A novel 17 apoptosis-related genes signature could predict overall survival for bladder cancer and its associations with immune infiltration

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ABSTRACT

Background: Apoptosis-related genes (ARGs) were used to develop a novel signature for forecasting overall survival (OS) and examining their relationships with immune infiltration in bladder cancer (BC).

Methods: Gene expression matrices as well as related clinical data were acquired for BC samples from online datasets. According to differentially expressed ARGs acquired from normal bladder tissues and cancer samples, functional enrichment analyses were conducted. With the assistance of LASSO and Cox regression analysis, a novel model was successfully established and evaluated by external and internal validations.

Results: Eventually, 17 ARGs (SLC5A6, GULP1, TAP1, MMP9, P4HB, FOXL2, CIDEC, EN2, NES, EPHA7, SUSD2, TMPRSS3, HOXB7, SATB1, MEST, PCDHGC3, ASPM) were utilized to construct the signature. Our constructed model was successfully established and evaluated by external and internal validations. A prognostic nomogram was also constructed based on TCGA dataset to predict OS prognosis in BC suffers. Besides, this ARG based model was markedly associated with clinical characteristics like tumor stage (P = 3.98e−06), race (P = 8.255e−06), N stage (P = 0.002), T stage (P = 3.679e−05) and M stage (P = 0.002). As for immune infiltration, our established model was significantly associated with seven tumor-infiltrating immune cells.

Conclusions: A prognostic signature was successfully developed by us according to 17 ARGs in BC using external and internal verifications, enabling clinicians to predict BC suffers' OS and promote specific individualization of patient care.

1. Introduction

Bladder cancer (BC), as the most recent data demonstrate, is one of the most prevalent malignant tumors in the urinary system that originates from the bladder urothelium [1, 2]. There are regional, racial, and gender differences in the incidence of BC, which can be seen in all ages. The high incidence age is 50–70 years old. Moreover, as the age increases, the incidence rate also gradually increases [3, 4]. BC carcinogenesis and progression are multi-factor, complex, and multi-step pathological processes. So, its particular pathophysiology is still unclear [5, 6]. Studies have demonstrated the significance of both internal genetic variables and external environmental influences. Various risk factors have been identified such as smoking, long-term exposure to industrial chemicals, chronic inflammation of the bladder, heredity and genes etc [7, 8]. For individuals with non-muscle-invasive bladder cancer (NMIBC), transurethral resection of the bladder tumor (TURBt) is currently the recommended first treatment, whereas chemotherapy is still the standard therapy for muscle-invasive bladder cancer (MIBC). However, these treatments still cannot sufficiently improve BC patients’ survival [9]. Hence, the development of novel therapies for BC patients is therefore urgently needed.

Over the decades, a growing number of treatments had been developed and applied in BC [10, 11]. Bacillus Calmette-Guerin, as an efficient method, was found to be a highly effective treatment among BC patients...
As immune checkpoint inhibitors, PD-1/PD-L1 inhibitors were designed to block molecules that influenced immune responses and decreased the strength of immune responses, ultimately affecting T cell activation [13, 14]. Cytotoxic T cell antigen, as another vital immune checkpoint inhibition, could mobilize the immune system against BC [15, 16]. There were several other methods to treat BC, including the usage of ganciclovir, rapamycin (mTOR) kinase inhibitors, cyclooxygenase-2 (COX-2) and interleukin-12 (IL-12) [17, 18, 19]. Recent studies also showed that various chemotherapy drugs may induce the apoptosis of tumor cells when applied to patients [20, 21]. Due to the BC patients’ individual needs and genetic instability, the search for different therapy methods was still ongoing.

