Identification of Robust Immune-Associated lncRNAs Signature for Immune Checkpoint Blockade and Prognosis in Breast Cancer

Qianhui Xu

Yuying Children's Hospital of Wenzhou Medical College: Wenzhou Medical University Second Affiliated Hospital

Yuxin Wang

Zhejiang University School of Medicine

Wen Huang (✉ qq2627897841@126.com)

Yuying Children's Hospital of Wenzhou Medical College: Wenzhou Medical University Second Affiliated Hospital <https://orcid.org/0000-0002-0010-3056>

Primary research

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Abstract

Background: There have numerous evidences to support that long non-coding RNAs (lncRNAs) may be crucial parts in cancer immunity. We aimed to establish a novel and robust immune-associated lncRNAs signature to improve prognostic precision in patients with breast cancer (BRCA).

Methods: BRCA cases were obtained from the The Cancer Genome Atlas (TCGA) database. Immune-related lncRNAs presenting significant association with prognosis were screened through stepwise univariate Cox regression and LASSO algorithm, and multivariate Cox regression. Kaplan-Meier analysis, ROC analyses, and proportional hazards model further conducted. The prediction reliability was further estimated in the internal validation set and combination set. Gene set enrichment analysis (GSEA) was applied for functional annotation. The correlation between immune checkpoint inhibitors and this signature was employed.

Results: 13 immune-related lncRNAs were systematically identified to establish immune-related lncRNAs predictive prognosis signature. The risk model we built showed significant correlation with BRCA patients' prognosis. The value of ROC for this lncRNAs model was up to 0.821. This immune-related lncRNAs signature can serve as an independent prognostic biomolecular factor. Our lncRNAs signature involved in chondrocyte development, endoderm development and so forth. This lncRNAs risk model was associated with tumor immune infiltration (i.e., B cells, Dendritic, Neutrophils, CD8 T cells and CD4 T cells, etc.) and immune checkpoint blockade (ICB) therapy key molecules (i.e., PD1).

Conclusion: The immune-related lncRNA signature we established possesses latent prognostic value for patients with BRCA and may have the capability to predict the clinical outcome of ICB treatment, which could provide guidance for immunological decision in patients with BRCA.

Background

Breast cancer (BRCA) whose mortality rate ranks second among female malignancies is one of the most common malignancy in women worldwide [1–3]. On the basis of the latest global cancer statistics, there was almost 2.1 million newly diagnosed breast cancer patients in 2018, amount to nearly one forth women cancer cases[1]. In the wake of the progress of the era, there has been numerous advanced innovations been exploiting in the early diagnosis and the systematical management of BRCA [4]. Nevertheless, patients with BRCA are still facing a formidable challenge in the light of their undesirable overall survival. Moreover, BRCA characteristic with incredibly high inter- and intra- tumor heterogeneity, and its pathological manifestations and etiology vary with each individual[5]. Besides, infiltrating immune cells in the tumor microenvironment possess a crucial role for prognostic survival in breast cancer[6]. Recent researches reported that the number of T cells had significant correlation with prognosis in patients with triple-negative breast cancers [7, 8]. Immune checkpoint inhibitors have dramatically yielded benefit in a great body of malignancies. breast tumors were immunologically quiescent, which is the most formidable challenge for immune checkpoint blockade therapy in breast cancer. Promisingly, the

results of preclinical trials and recent clinical data bring us some encouragement. Though we have made great achievement in the identification and validation of prognostic and predictive biomolecular indicators for BRCA, robust predictive biomarkers for ICB therapy outcome have not been exploited in BRCA[9]. As such, the most effective strategy for accurate prognostic determinations of how a given tumor will develop or respond to ICB treatment may be one based on molecular risk allocation, stratifying patients based on particular molecular signatures associated with prognosis, increasing efficacy accordingly.

Long noncoding RNA (lncRNA) whose RNA transcripts length longer than 200 bp is not able to code protein [10]. To this day, more and more studies have arrived at an agreement that lncRNAs serve as a crucial regulator in cancer immunity, including antigen release, and immune activation [11, 12]. An independent research found that lncRNA GAS5 expression level was downregulated in HCC patients and lncRNA GAS5 interference promoted tumor cell migration and dampen NK cell cytotoxicity [13]. Analogously, lncRNA TCONS_00019715 may function to facilitate tumoricidal activities through regulating macrophage polarization to the M1 phenotype[14]. Several researches have suggested the latent role of the lncRNAs risk model for prognostic prediction for patients with BRCA[15–18]. Nevertheless, the existing immune-related lncRNA risk score signature for BRCA patients' responsiveness to ICB is lacking.

