Original Article

Development of novel gene signatures for the risk stratification of prognosis and diagnostic prediction of osteosarcoma patients using bioinformatics analysis

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Background: Osteosarcoma (OS) is a common malignant bone cancer in children and teenagers that originates from osteoblast cells. Although many biomarkers have been reported in OS, they have not improved the prognosis of this disease. This study sought to identify effective biomarkers for the early diagnosis and prognosis of OS using a comprehensive bioinformatics analysis.

Methods: OS-associated microRNAs (miRNAs) were screened in the Human microRNA Disease Database (HMDD). The differentially expressed genes (DEGs) related to OS were screened using 3 data sets (GSE16088, GSE36001, and GSE56001) from the Gene Expression Omnibus (GEO) database. By comparing the targets of these miRNAs with DEGs in response to OS, we identified OS-associated candidate genes. The gene expression and clinical data of 96 OS samples with complete clinical information was downloaded from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) database. Comprehensive bioinformatics analyses, including univariate, multivariate Cox, and Kaplan-Meier (KM) analyses were conducted based on these data to identify the prognostic genes and construct prognostic signature for OS survival and recurrence. Logistic regression analysis was performed based on the GSE42352 data set (including 103 OS and 15 normal samples) to develop a diagnostic model for OS.

Results: By comparing the DEGs and predicted targets of the 28 OS survival-associated miRNAs, we identified 267 OS-associated candidate genes. Additionally, 14 genes were found to be significantly associated with the survival of OS patients. Finally, 3 genes [i.e., *signal transducer and activators of transcription factor 4* (*STAT4*), *heat shock protein family E member 1* (*HSPE1*), and *actin-related protein 2/3 complex subunit 5* (*ARPC5*)] were integrated into a prognostic index. The 3-gene signature was an independent factor for OS survival [hazard ratio (HR) =1.699; P<0.001] and recurrence (HR =2.532; P=0.004) and was found to have an excellent predictive performance [area under the receiver operating characteristic (ROC) curve (AUC) >0.7]. Additionally, 2 genes (i.e., *STAT4* and *HSPE1*) were identified to be associated with OS diagnosis (P<0.05). This 2-gene diagnostic signature for OS presented a good discriminative power (AUC =0.981) and the error between the predicted and actual value was 0.029.

Conclusions: We constructed a 3-gene prognostic signature and a 2-gene diagnostic signature that have the potential to assist in prognosis predicting and diagnosis of OS in clinic.

Keywords: Prognostic signature; diagnosis; recurrence; osteosarcoma (OS); biomarker

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Introduction

Osteosarcoma (OS) is a common malignant bone cancer in children and teenagers that originates from osteoblast cells. The majority of patients with early stage OS and localized tumors can be cured; however, the 5-year survival rate for OS patients with metastasis or recurrence is extremely low at 14% (1). About 30% of patients relapse within 2 years (2). Additionally, 80–90% of OS patients are diagnosed with a high grade, which poses a further significant challenge for OS treatment (3). Currently, biomarker identification has been applied in the individualized management of tumor patients, such as in the direction of therapy and prognosis. Thus, novel biomarkers for the early diagnosis and prognosis of OS urgently need to be identified to improve the management of OS.