With the help of bioinformatics analysis and the availability of many online databases, various useful data had be mined in cancers and there

Figure 1. Differentially expressed apoptosis-related genes (ARGs); (A) Heatmap of differentially expressed ARGs; (B) Volcano map of differentially expressed ARGs; (C) GO enrichment of 520 differently expressed ARGs; (D) KEGG pathway analysis of 520 differently expressed ARGs.
was an increasing application of riskscore models in tumor prognosis [22, 23]. Zhang et al. developed a four-glycolytic gene related model for forecasting BC suffers’ prognosis and its relationships with the cell cycle [24]. Tang et al. also established a nine BC-specific methylation site riskscore formula to evaluate these patients’ overall survival (OS) [25].

Currently, no signature for BC had been performed based on apoptosis-related genes (ARGs). Therefore, the present article was conducted to construct a prognostic signature with identified ARGs and explore its associations with immune infiltration in BC.

2. Materials and methods

2.1. ARGs related matrix acquired from public online databases

A matrix of gene expression and clinical data was derived from The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/) online website, containing 411 BC and 11 normal samples. Human ARGs were gathered from GSEA (http://www.gsea-msigdb.org/gsea/index.jsp) and displayed in Supplement Table S1. Under the cut-off values of false

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**Figure 2.** Construction a prognostic model index (riskscore) by means of univariate Cox regression analysis, LASSO Cox regression analysis and multivariate Cox regression analysis; (A) The forest plot of 56 differentially expressed ARGs were identified to be significantly related to OS of BC patients screened out by univariate Cox regression. Green points represent negative correlations, whereas red points represent positive correlations; (B–C) LASSO coefficients profiles of the prognostic ARGs; The partial likelihood deviance plot presented the minimum number corresponds to the covariates utilized for multivariate Cox analysis; (D) The forest plot of 17 differentially expressed ARGs were identified to be significantly related to OS of BC patients screened out by multivariate Cox regression.
discovery rate (FDR) < 0.05 and |log2 fold change (FC)| ≥ 1, we found differentially expressed ARGs among normal bladder and BC samples. Besides, the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) GSE13507 dataset was applied as the external validation group.

2.2. Functional enrichment analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were carried out, according to differently expressed ARGs, to identify the key biological characteristics of these genes. The “enrichplot” and “ggplot2” R packages were utilized to visualize molecular function (MF), biological processes (BP), and cellular components (CC) under the consideration of P < 0.05. These two R packages were also applied to present the top 30 enriched KEGG terms, under the consideration of P < 0.05.

2.3. A prognostic signature construction according to ARGs for BC

Univariate Cox regression analysis was utilized to identify prognostic ARGs. In order to prevent overfitting, LASSO analysis was then applied. Multifactor analysis was applied to obtain every gene's weight and their correlation coefficients. As a result, the formula of our established signature was displayed as following: Riskscore = \( \sum_{i=1}^{n} \exp i \beta \).

2.4. Evaluation of the established signature based on ARGs for BC

We evaluated our constructed signature internally and externally in this article. The entire TCGA-BC dataset was randomly split into two subgroups for internal validation, containing internal test 1 group as well as test 2 group. As for external validation, the TCGA dataset was regarded as the training group and the GSE13507 dataset was regarded as the external testing group. Based on their median riskscore, BC sufferers were classified into high-risk and low-risk subgroups. The Kaplan-Meier (K-M) analysis was applied to examine the survival differences among these two subgroups. With the use of the R “survivalROC” package, ROC curves and their area under the curve (AUC) values were used to assess the sensitivity and specificity of our established signature.

2.5. Cox regression analyses of univariate and multivariate

We conducted univariate and multifactor analysis to see if our constructed 17 ARGs based signature might function as an independent predictive biomarker for OS. Seven clinicopathological factors (N stage, age, T stage, gender, stage, race, and M stage) associated with our established riskscore were utilized to complete this analysis, under the consideration of P < 0.05 to be significant.

2.6. Nomogram construction and evaluation

A novel nomogram was conducted to forecast the likelihood of OS prognosis for BC patients, according to seven clinical factors (N stage, age, T stage, gender, stage, race, and M stage) associated with our established riskscore. The prognostic nomogram's accuracy was determined using the C-index as well as ROC curves. Additionally, calibration curves were used to evaluate the prognostic nomogram's expected and actual findings.