Here, we employed a systematic analyze to recognize and confirm a robust and novel biomolecular risk model based on the immune-associated lncRNAs classifier for BRCA prognosis. Then, we explored the latent role of this immune-correlated lncRNAs risk score model in immune checkpoint inhibitor treatment. Our findings established an immune-correlated lncRNAs signature on account of lncRNA expression level which could precisely forecast BRCA survival through comprehensive analysis of genomic files and thus put promising insights into the approaches of predicting prognosis and responsiveness to ICB in BRCA patients.

Methods

2.1 Patients and Datasets

We downloaded pancreatic ductal adenocarcinoma cases from The Cancer Genome Atlas (TCGA) portal (<http://cancergenome.nih.gov>). Patients without intact genomics or clinical data were excluded ($n = 8$), leaving 1097 BRCA samples in the final cohort. The analysis process flow chart was presented in Fig. 1. Altogether 1097 BRAC cases were stochastically assigned into the training and validation set at the rate of 1:1 for systematic analysis employing R project “caret” package. Both training and validation set need to comply with the following requirements: (1) cases were stochastically classifier as train and test group; (2) samples in two sets had similar clinicopathological characteristics. The testing cohort with 544 patients and the combination group were further employed to confirm results derived from the training group. There was no necessity to obtain Ethics Committee approval, owing to all information were

2.2 Immune-Related lncRNAs

The lncRNA profile was determined applying a constructed mining method refer to published articles[19]. Briefly, genes were recognized as protein-coding genes or non-coding genes based on their Ensembl IDs or Refseq IDs, and only the long non-coding genes in NetAffx Annotation files were retained. We downloaded the immune gene data from the ImmPort data project, and 2,483 immune-associated genes were gained [20, 21]. We employed the Pearson correlation to analyze the correlation between immune-related genes and lncRNAs. The square of correlation coefficient $P < 0.005$ and $|R| > 0.4$ was set for immune-associated lncRNAs. To visualize coexpression networks, we employed Cytoscape software 3.7.2.

2.3 Identification of Predictive Immune -Correlated lncRNAs

To evaluate the prognosis of immune-correlated lncRNAs, we employed this lncRNAs signature to assemble a unitive risk score model in BRCA. Firstly, we employed univariate Cox regression analysis for 1545 immune-correlated lncRNAs in the training group. The results with $p < 0.01$ was considered to be statistical significance. And 19 immune-related lncRNAs were filtered out. Secondly, these recognized lncRNAs were further screened and confirmed via the least absolute shrinkage and selection operator (LASSO) algorithm using R project “glmnet” package. Then, a multivariate Cox regression model was analyzed. Finally, we identified 13 immune-correlated lncRNAs and calculated their corresponding coefficients to construct the prognostic lncRNAs risk score signature in BRCA. Finally, this immune-related lncRNA prognosis risk model was established based on linearly combining the formula below with the expression level multiplied regression analysis (β). Risk score = $\beta_{\text{lncRNA1}} \times \text{lncRNA1 expression} + \beta_{\text{lncRNA2}} \times \text{lncRNA2 expression} + \dots + \beta_{\text{lncRNA n}} \times \text{lncRNA n expression}$. Here, β was the regression coefficient of the multivariate Cox regression analysis[22]. Besides, we compared these lncRNAs expression levels in BRCA and normal tissue specimens utilizing TCGA transcriptomic profiles.

2.4 Validation of the immune-correlated lncRNAs risk model

Based on their respective risk score, patients together with their clinical data were allocated. We employed the median risk score to assign cases into high-risk and low-risk sets for further research. Kaplan–Meier survival curves were analyzed in both sets. Then, the time-dependent receiver operating characteristic (ROC) curves were plotted to evaluate the predictive survival performance. Moreover, multivariate Cox regression analysis was performed to validate whether the signature could be used as an independent biomolecular indicator to predict survival. The predictive precision of this immune-related lncRNA risk score model was further confirmed in the testing set and combination cohort. $P < 0.05$ was deemed statistically significant, and each test was two-sided.