Numerous factors have been reported to contribute to the progression of OS, including gender, age, and familial and genetic factors (4). Additionally, there is accumulating evidence that genetic factors have important implications in OS pathogenesis (5-7). Various genes are dysregulated and have been identified as promising biomarkers for detection or prognosis. For example, *TGF*-β (8,9) was recognized as a promising diagnostic marker in OS and it presented a higher level in serum of OS patients compared with healthy individuals. *Ezrin* (10,11) was identified as a diagnostic and prognostic marker in OS. It was up-regulated in high-grade OS patients and points to a worse prognosis. Moreover, *Cyclin E1* (12), *MMP-9* (13), *HIF-1* (14) and *APE1* (15) were identified as prognostic markers of OS, and their high expression were correlated with adverse prognosis. In addition, *high-mobility group box 2* was found to be upregulated in OS and negatively associated with the survival of OS patients (16). Xi *et al.* demonstrated that the upregulation of *Transducin (beta)-like 1 X-linked receptor 1* promotes the initiation and recurrence of OS (17). Recently, using high-throughput gene expression databases such as the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), and the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) databases, researchers have detected various molecular signatures associated with OS prognosis. For example, Zhang *et al.* identified 2 metastasis- and recurrence-related genes in the GEO database (18). Although many biomarkers have been reported in OS, they have not improved the prognosis of this disease, which possibly due to the researches being flawed, too heterogeneous or lacking valid evaluation (19). Development of effective markers for diagnosis, prognosis and predicting recurrence of OS patients, may prove a much-needed strategy for early diagnosis and develop promising therapeutic targets. Thus, in the present study, we first screened out OS-associated microRNAs (miRNAs) in the Human microRNA Disease Database (HMDD). By comparing the targets of these miRNAs with the differentially expressed genes (DEGs) in response to OS, we identified OS-associated candidate genes. After conducting a comprehensive bioinformatics analysis, including univariate and multivariate Cox, and Kaplan-Meier (KM) analyses, we identified 3 prognosis-related genes in OS. The 3 genes were integrated into a prognostic index (risk-score model) for OS survival and recurrence stratification. Next, 2 of the 3 genes were found to be associated with OS diagnosis and incorporated into a logistics model for diagnostic prediction. We present the following article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1706/rc).

Methods

Research design

This study screened prognosis-related genes and constructed gene signatures for early diagnosis and prognostic prediction of OS based on the data obtained from public databases using a comprehensive bioinformatics analysis.

Selection of potential prognosis-related genes in OS

The HMDD, an online public repository, contains information about the miRNAs related to various human diseases. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). In this study, 180 miRNAs related to OS and 67 miRNAs associated with the prognosis of OS were downloaded from the HMDD. A Venn diagram was used to compare the 180 miRNAs and the 67 miRNAs. Ultimately, 28 miRNA hub genes associated with prognosis of OS were identified. The target genes of the 28 miRNAs were predicted by TargetScan and miRDB. The DEGs related to OS were screened using 3 data sets from the GEO repository; that is, GSE16088 (which comprised 9 normal and 14 tumor samples), GSE36001 (which comprised 6 normal and 19 tumor samples), and GSE56001 (which comprised 6 normal and 6 tumor samples). The DEGs in each data set were identified using Limma package in R and integrated using the robust rank aggregation (RRA) method. Genes that had a |$\log_2$fold change (FC)| ≥0.5 and a P value <0.05 were recognized as the DEGs. The potential prognosis-related OS genes were identified by comparing the DEGs with the predicted target genes of the 28 miRNAs from TargetScan and miRDB.

Construction of a prognosis signature for OS survival and recurrence

The TARGET database was used to download the gene expression and clinical data for OS. There were 96 samples with complete clinical information for OS. A univariate Cox analysis was performed to detect the prognosis-associated genes. Next, the samples were divided into two groups (high- and low-expression groups) according to the median expression of the prognosis-associated gene. KM curves were plotted to analyze the prognostic value of the prognosis-associated genes. The proportional hazard (PH) assumption was assessed by calculating Pearson's coefficients to determine whether the effect of the factor (here is the expression of the genes) on survival was independent of time (20). Genes that met the PH assumption were analyzed by forward and backward stepwise multivariate Cox regression, and 3 genes were ultimately identified.

Evaluation of the gene signature

Risk scores were calculated based on the expression of the core genes that met these criteria. The optimal cutoff of risk score was calculated using X-tile software, and the OS samples were then stratified into high- and low-risk groups. The "survivalROC" package in R was installed to plot the receiver operating characteristic (ROC) curves. The areas under the ROC curves (AUCs) were calculated to evaluate the prognostic value of the risk score for 3-, 5-, 10-, and 15-year survival. Univariate and multivariate Cox analyses were performed to examine the prognostic value of the risk score and X-tile classification. A nomogram was established by integrating the genes. The ROC and calibration curves were plotted to analyze the predictive performance of the nomogram. The gene expression and survival information of 53 OS samples in GSE21257 data set was downloaded to validate the nomogram.

Gene set enrichment analysis (GSEA)

To explore the biological function of the nomogram model, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were analyzed using the GSEA method in the TARGET database. When the P value was <0.05, a pathway was considered significantly enriched.