2.7. Application of clinical features in testing our established signature as well as tumor-infiltrating immune cells (TIICs) estimation

We included gender, age, grade, stage, T stage, race, M stage, and N stage to further test the precision of our established model for predicting OS in BC suffers. As previously described, we calculated the expressions of TIICs in all BC samples [26] and further explored whether or not these TIICs were highly involved with high- and low-risk subgroups based on the established model, when P-values below 0.05.

3. Results

3.1. Differentially expressed apoptosis-related genes in BC

An overview of our study's workflow was displayed in Supplement Figure S1. A matrix of gene expression and clinical data was derived from TCGA online website, containing 411 BC and 11 normal samples. Based on the “limma” package, 520 differentially expressed ARGs were initially screened out. Figure 1A and Figure 1B respectively showed these genes' heatmap as well as volcano plot, under the threshold of FDR < 0.05 and |log2 FC| > 1.

3.2. Functional annotations and pathway enrichment

Based on 520 differentially expressed ARGs, GO and KEGG enrichment analyses were carried out to identify the key biological characteristics of these genes, and the results were summarized in Supplement Table S2 and Table S3. The significance of these genes' heatmap as well as volcano plot, under the threshold of FDR < 0.05 and |log2 FC| > 1.

Table 1. Multivariate Cox regression analysis to filter key ARGs for BC patients.

| Gene     | Coefficients | HR   | HR.95L | HR.95H | P value |
|----------|--------------|------|--------|--------|---------|
| SLC5A6   | 0.011386574  | 1.011451647 | 0.998272753 | 1.024804526 | 0.088826719 |
| GULF1    | 0.260802756  | 1.297971623 | 1.06889252 | 1.576145685 | 0.00877968 |
| TAP1     | -0.007291208 | 0.992735308 | 0.987282872 | 0.998217856 | 0.009466041 |
| MPP9     | 0.000199264  | 1.000199284 | 0.999990639 | 1.000407973 | 0.001603245 |
| P4HB     | 0.00193458   | 1.00194056  | 0.999990639 | 1.000407973 | 0.001603245 |
| FOXL2    | 0.1289067    | 1.13756748  | 1.048511899 | 1.234175028 | 0.001940534 |
| CIDEC    | 0.0997246    | 1.108466703 | 1.104713577 | 1.165789798 | 0.000270452 |
| EN2      | -0.551675191 | 0.57984118  | 0.386538645 | 0.858278231 | 0.060708776 |
| NIS      | 0.017617532  | 1.017773636 | 1.000290941 | 1.035561887 | 0.046275679 |
| EPHA7    | 0.199638616  | 1.222091442 | 1.102429736 | 1.352237512 | 0.000127344 |
| SUSD2    | 0.032477302  | 1.030310464 | 1.007418106 | 1.09529293 | 0.011168349 |
| TMRP5S3  | 0.03562227   | 1.036254362 | 1.003990646 | 1.069619014 | 0.027720597 |
| HOX17    | -0.015497461 | 0.984622007 | 0.964193242 | 1.005483606 | 0.147409444 |
| SATB1    | -0.128122067 | 0.879745985 | 0.807641316 | 0.95828802 | 0.303319476 |
| MEST     | 0.00354523   | 1.003551522 | 0.999256925 | 1.007864576 | 0.105181088 |
| PCDHGC3  | 0.091854627  | 1.096205452 | 1.017064652 | 1.181504431 | 0.016281673 |
| ASPM     | 0.082039691  | 1.085498894 | 1.102123497 | 1.153806796 | 0.008418366 |