2.5 Development of nomogram

To estimate the prognostic capability of risk score, stage and age for 1/3/5-year OS, receiver operating characteristic (ROC) curves was carried out to assess the area under the curve (AUC) values [23]. To open a quantitative method to forecast the survival of BRCA patients, we constructed and plotted a

nomogram that including this thirteen-lncRNAs risk score and other clinical characteristics to assess 1-, 3-and 5-year OS possibility.

2.6 Function of immune-correlated lncRNA signature on BRCA

We carried out Gene set enrichment analysis to investigate underlying mechanisms significantly correlated with our 13 immune-correlated lncRNA risk model. We analyzed the gene sets of “c5.go.v7.2.symbols.gmt[*gene ontology*]” from the Molecular Signatures Database through GSEA[24]. To achieve a normalized enrichment score for each analysis, gene set permutations with 1,000 times were carried out. A nominal $p < 0.05$ were deemed significant results.

2.7 Association with Tumor-infiltrating immune cells

CIBERSORT consisted of 22 TIIC subsets utilizes a deconvolution strategy to reveal the abundance of tumor-infiltrating immune cells (TIICs) [25]. The correlation between 22 TIIC subsets with the immune-related lncRNA risk model was carried out to investigate whether our as-constructed immune-correlated lncRNAs signature serve as key roles in immune infiltration of BRCA.

2.8 Immune checkpoint blockade

According to previous research, immune checkpoint blockade therapy-correlated crucial genes expression might be associated with responsiveness to immune checkpoint blockade treatment[26]. We employed six key genes of immune checkpoint inhibitors therapy: programmed death 1 (PD-1, also known as PDCD1), programmed death ligand 1 (PD-L1, also known as CD274), programmed death ligand 2 (PD-L2, also known as PDCD1LG2), T-cell immunoglobulin domain and mucin domain-containing molecule-3 (TIM-3, also known as HAVCR2), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and indoleamine 2,3-dioxygenase 1 (IDO1) in BRCA[27–29]. To explore the latent role of our as-constructed lncRNAs risk model in ICB treatment in patients with BRCA, we analyzed the association between our risk score model and these six immune checkpoint blockade key genes expression.

2.9 Statistical analysis

Statistical significance was set at a threshold of a two-tailed $P < 0.05$. Construction of the immune–lncRNA coexpression network was completed using CYTOSCAPE software [30] (version 3.4.0; The Cytoscape Consortium, San Diego, CA, USA). R software (version 4.0.2; R Foundation) was adopted for all analyses. GSEA (<http://www.broadinstitute.org/gsea/index.jsp>) was utilized to distinguish between two sets of functional annotations.

Results

3.1 Establishment of a Coexpression Network

We obtained 14,142 lncRNAs from TCGA- BRCA. Altogether 2,483 immune-correlated genes were downloaded in the ImmPort portal(<https://www.immport.org>). The lncRNA and immune-related gene coexpression network was assembled to visualize these immune-correlated lncRNAs. Ultimately, 1545 immune-correlated lncRNAs were screened ($P < 0.005$ and $|R| > 0.4$) in our research (Table S1).

