse assembly, response to chemokine, and cellular response to chemokine. The enriched cell component terms of DEGs included collagen-containing extracellular matrix, ion channel complex, transmembrane transporter complex, and intrinsic component of synaptic membrane. Moreover, these DEGs were also found enriched in the molecular function such as extracellular matrix structural constituent, CXCR chemokine receptor binding, and cytokine activity (Fig. 2C). Then we conducted the KEGG analysis of the TIL-related DEGs, and DEGs were related to interaction between cytokines and receptors, circadian entrainment, chemo-

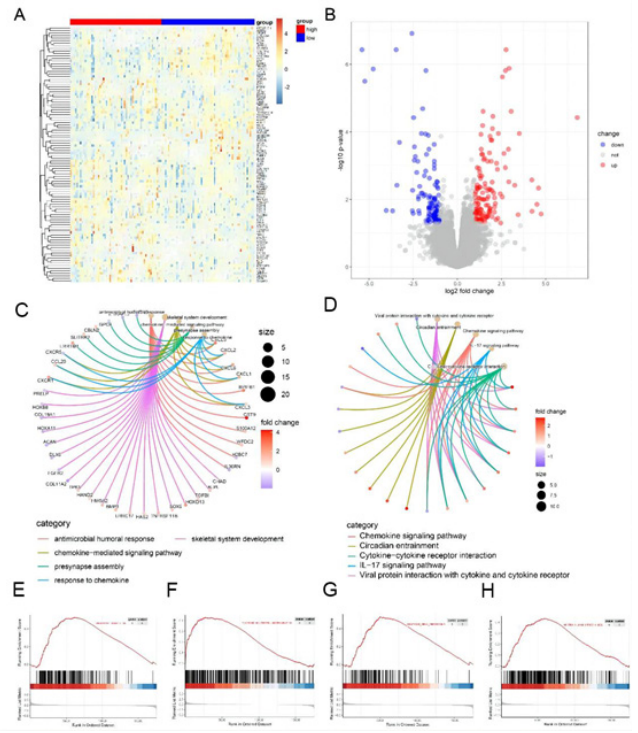

Fig. 2. Identification of TIL-related DEGs and enrichment analysis in TNBC. (A) Heatmap and (B) Volcano plot of the DEGs between high or low immune risk groups. (C) GO enrichment analysis of the TIL-related DEGs. (D) KEGG enrichment analysis of the TIL-related DEGs. (E-H) GSEA of the TIL-related DEGs.

kine or IL-17 signalings (Fig. 2D). To further reveal the impact of the TIL-related DEGs on TNBC development, GSEA revealed that these genes mainly affected the biological functions related to synaptic membrane secretory granule lumen, collagen-containing extracellular matrix, transmembrane transporter complex, ion channel complex (Fig. 2E-H).

3.3. Establishment of a Lasso Cox risk model with TIL-related DEGs.

The prognostic risk model was established based on the TIL-related DEGs in TNBC. Data sampling was conducted for the cross-validation of training dataset and validation dataset. Totally 6 key genes were obtained, namely SLITRK3, PCDHGB3, NELL2, SRRM4, ASIC2, and B4GALNT2, and an immune-related six-gene prognostic signature in TNBC was constructed (Fig. 3A-B).

ROC curves assessed the ability of this six-gene prognostic signature for OS prediction in TNBC. As a result, the AUC value was 0.51, 0.76 and 0.9 at 1, 3, and 5 years, respectively, indicating the better ability of our model for the long-term survival evaluation in TNBC patients (Fig. 3C). Additionally, KM survival analyses indicated the worse survival outcomes of high-risk TNBC patients, albeit the difference between groups did not show statistical significance (P=0.0744) (Fig. 3D).

3.4. Analysis of the mutation characteristics

The relation between genomic mutation and risk score was explored. We found that the missense mutation was the major mutation type in both two risk groups. The mutation of top 30 genes in different TNBC samples was shown in the waterfall plot. The results showed top thirty genes with highest mutation frequency were totally the same in the two groups. However, the mutation types of these genes were different between the two groups (Fig. 4A-D). TNBC samples in the high prognostic risk group showed relatively higher mutation burden. With analysis of GISTIC 2.0, genes with significant differences in the CNV were shown in the bubble plot. Genes were ranked by the frequency of CNV amplification or deletion, and the top 10 genes with the most frequently changed CNV were presented (Fig. 4E-F).