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Figure 3. Evaluation and external verification of 17 ARGs established signature; (A) Kaplan-Meier survival curves of OS among BC patients from high- and low-risk groups stratified by 17 ARGs signature in the TCGA dataset; (B–C) 3-year and 5-year ROC in the TCGA dataset; (D) Kaplan-Meier survival curves of OS among BC patients from high- and low-risk groups stratified by 17 ARGs signature in the GSE13507 dataset; (E–F) 3-year and 5-year ROC in the GSE13507 dataset; (G–H) The distribution of risk scores for samples, patients' survival status and heatmap of 17 different ARGs in the TCGA and the GEO datasets.
(BP) of GO analysis were displayed as follows: regulation of neuron death, neuron apoptotic process, regulation of apoptotic signaling pathway, and so on. The significantly enriched cellular components (CC) of GO analysis were shown below: neuronal cell body, chromosomal region, condensed chromosome and so on. The significantly enriched molecular functions (MF) of GO analysis were presented as follows: cytokine receptor binding, receptor ligand activity, scavenger receptor activity, and so on (Figure 1C). In addition, the significantly enriched KEGG terms were Human cytomegalovirus infection, MAPK signaling pathway, Human T-cell leukemia virus 1 infection, Cellular senescence, Hepatitis B and so on (Figure 1D). All of these indicated the significantly functional annotations and pathway enrichment in BC, according to the differently expressed ARGs.

3.3. A prognostic signature construction (riskscore) according to ARGs for BC

With the help of univariate Cox, LASSO and multivariate Cox regression analysis, a novel model (riskscore) was successfully established. Univariate analysis showed that 520 differentially expressed ARGs were filtered to 56 differentially expressed ARGs (Figure 2A). In order to prevent overfitting, the LASSO analysis was then applied and 28 genes were obtained (Figure 2B-2C). According to multivariate Cox regression analysis, 17 ARGs (SLCSA6, GULP1, TAP1, MMP9, P4HB, FOXL2, CIDEC, EN2, NES, EPH7, SUSD2, TMPRSS3, HOXB7, SATB1, MEST, PCDHGC3, ASPM) were finally identified to develop the model (Figure 2D, Table 1). Hence, the riskscore formula was calculated and shown as following:

\[
\text{Riskscore} = (0.01139 \times \text{Exp SLC5A6}) + (0.26809 \times \text{Exp GULP1}) + (-0.00729 \times \text{Exp TAP1}) + (0.00020 \times \text{Exp MMP9}) + (0.00109 \times \text{Exp P4HB}) + (0.12889 \times \text{Exp FOXL2}) + (0.09972 \times \text{Exp CIDEC}) + (-0.55168 \times \text{Exp EN2}) + (0.01761 \times \text{Exp NES}) + (0.19964 \times \text{Exp EPH7}) + (0.03248 \times \text{Exp SUSD2}) + (0.03560 \times \text{Exp TMPRSS3}) + (-0.01550 \times \text{Exp HOXB7}) + (-0.12812 \times \text{Exp SATB1}) + (0.03355 \times \text{Exp MEST}) + (0.09185 \times \text{Exp PCDHG3}) + (0.08204 \times \text{Exp ASPM})
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3.4. Internal and external evaluation of our established signature (riskscore) for BC

We evaluated our constructed signature internally and externally in this article. As for external validation, the TCGA dataset was regarded as the training group and the GSE13507 dataset was regarded as the external testing group. The entire TCGA-BC dataset was randomly split into two subgroups for internal validation, containing internal test 1 group as well as test 2 group. Based on their median riskscore, BC suffers into two subgroups for internal validation, containing internal test 1 group and the GSE13507 dataset was regarded as the testing group. Similarly, internal validation indicates that the model is built with credibility (Figure 4). The 3-year and 5-year AUC for verification was displayed in Table 2. All in all, internal and external validations indicated the stabilities and credibility of our established signature based on ARGs in BC.