3.2 Identification of Immune-Correlated lncRNA Signature.

Cases from TCGA-BRCA cohort were randomly assign into training set and internal testing group at the ratio of 1:1 (Tables S2, S3). On the basis of the results of univariate Cox model, we found 19 immune-related lncRNAs possess valuable prognostic performance in BRCA patients ($P < 0.05$, Table S4). Next, we applied LASSO Cox regression algorithm to validate further variables and 18 immune-related lncRNAs were recognized (Figs. 2A,2B and Table S5). Multivariate proportional hazards model was carried out, then 13 immune-related lncRNAs finally recognized as the biomolecular predictors of prognosis in BRCA patients. The forest plot of each immune-related lncRNAs together with survival was shown in Fig. 2C. Table 1 presented that AC005332.6, AP001207.3, LINC00668, AC110995.1, AL161719.1 and SP2 – AS1 were unfavorable lncRNAs, whereas AL359752.1, AL606834.1, AL358472.3, ST7 – AS1, LINC01871, AP005131.2 and PCAT18 were protective lncRNAs. Comparison of genomics files in TCGA presented that the expression level of most lncRNAs was notably differentially expressed in BRCA (Figs. 2D-H). We further assembled immune lncRNAs co-expression networks (Figures S2A, S2B). Subsequently, 13 immune-correlated lncRNA signature was developed utilizing a risk score method[31–33]. Subsequently, the risk score was calculated as follows: $(0.022756 \times \text{AC005332.6 expression}) + (-0.20086 \times \text{AL359752.1 expression}) + (-0.73265 \times \text{AL606834.1 expression}) + (0.060771 \times \text{AP001207.3 expression}) + (-0.31622 \times \text{AL358472.3 expression}) + (-0.39027 \times \text{ST7-AS1 expression}) + (0.019412 \times \text{LINC00668 expression}) + (-0.25798 \times \text{LINC01871 expression}) + (-0.46171 \times \text{AP005131.2 expression}) + (0.259619 \times \text{AC110995.1 expression}) + (-0.11071 \times \text{PCAT18 expression}) + (0.180171 \times \text{AL161719.1 expression}) + (0.281635 \times \text{SP2-AS1 expression})$.

3.3 Confirmation of Immune-Correlated lncRNA Signature

Every case gained a risk score in the group of BRCA by adopting this 13 immune-related lncRNA model, and all patients were randomized into high-risk or low-risk set based on the median threshold. Figure 3A presented the allocations of 13 lncRNAs expression levels together with cases and groups. The distributions of risk score as well as survival time in the training group, which indicated that cases in high-risk set commonly had poorer prognosis in BRCA(Figs. 3B, 3C). Besides, Kaplan–Meier curves displayed that cases with low risk presented significant better prognosis compared with high-risk cases ($P = 4.829e - 10$) in the training set (Fig. 3D). To further estimate the value of this immune-related lncRNAs classifier in forecasting survival of BRCA, we analyzed ROC curves. Figure 3E shown that the value of the area under the curve (AUC) for this immune-related lncRNAs signature reached 0.821. Furthermore, the hazard ratio (HR) for risk score in univariate proportional hazards model analysis was 1.049 (95% CI: 1.032 – 1.067; Fig. 3F). And we obtained accordant results employing multivariate Cox model (HR = 1.042, 95% CI: 1.023 – 1.062; Fig. 3G).

Building a 13 lncRNA Model

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Next, we further validated these findings in the internal validation group and the whole set to confirm the prognostic value of this lncRNA risk model among distinct populations. The allocations of lncRNA expression level, survival time, and risk score in the internal testing set and the whole cohort were presented Figs. 4A-C and Figures S2A-C. Moreover, we analyzed Kaplan–Meier curves and found that the BRCA cases in low-risk set presented a notably better prognosis than the high-risk score cases in the internal validation cohort and the combination set (Fig. 4D and Figure S2D; all $P < 0.05$). The values of area under the ROC (AUC) reached both up to 0.8 or more in the internal validation group and the whole cohort (Fig. 4E; Figure S2E), indicating an outstanding performance of this immune-related lncRNA model to predict survival in BRCA. In line with the findings obtained in the training group, this immune-related lncRNA risk score was an independent predictive biomolecular indicator in both univariable as well as multivariable regression analysis of the test group and the whole set (Figs. 4F-G and Figures S2 F-G).

3.5 Validation of predictive value of this lncRNAs signature

To confirm whether the survival prediction was best by this lncRNAs signature among all clinical parameters, age and stage were collected as the candidate predictive biomolecular indicators. We analyzed the AUC curve for 1-, 3-, and 5-year prognosis and found that our risk model possesses the highest AUC among these factors (Figs. 5A, B, and C). We assembled a nomogram on the basis of age, stage, and risk signature to forecast prognosis of BRCA cases (Fig. 5D). By and large, the risk score model we established can provide the most helpful and precise guidance to predicting survival among these prognostic indicators.