3.5. Clinical prognosis analysis of the six-gene prognostic risk model

Univariate Cox regression analysis evaluated the relation between the hub gene expression or clinical characteristics and overall survival of TNBC patients. As a result, higher levels of SLITRK3, NELL2, and B4GALNT2 were associated with reduced survival of TNBC patients (P<0.05). Although the Hazard Ratio (HR) of the 6 hub genes did not show obvious increase, the elevated prognostic risk score predicted shorter overall survival of the TNBC patients (P<0.001) (Fig. 5A). The relation of risk score and different TNM stages was also analyzed, and the risk score showed no statistical difference between the T1&T2 and T3&T4 groups, between the N0&N1 and N2&N3 groups, or between the M0 and M1 groups (Fig. 5B-D). For multivariate Cox regression analysis, concomitant variables (P<0.1) including SLITRK3, PCDHGB3, NELL2, B4GALNT2 and T staging were incorporated. The results showed that these factors did not show independent prognostic value in TNBC (Table 2). Next, a nomogram was constructed using the risk factors mentioned above and calibration curve was generated to evaluate the consistency of the actual and the predicted 1-, 3-, and 5-year OS (Fig. 5E-F). The C-index of the model was 0.723, which indicated great performance in the survival

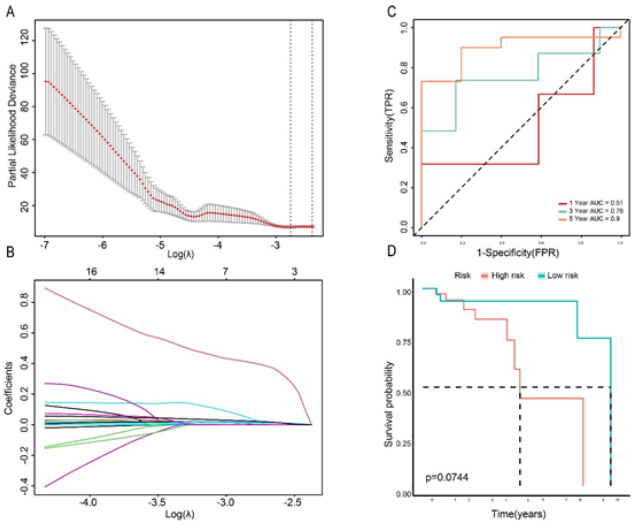

Fig. 3. Establishment of prognostic risk model based on Lasso Cox regression. (A) The coefficient selection in the LASSO Cox regression model. Vertical dashed lines are plotted at the best lambda. (B) Ten cross-validations of adjusted parameter selection in the LASSO model. Each curve corresponds to one gene. (C) Time-dependent ROC curves showed the predictive ability of the model for survival outcomes of TNBC patients at 1 year, 3 years and 5 years. (D) KM curves showed the survival outcomes of TNBC patients in the high- or low-risk groups.

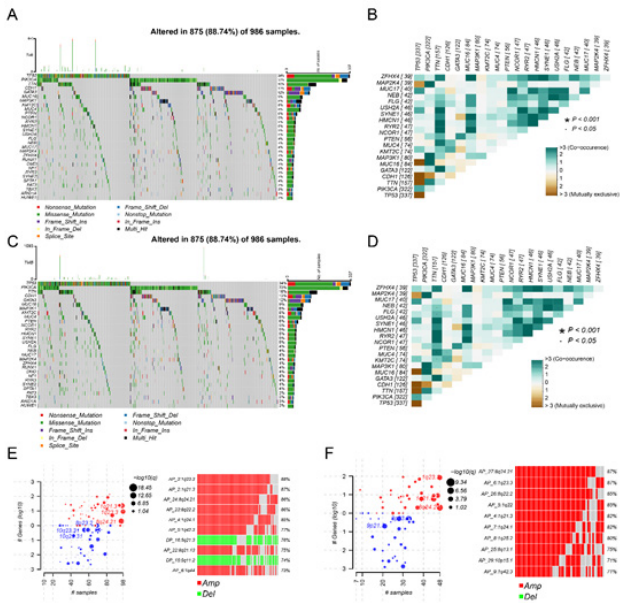

Fig. 4. Mutation characteristic analysis of prognostic model. (A) The mutation types of the top 30 genes with the highest mutation frequency in the high-risk group. (B) Gene expression correlation analysis in the high-risk group. (C) The mutation types of the top 30 genes with the highest mutation frequency in the low-risk group. (D) Gene expression correlation analysis in the low-risk group. (E) CNV analysis in the high-risk group. (F) CNV analysis in the low-risk group. Red color indicated that the copy number increased, and blue color indicated that the copy number decreased.

prediction for TNBC patients.