3.5. Our established signature would function as an independent predictive biomarker for OS in BC

We conducted univariate and multifactor analysis to see if our constructed 17 ARGs based signature might function as an independent predictive biomarker for OS. Seven clinicopathological factors (age, gender, stage, race, N stage, T stage, and M stage) associated with our established riskscore were utilized to complete this analysis. Both P values of our established riskscore in univariate and multifactor analyses were below 0.05 (Figure 5 and Table 3), showing that our established signature would function as an independent predictive biomarker for OS in BC.

3.6. Nomogram construction and evaluation according to clinical factors and our established signature

A novel nomogram was conducted to forecast the likelihood of OS prognosis for BC patients, according to seven clinical factors (age, gender, stage, race, N stage, T stage, and M stage) associated with our established riskscore (Figure 6A). The prognostic nomogram’s accuracy was determined using the C-index and their one-, three-, five-year AUCs of ROC curves, with the values of 0.732, 0.776, 0.780, 0.781, respectively (Figure 6B–D, Table 4). Additionally, calibration curves were used to evaluate the prognostic nomogram’s expected and actual findings (Figure 6E-6G).

3.7. Relationships among our established signature and clinicopathologic factors

The outcomes of us shed light on that the 17 ARGs based signature was strongly linked to race (P = 8.255e–06), M stage (P = 0.002), T stage (P = 3.679e–05), tumor stage (P = 3.98e–06), and N stage (P = 0.002) (Figure 7, Table 5). All of these indicated the significant relationships among our established signature and clinicopathologic factors.

3.8. Application of clinical features in testing our established signature

We included gender, age, grade, stage, T stage, race, M stage, and N stage to further test the precision of our established model for predicting OS in BC suffers. Our established signature was proven to be able to predict OS in Female (P < 0.001), Male (P < 0.001), Age > 65 (P < 0.001), Age ≤ 65 (P ≤ 0.001), M0 (P < 0.001), High Grade (P < 0.001), N0 (P < 0.001), N1-N3 (P < 0.001), WHITE (P < 0.001), Stage III–IV (P < 0.001), Stage I–II (P = 0.004), T3–4 stage (P < 0.001), T1–2 stage (P = 0.12), Low Grade (P = 1.000), M1 (P = 0.128), ASIAN (P = 0.311), AFRICAN (P = 0.081) had no significant difference in statistics, with P value ≥ 0.05 (Figure 8). All of these indicated that our established signature also could predict OS in several clinicopathologic parameters.

3.9. Tumor-infiltrating immune cells (TIICs) estimation in our established signature

The outcomes indicated that 7 out of 22 TIICs (including T cells CD4 memory activated, Macrophages M0, T cells CD8, Macrophages M2, Mast cells activated, T cells follicular helper, Neutrophils) were firmly
Figure 4. Internal verification of 17 ARGs established signature; (A) Kaplan-Meier survival curves of OS among BC patients from high- and low-risk groups stratified by 17 ARGs signature in the internal verification dataset 1 (test 1); (B–C) 3-year and 5-year ROC in test 1; (D) Kaplan-Meier survival curves of OS among BC patients from high- and low-risk groups stratified by 17 ARGs signature in the internal verification dataset 2 (test 2); (E–F) 3-year and 5-year ROC in test 2; (G–H) The distribution of risk scores for samples, patients’ survival status and heatmap of 17 different ARGs in test 1 and test 2.
associated with our established signature (all \( P < 0.05 \); Figure 9A-9G). All 22 TIICs in high- and low-risk subgroups classified by our established signature were detailed by the radar chart in Figure 9H. All of these indicated the significant relationships among our established signature and TIICs.

### 4. Discussion

As a worldwide disease, BC ranks the fourth most commonly diagnosed cancer and the eighth mostly estimated death in men in USA, 2022 [27]. As reported, there are about 430,000 new cases diagnosed globally each year. For women, the global standardized incidence rate (SIR) is 2.4
(per 100,000 people/years), while for males, it is 9.6 (per 100,000 people/years) [28]. Similar to that of Europe, its global SIR is 4.3 for female and 20.2 for male [29, 30]. Of all European countries, the highest SIR is in Greece with 4.5 in women and 40.4 in men, while the lowest is in Austria with 3.0 in women and 9.9 in men [31].