Construction of a signature for OS diagnosis

The GSE42352 data set (which comprised 15 normal and 103 tumor samples) was used to determine whether the 3 prognosis-associated genes could be also applied for OS diagnosis. A logistic regression was conducted to assess the diagnostic value of the genes. The goodness of fit for the logistic model was evaluated using the Akaike information criterion (AIC); a lower AIC value indicated a better fit. A nomogram was constructed for diagnosis, and the predictive performance was evaluated by the Hosmer-Lemeshow test. A principal component analysis (PCA) with a 95% confidence ellipse was performed to examine the discriminative performance of the diagnosis model. The GSE19276 (which comprised 5 normal and 44 tumor samples) and GSE36001 (which comprised 6 normal and 19 tumor samples) data sets were used to validate the diagnosis signature. The accuracy and diagnostic utility of the diagnosis signature were evaluated based upon the AUC.

Statistical analysis

KM analysis was performed for survival analysis with two-sided log-rank tests for comparison. Univariate and multivariate Cox analyses were performed to examine the prognostic value of the prognostic gene signature. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. A logistic regression was conducted to assess the diagnostic value of the genes and establish the diagnostic model. The accuracy and prognostic/diagnostic utility of the prognostic/diagnostic signatures were evaluated based upon the AUC as calculated by the "survivalROC" package in R. The value of AUC ranges from 0.5 to 1. The closer AUC is to 1.0, the higher the accuracy of the detection prognostic/diagnostic signature is. Statistical analyses were conducted in R version 4.0.4 (The R Foundation for Statistical Computing, Vienna, Austria) and SPSS 22.0 (IBM Corp., Armonk, NY, USA). A P value <0.05 was considered significant.