4. Discussion

TILs as an immunomodulatory factor in tumor microenvironment (TME) regulate immune escape of tumor cells, affecting cancer progression. Accumulating evidence has revealed the role of TILs as biological prognostic biomarkers in TNBC [18]. In this study, we identify the immune infiltration pattern of the TILs in TNBC and establish an immune-related risk model using random forest method. TNBC samples were allocated to the high or low-immune risk groups, and a total of 243 DEGs between groups were identified. Based on Lasso cox regression, a 6-gene prognostic risk signature was established, with great performance for OS prediction for TNBC.

TILs are critically involved in antitumor immunity, and a variety of immune cells including, B cells, NK cells, T cells, and others participate in the immune responses within the TME [19]. In the current work, we investigated the infiltration levels of 22 types of immune cells, and activated CD4 memory, CD8 T cells, Monocytes, resting Mast cells, resting NK cells, and M2 Macrophages were found enriched in the tumor cells in TNBC, which are reported to affect TNBC patient prognosis and response to therapy [20, 21], indicating the immunoreactive TME in TNBC. A random forest model was established to predict the immune infiltration risk in TNBC, and the predictive accuracy was confirmed by the ROC curve (AUC=0.86). The higher risk score indicated worse survival outcomes in TNBC patients. The establishment of an immune-related risk model benefits comprehensive characterization of BC immune infiltrates, which may contribute to optimized patient selection and stratification.

Furthermore, the TIL-related DEGs were identified. Enrichment analyses indicated that TIL-related DEGs were linked with the chemokine-related signaling pathway, CXCR chemokine receptor binding, extracellular matrix

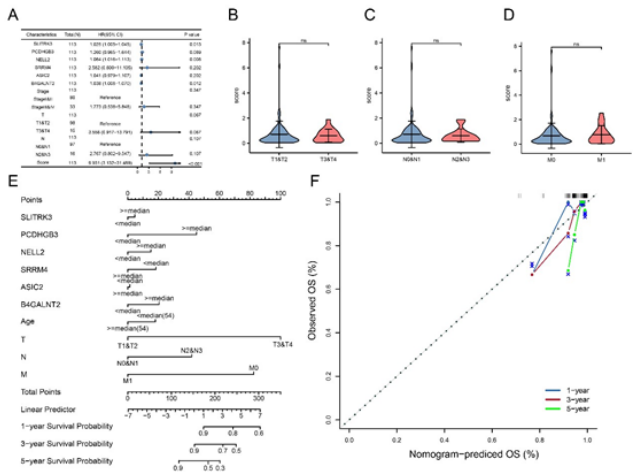

Fig. 5. Prognostic analysis and nomogram construction. (A) The univariate cox regression analysis of the relation of 6 hub genes, risk score of the prognostic model and clinicopathologic factors (TNM staging) and OS of TNBC patients. (B) Nomogram was constructed for the prediction of 1-, 3- and 5-year OS probability. (C) The calibration curve was used to evaluate the predictive ability of the nomogram for 1-, 3- and 5-year OS.

Table 2. Univariate and Multivariate Cox regression analysis.

Characteristics	Total	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
SLITRK3	113	1.025 (1.005-1.045)	0.013	0.930 (0.75-1.15)	0.507
PCDHGB3	113	1.260 (0.965-1.644)	0.089	1.332 (0.94-1.89)	0.108
NELL2	113	1.064 (1.016-1.113)	0.008	1.035 (0.95-1.12)	0.418
SRRM4	113	2.582 (0.600-11.105)	0.202		
ASIC2	113	1.041 (0.979-1.107)	0.202		
B4GALNT2	113	1.038 (1.008-1.070)	0.012	0.922 (0.69-1.23)	0.583
Stage	113				
StageI&II	80	Reference			
StageIII&IV	33	1.773 (0.538-5.848)	0.347		
Score	113	9.931 (3.132-31.489)	<0.001	1519.166 (0.001-3851261726.52)	0.330

(ECM), IL-17 pathway as well as interaction between cytokines and receptors. TME consists of not only immune cells and non-cancerous host cells, but also the non-cellular components such as ECM and chemokines, and cytokines [22]. Chemokines as well as chemokine receptors are suggested as potential targets for tumor immunotherapy [23], and development of the chemokine receptor inhibitors has been revealed to enhance the anti-tumor immune response and inhibit TNBC tumor growth or enhance the response to chemotherapy and radiotherapy [24, 25]. IL-17 is reported to promote TNBC cell migration, and inhibition of IL-17 decreases the CD8+ T cells in mouse tumors, which indicates that IL-17 contributes to the immunosuppressive TME in TNBC [26]. Therefore, exploration of the TIL-related DEGs in TME of TNBC might provide promising targets for TNBC therapy.