In the present article, we thoroughly analyzed the TCGA BC dataset's gene expression matrix of ARGs and developed a predictive signature for forecasting BC patients' OS prognosis. 520 differentially expressed ARGs were firstly identified among 411 BC and 11 normal samples. GO and KEGG enrichment analyses were carried out to identify the key biological characteristics of these genes. With the help of LASSO and Cox regression analysis, a novel signature containing 17 key ARGs (SLC5A6, GULP1, TAP1, MMP9, P4HB, FOXL2, CIDEC, EN2, NES, EPHA7, SUSD2, TMPRSS3, HOXB7, SATB1, MEST, PCDHGC3, ASPM) was successfully constructed and validated by internal and external datasets. Our constructed signature significantly distinguished high-risk from low-risk BC patients of OS by internal and external validations and was also proven to be able to serve as an independent prognostic biomarker. Furthermore, a prognostic nomogram was also constructed based on TCGA dataset to predict OS prognosis in BC suffers. Besides, this ARG based model was markedly associated with clinical characteristics like tumor stage, race, N stage, T stage and M stage. As for immune infiltration, our established

Figure 7. Association between clinicopathologic characteristics (race, stage, T, N, M) and our established riskscore; (A) Distribution of riskscores in race; (B) Distribution of riskscores in stage; (C) Distribution of riskscores in T stage; (D) Distribution of riskscores in N stage; (D) Distribution of riskscores in M stage.

Table 5. Clinical correlation analysis between these 17 prognostic ARGs, our established riskscore and clinical features.

| id  | Age     | Gender | Race  | Stage | T     | M     | N     |
|-----|---------|--------|-------|-------|-------|-------|-------|
| SLCSA6 | 52.229 (0.350) | −0.074 (0.941) | 5.953 (0.051) | −2.267 (0.024) | −1.922 (0.055) | 2.816 (0.245) | 10.152 (0.006) |
| GULP1 | 52.444 (0.342) | 0.09 (0.928) | 10.124 (0.006) | −3.876 (1.28e−04) | −3.223 (0.001) | 2.52 (0.284) | 6.428 (0.040) |
| TAP1 | 38.31 (0.865) | 0.134 (0.893) | 17.816 (1.353e−04) | −2.267 (0.024) | −1.922 (0.055) | 3.256 (0.196) | 3.223 (0.001) |
| MMP9 | 48.183 (0.506) | 0.916 (0.360) | 18.667 (8.843e−05) | −1.156 (0.249) | −1.191 (0.235) | 22.799 (1.12e−05) | 3.187 (0.203) |
| P4HB | 44.294 (0.638) | −0.541 (0.589) | 1.505 (0.471) | 0.089 (0.929) | −0.216 (0.829) | 3.075 (0.215) | 1.415 (0.493) |
| FOXL2 | 67.706 (0.039) | 1.49 (0.139) | 4.279 (0.118) | −1.715 (0.087) | −1.179 (0.239) | 0.794 (0.672) | 3.256 (0.196) |
| CIDEC | 42.556 (0.730) | 1.4 (0.165) | 4.857 (0.088) | −2.232 (0.026) | −2.422 (0.016) | 10.308 (0.006) | 1.504 (0.472) |
| EN2 | 44.948 (0.638) | 1.505 (0.471) | 0.089 (0.929) | −0.216 (0.829) | 3.075 (0.215) | 1.415 (0.493) | 3.256 (0.196) |
| NES | 47.607 (0.530) | 0.006 (0.995) | 10.673 (0.005) | −3.318 (0.001) | −1.88 (0.061) | 8.424 (0.015) | 0.599 (0.741) |
| EPHA7 | 53.195 (0.316) | −1.755 (0.081) | 0.891 (0.641) | −0.788 (0.432) | −0.489 (0.626) | 2.99 (0.224) | 4.158 (0.125) |
| SUSD2 | 51.76 (0.367) | 0.995 (0.322) | 8.18 (1.01e−04) | −3.452 (6.33e−04) | −2.764 (0.006) | 13.019 (0.001) | 8.96 (0.011) |
| TMPRSS3 | 45.092 (0.632) | 1.204 (0.231) | 1.387 (0.144) | −0.614 (0.540) | −0.944 (0.346) | 1.634 (0.442) | 8.872 (0.012) |
| HOXB7 | 56.951 (0.203) | −2.347 (0.020) | 11.965 (0.003) | 3.22 (0.002) | 3.135 (0.002) | 5.213 (0.074) | 1.518 (0.468) |
| SATB1 | 50.696 (0.406) | −2.646 (0.009) | 12.522 (0.002) | 1.597 (0.112) | 1.95 (0.053) | 18.976 (7.575e−05) | 1.458 (0.482) |
| MEST | 37.952 (0.874) | −0.376 (0.707) | 0.373 (0.830) | −1.478 (0.141) | −0.886 (0.376) | 2.975 (0.354) | 7.21 (0.027) |
| PCDHGC3 | 56.277 (0.221) | −0.719 (0.473) | 3.068 (0.216) | −1.387 (0.048) | −1.509 (0.123) | 0.67 (0.967) | 3.236 (0.198) |
| ASPM | 57.006 (0.182) | −1.721 (0.086) | 19.077 (7.204e−05) | −1.25 (0.212) | −0.956 (0.340) | 0.495 (0.781) | 0.264 (0.876) |
| riskScore | 64.185 (0.071) | 1.955 (0.054) | 23.409 (8.25e−06) | −4.698 (3.98e−06) | −4.193 (3.679e−05) | 12.686 (0.002) | 12.371 (0.002) |
model was significantly associated with seven tumor-infiltrating immune cells, indicating the significant relationships among our established signature and TIICs. Taken together, a prognostic signature was successfully developed by us according to 17 ARGs in BC using external and internal verifications, enabling clinicians to predict BC suffers’ OS and promote specific individualization of patient care.