Results

Screening for OS-associated genes

We downloaded 180 miRNAs related to OS and

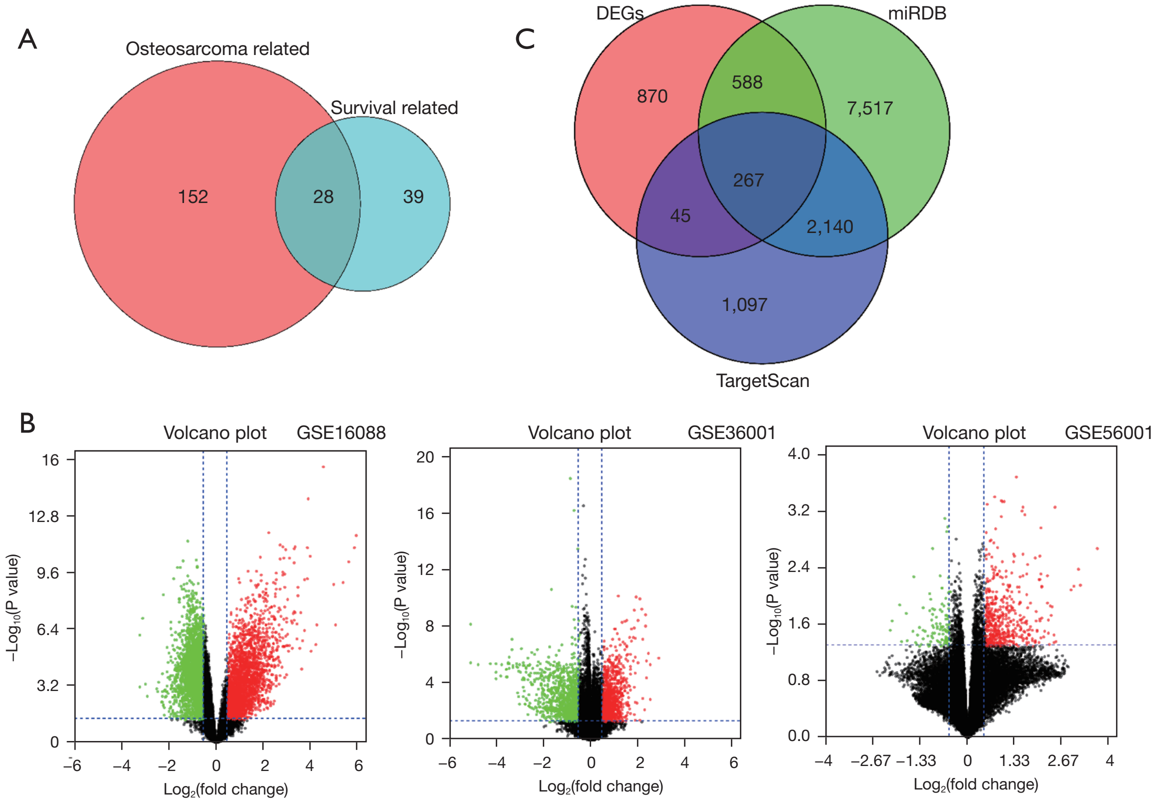

Figure 1 Identifying OS-associated genes. (A) Twenty-eight shared miRNAs were identified by comparing 180 miRNAs related to OS and 67 miRNAs related to survival in the HMDD database. (B) DEGs were identified between the OS and normal samples in GSE16088, GSE36001, and GSE56001 data sets (|log$_2$FC| ≥0.5 and P value <0.05). (C) Target genes of the 28 miRNAs were predicted by miRDB and TargetScan. A total of 267 OS-associated candidate genes were identified by comparing the target genes and DEGs. DEGs, differentially expressed genes; OS, osteosarcoma; miRNAs, microRNAs; HMDD, Human microRNA Disease Database; FC, fold change.

67 miRNAs related to survival from the HMDD database. After comparing the 2 types of miRNAs, 28 common miRNAs were obtained (see *Figure 1A*). To screen out the OS-associated genes, the following steps were performed. First, 3 data sets from the GEO database (i.e., GSE16088, GSE36001, and GSE56001) were downloaded to identify the DEGs (see *Figure 1B*). To minimize the batch effect, the RRA method was used to integrate the DEGs. A total of 1,770 DEGs were detected, including 1,019 upregulated and 751 downregulated genes that met the criteria of a |log$_2$FC| ≥0.5 and a P value <0.05. Second, the target genes of the 28 miRNAs were predicted by miRDB and TargetScan. A total of 10,512 and 3,549 target genes were detected by miRDB and TargetScan, respectively. Third, the similarities between the 1,770 DEGs and the 2 groups of the target genes were analyzed by a Venn diagram. Ultimately, 267 intersected genes were identified, which were defined as the OS-associated genes (see *Figure 1C*).

Construction of a prognostic model for OS

We used 96 OS samples with gene expression and complete survival information in the TARGET database to establish the prognostic model. A univariate Cox analysis was conducted to analyze the association between the 267 OS-associated genes and OS survival, and 34 genes were found to be associated with the outcomes of OS (P<0.05; see Figure S1). Subsequently, the prognostic value of the 34 genes were further analyzed by KM curves. The 96 OS samples were divided into high- and low-expression groups based on the cutoff of the median expression of each gene. The survival differences between the high- and low-expression groups of 14 genes were significant (P<0.05; see Figure S2). Next, the PH assumption was tested for the following multivariate Cox analysis. The PH assumptions for *DEK* and *RH0BTB1* were refuted, as the scaled Schoenfeld residuals of the 2 genes were significantly associated

Table 1 Information for the 3 survival-associated genes identified by a stepwise multivariate Cox analysis

ID	Coef	P value	HR	Low 95% CI	High 95% CI
STAT4	−0.0101	0.0112	0.9899	0.9822	0.9977
HSPE1	0.1698	0.1362	1.1851	0.9478	1.4818
ARPC5	−0.0037	0.0526	0.9963	0.9926	1.00004

STAT4, *signal transducer and activators of transcription factor 4*; *HSPE1*, *heat shock protein family E member 1*; *ARPC5*, *actin-related protein 2/3 complex subunit 5*; Coef, coefficient; HR, hazard ratio; CI, confidence interval.