Next, the prognostic risk model based on the TIL-related DEGs was established. Six hub genes, including SLITRK3, PCDHGB3, NELL2, SRRM4, ASIC2, and B4GALNT2 were identified according to Lasso cox regression analysis. The six-gene prognostic signature showed better predictive ability for the long-term prognosis of TNBC patients. SLITRK3 is previously reported as a neuronal transmembrane protein related to synapse development and also as a novel cancer biomarker of lung cancer, gastrointestinal stromal tumor and brain tumors [27, 28]. PCDHGB3 belongs to the protocadherin gamma gene cluster and may affect cell-cell connections [29]. However, its role in cancer progression is rarely investigated. NELL2 is a member of the family of multimeric and multimodular extracellular glycoproteins, and has been revealed to be downregulated in diverse malignancies such as renal cell carcinoma, gastric cancer as well as breast cancer, and NELL2 silencing facilitates cancer cell migration [30, 31]. SRRM4 is lowly expressed in tumor samples, which is linked with the increased expression of mitotic gene expression, and the overexpression of SRRM4 inhibits tumor cell proliferation [32]. However, a study by Deshpande et al. shows that SRRM4 is upregulated in systemic breast cancer cells, which is related to enhanced breast-to-brain metastases and reduced survival outcomes [33]. ASIC2 belongs to the degenerin/epithelial sodium channel (DEG/ENaC) superfamily. It is reported to be expressed in the A549 lung cancer cells and involved in the extracellular acidosis-mediated proliferation and migration of A549 cells [34]. B4GALNT2 is highly expressed in TNBC samples, and silencing of B4GALNT2 suppresses TNBC cell proliferation, migration and invasion and increases TNBC cell apoptosis [35-37]. The prognostic prediction ability of the six hub genes in TNBC was also explored. SLITRK3, NELL2, SRRM4, and B4GALNT2 were revealed as risk factors for OS in TNBC. The nomogram was constructed with 6 hub genes as well as clinicopathological risk factors, with great performance in OS prediction. Risk score also showed prognostic value in TNBC.

5. Conclusion

To conclude, we identified 6 hub genes associated with immune cell infiltration in TNBC. The 6-gene prognostic signature was constructed and showed great prognostic potency, especially for the long-term prognosis prediction of TNBC patients. The findings of our study might benefit the clinical decision-making and design of individualized treatment plans.

Conflict of Interests

The authors declare no competing interests.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Informed Consent

Not applicable.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

WY, ZN, ZB and CY were responsible for the study design; WY and CY were responsible for data analysis; ZN and ZB were responsible for the interpretation of the analysis results; WY wrote the first draft of the paper.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 71 (3): 209-249. doi: 10.3322/caac.21660
2. Barzaman K, Karami J, Zarei Z, Hosseinzadeh A, Kazemi MH, Moradi-Kalbolandi S, Safari E, Farahmand L (2020) Breast cancer: Biology, biomarkers, and treatments. Int Immunopharmacol 84: 106535. doi: 10.1016/j.intimp.2020.106535
3. Derakhshan F, Reis-Filho JS (2022) Pathogenesis of Triple-Negative Breast Cancer. Annu Rev Pathol 17: 181-204. doi: 10.1146/annurev-pathol-042420-093238
4. Garrido-Castro AC, Lin NU, Polyak K (2019) Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment. Cancer Discov 9 (2): 176-198. doi: 10.1158/2159-8290.cd-18-1177
5. Li Y, Zhang H, Merkher Y, Chen L, Liu N, Leonov S, Chen Y (2022) Recent advances in therapeutic strategies for triple-negative breast cancer. J Hematol Oncol 15 (1): 121. doi: 10.1186/s13045-022-01341-0
6. Merlo LM, Pepper JW, Reid BJ, Maley CC (2006) Cancer as an evolutionary and ecological process. Nat Rev Cancer 6 (12): 924-935. doi: 10.1038/nrc2013
7. Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, Zitvogel L (2016) Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. Ann Oncol 27 (8): 1482-1492. doi: 10.1093/annonc/mdw168
8. Deepak KGK, Vempati R, Nagaraju GP, Dasari VR, S N, Rao DN, Malla RR (2020) Tumor microenvironment: Challenges and opportunities in targeting metastasis of triple negative breast cancer. Pharmacol Res 153: 104683. doi: 10.1016/j.phrs.2020.104683