Currently, a growing number of studies had built prediction models in BC by TCGA data mining. For example, Tang et al. established a nine BC-specific methylation site riskscore formula to evaluate these BC patients’ OS prognosis, exerting a prognosis predicting value [25]. Wang et al. developed a novel signature based on pyroptosis-related lncRNAs in BC, playing vital roles in cancer immunology and serving as potential therapeutic targets [32]. MAN1B1 was also revealed as a new biomarker for BC and strongly linked with immune cells infiltration degree [33]. As for this study, it was the first time for us to build a predictive signature for BC based on ARGs and to explore the correlations between our established signature and clinicopathological features. Besides, we also validated this signature internally and externally.

Nomogram, as a reliable tool, had long been applied in a predictive statistical model quantifying the risks of a clinical event by an intuitive graph and a growing number of researches had successfully used nomograms to quantify certain cancers risks in combination of key prognostic factors [34, 35, 36]. In this article, a novel nomogram was conducted to forecast the likelihood of OS prognosis for BC patients, according to seven clinical factors (N stage, age, T stage, gender, stage, race, and M stage) associated with our established riskscore. The prognostic nomogram’s accuracy was determined using the C-index and their one-, three-, five-year AUCs of ROC curves, with the values of 0.732, 0.776, 0.780, 0.781, respectively. Its AUC values as well as C-index showed an excellent prognostic ability in predicting OS. Calibration plot

![Figure 8. Clinicopathological parameters stratified by our established riskscore for OS; (A) Age>65 stratified by riskscore for OS; (B) Age ≤ 65 stratified by riskscore for OS; (C) gender Female stratified by riskscore for OS; (D) gender Male stratified by riskscore for OS; (E) High grade stratified by riskscore for OS; (F) M0 stratified by riskscore for OS; (G) N0 stratified by riskscore for OS; (H) N1–3 stratified by riskscore for OS; (I) Race White stratified by riskscore for OS; (J) Stage I–II stratified by riskscore for OS; (K) Stage III–IV stratified by riskscore for OS; (L) T1–2 stratified by riskscore for OS; (M) T3–4 stratified by riskscore for OS.](image-url)
also displayed the satisfactory conformity between the predicted and actual values. Additionally, calibration curves were used to evaluate the prognostic nomogram's expected and actual findings.