3.6 Functional Analysis of Prognostic lncRNA Model

To explore the biological implications of this immune-associated lncRNAs risk model mediated in BRCA occurrence and progression, we employed out GSEA in both the low-risk and high-risk sets. Our findings displayed that the high-risk score of lncRNAs signature presented marked enrichment in pathways, such as chondrocyte development, endoderm development, endoderm formation, endoderm cell differentiation, laminin binding and so forth (Fig. 5E).

3.7 Correlation of the immune-related lncRNA signature with immune cell infiltration and ICB therapy-related molecule

We further explored whether this immune-related risk score model was correlated with TIICs. We found the as-constructed signature exhibited the marked negative association with immune infiltration of B cells ($r = -0.061$; $p = 0.043$), CD4 + T cells ($r = -0.089$; $p = 0.003$), CD8 + T cells ($r = -0.092$; $p = 0.002$), Dendritic cells ($r = -0.087$; $p = 0.004$), Neutrophils ($r = -0.087$; $p = 0.004$, Figs. 6A-E). These results strongly provide evidence to support that our prognostic lncRNA risk score model was significantly correlated with immune cell infiltration in BRCA.

Then, we explored six key immune checkpoint inhibitors-related genes: PDCD1, CD274, PDCD1LG2, CTLA-

Loading [MathJax]/jax/output/CommonHTML/jax.js the correlation between the ICB therapy key targets and

immune-related lncRNA signature to investigate the latent role of this lncRNAs risk score model in the ICB immunotherapy in patients with BRCA (Fig. 7A). The findings presented that this immune-related lncRNA risk score model was markedly negative correlated to PDCD1 ($r = -0.083$; $p = 0.006$, Figs. 7B), suggesting that the immune-related lncRNA signature play a potential role in the evaluation and measurement of response to ICB immunotherapy in BRCA.

Discussion

Globally, breast cancer as the most frequently diagnosed form of cancer is the primary cause of death due to cancer in women in most countries. The heterogeneity of BRCA exists from the tumor microenvironment to the phenotypes and genotypes of cancer cells, leading to patients with the identical pathological period possess various responsiveness to clinical treatments and clinical outcomes. As the only predictive indicator utilized routinely in clinical application to forecast the survival and guide clinical management, the TNM staging fails to forecast the clinical outcome for patients with the same clinical stage[34]. Thus, there is urgent need to exploit the novel and effective biomarkers for early diagnosis, treatment option, and survival evaluation to improve the prognosis for BRCA. In the past decades, more and more studies provided evidences to support that lncRNAs might play key roles in tumorigenesis, progression and invasion in breast cancer[15, 35]. According to previous researches, lncRNAs were reported as crucial regulators in regulating cancer immunity[12]. A recent research from Mineo Marco et al reported that INCR1 knockdown can improve CAR T cell therapy via sensitizing tumor cells to cytotoxic T cell-mediated clearing [36]. Another research showed encouraging potentiality for new clinical management decisions on the basis of epigenetic regulation targeting lncMALAT1, which can coordinate with the immune system[37]. More and more evidences has strongly supported that immune-related lncRNAs may be novel disease biomolecules for cancer clinic treatment and possess valuable prognostic significance for survival[38, 39]. Several immune-related lncRNA signatures have been explored in some tumors, such as bladder cancer, breast cancer, and colon cancer[40–42]. Nevertheless, the latent role of immune-correlated lncRNAs risk score model as a helpful predictive indicator needs further validated in cancer immune checkpoint therapy, especially in BRCA.

Here, our study assembled an immune-associated lncRNA signature and explored its predictive performance, as well as its role in immune cell infiltration and the assessment of responsiveness to immune checkpoint blockade treatment for BRCA patients. In our research, immune-associated lncRNAs were systematically identified through employing univariate Cox regression model as well as the biostatistics method. Subsequently, we employed LASSO algorithm analysis in lncRNA files derived from TCGA database, and final 13 significant immune-related lncRNAs were recognized. These 13 lncRNAs were included into developing the predictive risk score model. Subsequently, Kaplan–Meier curves, the timedependent ROC curves, and Cox regression analysis were employed to confirm the predictive performance of this immune-correlated lncRNAs risk score model, which can serve as an independent biomolecular indicator to forecast BRCA survival. Further validation was analyzed in both the internal testing group and combined cohort.