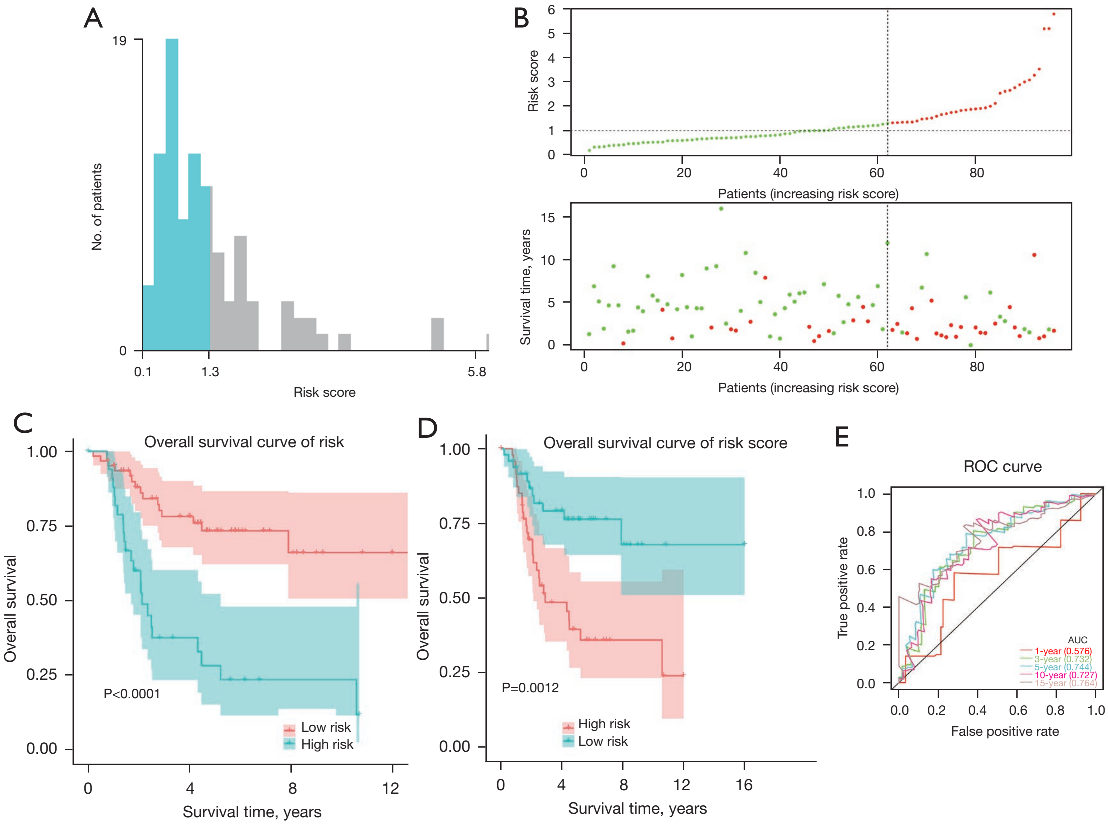

Figure 2 Evaluation of our 3-gene signature for OS survival. (A) X-tile was used to identify the optimal cutoff value for the risk score. (B) Risk score and survival time of each patient. (C) Survival curves for high- and low-risk groups according to X-tile stratification. (D) Survival curves for high- and low-risk groups according to the median risk score. (E) ROC curves of 1-, 3-, 5-, 10-, and 15-year survival for the prognostic signature. ROC, receiver operating characteristic; AUC, area under the ROC curve; OS, osteosarcoma.

with survival time. However, the PH assumption was supported for the remaining 12 genes. Next, 3 prognosis-associated genes were selected in the 12 genes by a stepwise multivariate Cox analysis. Finally, a prognostic signature comprised of 3 genes [i.e., *signal transducer and activators of transcription factor 4* (*STAT4*), *heat shock protein family E member 1* (*HSPE1*), and *actin-related protein 2/3 complex subunit 5* (*ARPC5*)] was developed for OS (see *Table 1*).

Evaluation of the 3-gene signature for OS survival

Based on the 3 prognosis-associated genes mentioned above, the risk score of each sample was calculated by summing the product of the expression level of each gene and its corresponding coefficient. The optimal cutoff value of the risk score was identified as 1.3 by X-title (see *Figure 2A*). Based on this cutoff value, 96 OS samples were stratified into