As for immune infiltration, it had been reported by various researches that the composition and function of TIICs could have latent prognostic value and vary with the host immune status [37, 38, 39]. Moreover, immune infiltration could be effectively targeted by immunotherapy and significantly linked to cancer patients' clinical outcomes [40, 41]. In our research, we further evaluated the associations among TIICs and our established signature in BC. The outcomes indicated that 7 out of 22 TIICs (including T cells CD4 memory activated, Macrophages M0, T cells CD8, Macrophages M2, Mast cells activated, T cells follicular helper, Neutrophils) were firmly associated with our established signature. All of these indicated the significant relationships among our established signature and TIICs.

As for the relationships among our established signature and clinicopathologic factors, the outcomes of us shed light on that the 17 ARGs based signature was strongly linked to race, M stage, T stage, tumor stage, N stage and was able to predict OS in Male, Female, Age >65, Age >65, High Grade, M0, N0, N1–3, Stage III-IV, Stage I–II, WHITE, T3–4 stage, T1–2 stage. All of these indicated a potentially clinical application of our established signature. In a word, we developed a 17 ARGs based model that could independently forecast the OS for BC, indicating that the usage of targeted ARGs therapy might be a promising future therapeutic approach for the treatment of BC. Further studies were required to explore the involved mechanisms of ARGs and provided new suggestions for BC therapies.

This article’s strength was that we firstly mined the potential roles and functions of ARGs in BC. Furthermore, this model was constructed and evaluated by internal and external validations of one external GSE13507 validation dataset and two internal TCGA test 1–2 groups, increasing the accuracy of our signature. Several shortcomings still existed in this study.

Firstly, the 17 ARGs for establishing our signature have not been experimentally verified and mined for their expression or roles in BC. Secondly, more data in the real world was needed to verify our established signature for future clinical applications in BC.

5. Conclusions

All in all, our results successfully singled out 17 ARGs containing SLC5A6, GULP1, TAP1, MMP9, P4HB, FOXL2, CIDEC, EN2, NES, EPHA7, SUSD2, TMPRSS3, HOXB7, SATB1, MEST, PCDHGC3 and ASPM as a robust prognostic model in BC using internal and external verifications, enabling clinicians to predict BC suffers’ OS and promote specific individualization of patient care. Additionally, the ARG-based signature we had developed might be used as an independent predictive biomarker for OS and was strongly correlated with immune infiltration in BC. More data in the real world was needed to verify our established signature for future clinical applications in BC.

Declarations

Author contribution statement

Yi Wang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Hong Cheng: Analyzed and interpreted the data; Wrote the paper.
Tengyue Zeng: Performed the experiments; Analyzed and interpreted the data.
Shuqiu Chen: Contributed reagents, materials, analysis tools or data.
Qianwei Xing: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Bingye Zhu: Conceived and designed the experiments; Analyzed and interpreted the data.

Figure 9. Correlations of our established 17 apoptosis-related riskscore signature and tumor-infiltrating immune cells (TIICs); (A) Macrophages M0; (B) Macrophages M2; (C) Mast cells activated; (D) Neutrophils; (E) T cells CD4 memory activated; (F) T cells CD8; (G) T cells follicular helper; (H) Radar chart.
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Data availability statement
Data associated with this study has been deposited at TCGA-BLCA and GEO under the accession number GSE13507.

Declaration of interest
The authors declare no conflict of interest.

Additional information
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