Subsequently, our pathway enrichment results suggested the latent impact of our immune-related lncRNA risk model on BRCA tumorigenesis and progression via endoderm development, endoderm formation, endoderm cell differentiation, laminin binding and so forth. Our results provide new evidence for strongly supporting that lncRNAs whose functions was still unclear may be novel biomarkers for predicting clinical outcomes in BRCA. Nonetheless, these findings require to be confirmed in further researches.

With the rise of immunotherapy, immune checkpoint blockade (ICB) treatment has markedly transformed anti-tumor immunopathological treatment[43]. Patients with breast cancer administrated monotherapy of ICB has obtained objective benefit, however, the prognosis of BRCA patients in the use of ICB as monotherapy was not better than traditional chemotherapy regimens[9]. Such biomolecules as immune checkpoint gene and tumor mutational burden cannot accurately forecast clinical outcomes from ICB treatment. Thus, exploiting biomolecular markers that can precisely forecast responsiveness to ICB is crucial to further advance precision immunotherapy[28, 44]. Several studies reported that lncRNAs associated with immune reaction could forecast responsiveness to clinical treatment[45, 46]. In this study, the association analyses shown that PDCD1, CD274, PDCD1LG2, CTLA-4, HAVCR2, and IDO1 were coexpressed. Besides, this immune-correlated lncRNA risk score was significantly correlated with the ICB treatment target genes (i.e., PDCD1). These findings indicated that this immune-related lncRNA risk score model may serve as a key part to measure the responsiveness to ICB treatment of BRCA patients. Recently, accumulating evidences have supported that numerous lncRNAs possess key roles in regulating immunity, such as immune cell infiltration, antigen presentation and so on[11, 12]. Here, our results shown that this immune-correlated lncRNAs risk model was markedly correlated with immune cell infiltration (i.e., B cells, CD4 + T cells, CD8 + T cells, dendritic cells and neutrophils etc.,) in BRCA, which indicated that as-constructed immune-correlated lncRNA risk score model might serve as a key role in immune cell infiltration in BRCA.

Compared with published researches that investigated the lncRNA prognostic performance in BRCA, the superiority of our research is that, as far as we know, this research is the first to exploit immune-related lncRNAs signature which may precisely predict responsiveness to ICB in BRCA.

Conclusion

By and large, these 13 immune-related lncRNAs risk scores model was been found significant correlated with BRCA prognosis, and it could serve as an independent prognostic biomolecular indicator to predict BRCA prognosis. Moreover, as-constructed immune-associated lncRNA signature was be observed to be significantly correlated to immune cell infiltration as well as responsiveness to ICB treatment in BRCA. Conclusively, this research provided a promising avenue to facilitate the individualized survival prediction and gauge responsiveness to ICB antitumor immunotherapy in BRCA, which may present valuable clinical applications in BRCA ICB immunotherapy. Nevertheless, our findings should be confirmed in future researches which explore BRCA occurrence and development mechanisms and the implication of these 13 immune-related lncRNAs.

Abbreviations

AUC: area under the curve

BRCA: breast cancer

CTLA-4: cytotoxic T-lymphocyte antigen 4

CI: confidence interval

CD274: Also known as PD-L1

DFS: Disease-free survival

GSEA: Gene set enrichment analysis

HR: hazard ratio

HAVCR2: Also known as TIM3

IDO1: indoleamine 2,3-dioxygenase 1

ICB: immune checkpoint blockade

LncRNAs: long non-coding RNAs

LASSO: least absolute shrinkage and selection operator

OS: overall survival

PD-1: Programmed Cell Death 1

PD-L1: Programmed Cell Death-Ligand 1

PD-L2: Programmed Cell Death-Ligand 2

PDCD1: Also known as PD-1

PDCD1LG2: Also known as PD-L2

ROC: receiver operating characteristic

TCGA: The Cancer Genome Atlas

TILs: tumor-infiltrating immune cells

TIM-3: T-cell immunoglobulin domain and mucin domain-containing molecule-3

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Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author contribution

Wen Huang conceived and designed the experiments. Qianhui Xu, and Yuxin Wang analyzed the data. Qianhui Xu wrote the manuscript. All authors read and approved the final manuscript.