9. Liu Y, Hu Y, Xue J, Li J, Yi J, Bu J, Zhang Z, Qiu P, Gu X (2023) Advances in immunotherapy for triple-negative breast cancer. Mol Cancer 22 (1): 145. doi: 10.1186/s12943-023-01850-7
10. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, Budczies J, Huober J, Klauschen F, Furlanetto J, Schmitt WD, Blohmer JU, Karn T, Pfitzner BM, Kümmel S, Engels K, Schneeweiss A, Hartmann A, Noske A, Fasching PA, Jackisch C, van Mackelenbergh M, Sinn P, Schem C, Hanusch C, Untch M, Loibl S (2018) Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 19 (1): 40-50. doi: 10.1016/s1470-2045(17)30904-x
11. Gao C, Li H, Liu C, Xu X, Zhuang J, Zhou C, Liu L, Feng F, Sun C (2021) Tumor Mutation Burden and Immune Invasion Characteristics in Triple Negative Breast Cancer: Genome High-Throughput Data Analysis. Front Immunol 12: 650491. doi: 10.3389/fimmu.2021.650491
12. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA (2015) Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 12 (5): 453-457. doi: 10.1038/nmeth.3337
13. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25 (1): 25-29. doi: 10.1038/75556
14. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M (1999) KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 27 (1): 29-34. doi: 10.1093/nar/27.1.29
15. Yu G, Wang LG, Han Y, He QY (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 16 (5): 284-287. doi: 10.1089/omi.2011.0118
16. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102 (43): 15545-15550. doi: 10.1073/pnas.0506580102
17. Friedman J, Hastie T, Tibshirani R (2010) Regularization Paths for Generalized Linear Models via Coordinate Descent. J Stat Softw 33 (1): 1-22.
18. Loi S, Michiels S, Adams S, Loibl S, Budczies J, Denkert C, Salgado R (2021) The journey of tumor-infiltrating lymphocytes as a biomarker in breast cancer: clinical utility in an era of checkpoint inhibition. Ann Oncol 32 (10): 1236-1244. doi: 10.1016/j.annonc.2021.07.007
19. Savas P, Salgado R, Denkert C, Sotiriou C, Darcy PK, Smyth MJ, Loi S (2016) Clinical relevance of host immunity in breast cancer: from TILs to the clinic. Nat Rev Clin Oncol 13 (4): 228-241. doi: 10.1038/nrclinonc.2015.215
20. Dieci MV, Miglietta F, Guarneri V (2021) Immune Infiltrates in Breast Cancer: Recent Updates and Clinical Implications. Cells 10 (2). doi: 10.3390/cells10020223
21. Han M, Li J, Wu S, Wu C, Yu Y, Liu Y (2023) Comparison of the tumor immune microenvironment phenotypes in different breast cancers after neoadjuvant therapy. Cancer Med 12 (3): 2906-2917. doi: 10.1002/cam4.5207
22. Xiao Y, Yu D (2021) Tumor microenvironment as a therapeutic target in cancer. Pharmacol Ther 221: 107753. doi: 10.1016/j.pharmthera.2020.107753
23. Mollica Poeta V, Massara M, Capucetti A, Bonecchi R (2019) Chemokines and Chemokine Receptors: New Targets for Cancer Immunotherapy. Front Immunol 10: 379. doi: 10.3389/fimmu.2019.00379
24. Meng Y, Zhou J, Liu X, Zeng F, Wen T, Meng J, Liu J, Xu H (2023) CXC Chemokine Receptor Type 4 Antagonistic Gold Nanorods Induce Specific Immune Responses and Long-Term Immune Memory to Combat Triple-Negative Breast Cancer. ACS Appl Mater Interfaces 15 (15): 18734-18746. doi: 10.1021/acsami.3c03130