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Table

Table 1: Multivariate Cox results of lncRNAs based on TCGA BRCA data.

id	coef	HR	HR.95L	HR.95H	pvalue
AC005332.6	0.022756	1.023017	1.000404	1.046142	0.046
AL359752.1	-0.20086	0.818027	0.633423	1.056431	0.123738
AL606834.1	-0.73265	0.480632	0.317527	0.727521	0.000532
AP001207.3	0.060771	1.062656	1.018321	1.108921	0.005191
AL358472.3	-0.31622	0.728897	0.507642	1.046586	0.086664
<i>ST7 - AS1</i>	-0.39027	0.676875	0.416899	1.098971	0.114496
LINC00668	0.019412	1.019601	1.004687	1.034737	0.009824
LINC01871	-0.25798	0.77261	0.642958	0.928406	0.005913
AP005131.2	-0.46171	0.630203	0.357934	1.10958	0.109665
AC110995.1	0.259619	1.296436	1.127503	1.490681	0.000268
PCAT18	-0.11071	0.895202	0.778816	1.028981	0.119251
AL161719.1	0.180171	1.197422	1.08536	1.321054	0.000326
<i>SP2 - AS1</i>	0.281635	1.325295	1.021514	1.719415	0.033989

Figures

Figure 1

Overall study design. Flow chart indicating the process used to select immune-lncRNAs included in the analysis.

Figure 2

Regression coefficient diagram based on LASSO regression. A) LASSO coefficient profiles of 19 immune-lncRNAs. A vertical line is drawn at the value chosen by 10-fold cross-validation. B) Ten-time cross-validation for tuning parameter selection in the lasso model. The vertical lines are plotted based on the optimal data according to the minimum criteria and 1-standard error criterion. The left vertical line represents the 18 lncRNAs finally identified. C) Forest plots showing the relationships of various lncRNA subsets with OS in training cohort. The unadjusted HRs are presented with 95% CIs. Differential lncRNAs expression of model lncRNA in TCGA database: D) AC005332.6; E) AC110995.1; F) AL161719.1; G) AL358472.3; H) AL359752.1; I) AL606834.1; J) AP005131.2; K) LINC01871; L) SP2-AS1; M) ST7-AS1. (all $P < 0.05$).

Figure 3

Verification of the lncRNA signature for predicting BRCA prognosis in the training cohort. A) Heatmap of the 13 immune-related lncRNAs expression in BRCA. The color from green to red shows a trend from low expression to high expression. B) Distribution of lncRNA risk score. C) The survival status and duration of BRCA cases. D) Kaplan–Meier curve showing OS in the low- and high-risk groups classified based on the median risk score. E) ROC analysis of the risk scores for overall survival prediction. The AUC was calculated for ROC curves, and sensitivity and specificity were calculated to assess score performance. F) Univariate Cox regression analyses of OS. G) Multivariate Cox regression analyses of OS.

Figure 4

Validation of lncRNA prognostic risk scores in the validation group. A–E) are validation cohort results that are consistent with the training group results (Figure 3). Cox regression results. F) Univariate results in the validation group. G) Multivariate results in the validation group. H) Risk score significantly increased for advanced stage cases in the entire group.

Figure 5

Confirmation of prognostic value of the lncRNA signature in BRCA patients. A-C) Areas under curves (AUCs) for predicting 1-, 3-, and 5-year survival with different clinical signatures. D) Nomogram was constructed by stage and risk signature for predicting survival of BRCA patients. ROC curves are used for evaluating the efficiency of the forest plot. E) GSEA delineation of the biological pathways related to the risk score values of the lncRNA model using the gene set “c5.go.v7.2.symbols.gmt[*gene ontology*]”.

Figure 6

Association between the immune-related long noncoding RNA (lncRNA) signature and tumor immune cell infiltration. A) correlation between this signature and B cells, B) correlation between this signature and CD4+T cells, C) correlation between this signature and CD8+T cells, D) correlation between this signature and Dendritic cells, E) correlation between this signature and Neutrophils.

Association between crucial immune checkpoint genes and our immune-related lncRNA signature. A) Correlation analyses of immune checkpoint inhibitors CD274, PDCD1, PDCD1LG2, CTLA4, HAVCR2, and IDO1 with our immune-related lncRNA signature. B) correlation between this signature and PDCD1.

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