25. Xie XF, Wu NQ, Wu JF, Zhang GL, Guo JF, Chen XL, Du CW (2022) CXCR4 inhibitor, AMD3100, down-regulates PARP1 expression and Synergizes with olaparib causing severe DNA damage in BRCA-proficient triple-negative breast cancer. Cancer Lett 551: 215944. doi: 10.1016/j.canlet.2022.215944
26. Tsai YF, Huang CC, Lin YS, Hsu CY, Huang CP, Liu CY, Chiu JH, Tseng LM (2021) Interleukin 17A promotes cell migration, enhances anoikis resistance, and creates a microenvironment suitable for triple negative breast cancer tumor metastasis. Cancer Immunol Immunother 70 (8): 2339-2351. doi: 10.1007/s00262-021-02867-x
27. Bollig-Fischer A, Bao B, Manning M, Dyson G, Michelhaugh SK, Mittal S, Bepler G, Mamdani H (2021) Role of novel cancer gene SLITRK3 to activate NTRK3 in squamous cell lung cancer. Mol Biomed 2 (1): 26. doi: 10.1186/s43556-021-00051-2
28. Wang CJ, Zhang ZZ, Xu J, Wang M, Zhao WY, Tu L, Zhuang C, Liu Q, Shen YY, Cao H, Zhang ZG (2015) SLITRK3 expression correlation to gastrointestinal stromal tumor risk rating and prognosis. World J Gastroenterol 21 (27): 8398-8407. doi: 10.3748/wjg.v21.i27.8398
29. Nicoludis JM, Vogt BE, Green AG, Schärfe CP, Marks DS, Gaudet R (2016) Antiparallel protocadherin homodimers use distinct affinity- and specificity-mediating regions in cadherin repeats 1-4. Elife 5: e18449. doi: 10.7554/eLife.18449
30. Tong L, Zhang W, Qu B, Zhang F, Wu Z, Shi J, Chen X, Song Y, Wang Z (2020) The tRNA-Derived Fragment-3017A Promotes Metastasis by Inhibiting NELL2 in Human Gastric Cancer. Front Oncol 10: 570916. doi: 10.3389/fonc.2020.570916
31. Nakamura R, Oyama T, Inokuchi M, Ishikawa S, Hirata M, Kawashima H, Ikeda H, Dobashi Y, Ooi A (2021) Neural EGFL like 2 expressed in myoepithelial cells and suppressed breast cancer cell migration. Pathol Int 71 (5): 326-336. doi: 10.1111/pin.13087
32. Head SA, Hernandez-Alias X, Yang JS, Ciampi L, Beltran-Sastre V, Torres-Méndez A, Irimia M, Schaefer MH, Serrano L (2021) Silencing of SRRM4 suppresses microexon inclusion and promotes tumor growth across cancers. PLoS Biol 19 (2): e3001138. doi: 10.1371/journal.pbio.3001138
33. Deshpande K, Martirosian V, Nakamura BN, Das D, Iyer M, Reed M, Shao L, Bamshad D, Buckley NJ, Neman J (2024) SRRM4-mediated REST to REST4 dysregulation promotes tumor growth and neural adaptation in breast cancer leading to brain metastasis. Neuro Oncol 26 (2): 309-322. doi: 10.1093/neuonc/noad175
34. Wu Y, Gao B, Xiong QJ, Wang YC, Huang DK, Wu WN (2017) Acid-sensing ion channels contribute to the effect of extracellular acidosis on proliferation and migration of A549 cells. Tumour Biol 39 (6): 1010428317705750. doi: 10.1177/1010428317705750
35. Asghari Esfeden B, Khanahmadi A. R, Lotfi, E (2024) Heritability of fertility, hatchability and their relationship with egg quality traits in Japanese quail. J Poult Sci Avian Dis 2(2): 31-35. https://jpsad.com/index.php/jpsad/article/view/33
36. Lima-Neto RGD and Mohammadi MR (2023) Chromoblastomycosis successfully treated with the combination of itraconazole and 5-flucytosine. Cell Mol Biomed Rep 3(3):145-151. doi: https://doi.org/10.55705/cmbr.2023.378135.1092
37. Yu P, Zhu L, Cui K, Du Y, Zhang C, Ma W, Guo J (2021) B4GALNT2 Gene Promotes Proliferation, and Invasiveness

and Migration Abilities of Model Triple Negative Breast Cancer (TNBC) Cells by Interacting With HLA-B Protein. Front Oncol 11: 722828. doi: 10.3389/fonc.2021.722828