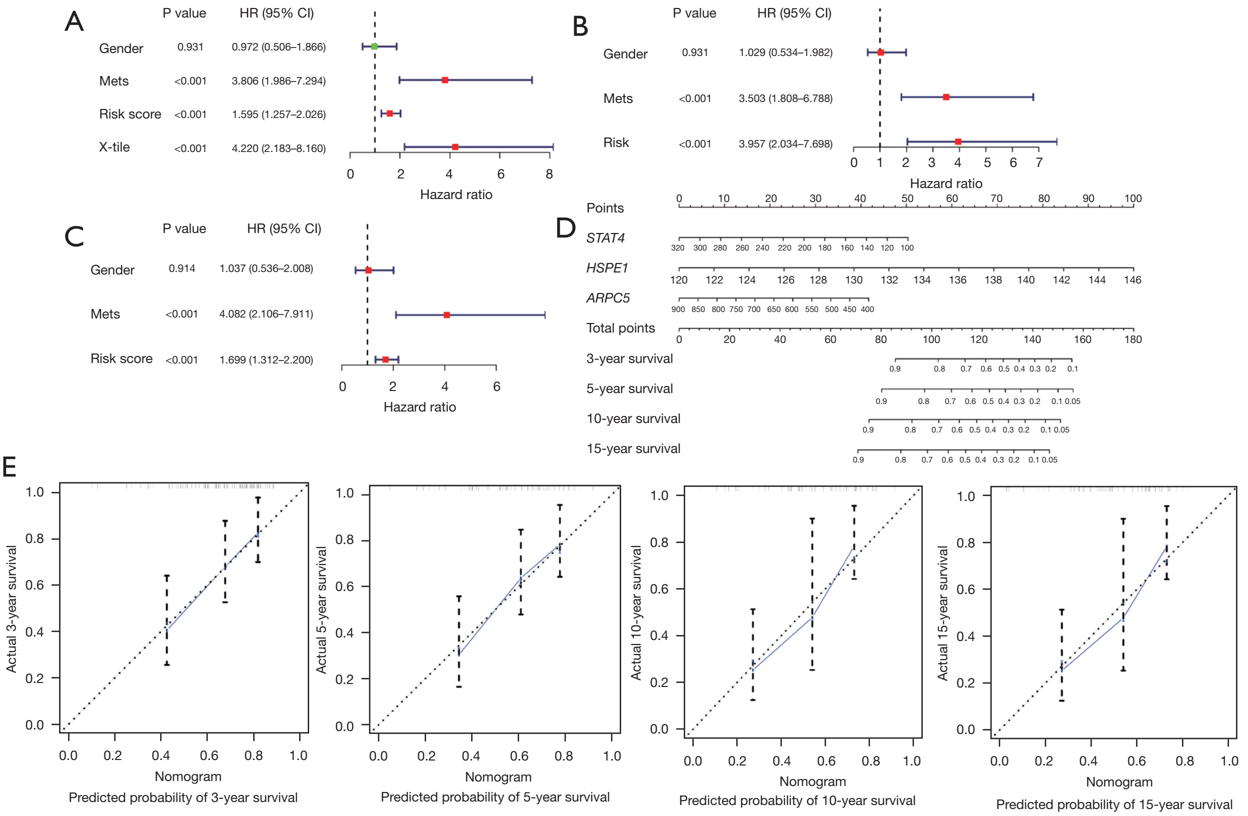

Figure 3 Examination of the predictive performance of our 3-gene signature. (A) Univariate Cox analysis of the 4 factors of gender, metastasis status, risk score, and risk stratification by X-tile. (B) Multivariate Cox analysis of the 3 factors of gender, metastasis status, and risk stratification by X-tile. (C) Multivariate Cox analysis of the 3 factors of gender, metastasis status, and risk score. (D) A nomogram was developed by integrating the expression of 3 prognosis-related genes of *STAT4*, *HSPE1*, and *ARPC5* to predict the 3-, 5-, 10-, and 15-year survival. (E) The calibration curves were plotted for 3-, 5-, 10-, and 15-year survival. HR, hazard ratio; CI, confidence interval; *STAT4*, *signal transducer and activators of transcription factor 4*; *HSPE1*, *heat shock protein family E member 1*; *ARPC5*, *actin-related protein 2/3 complex subunit 5*.

the following 2 groups: (I) the high-risk group, containing 34 samples; and (II) the low-risk group, containing 62 samples. As *Figure 2B* shows, the number of deaths increased as the risk score increased. Additionally, the survival rate of the high-risk group was significantly lower than that of the low-risk group (P<0.05; see *Figure 2C*). Similar results were obtained after risk stratification based on the median risk score (P<0.05; see *Figure 2D*). *Figure 2E* shows the ROC curves for 1-, 3-, 5-, 10-, and 15-year survival for the prognostic model. The AUCs for 3-, 5-, 10-, and 15-year survival were all >0.7, which suggests that the model has excellent discriminative performance.

Next, to examine the prognostic value of the risk score and the risk stratification obtained by X-tile, we performed univariate and multivariate Cox analyses using these 2 factors and 2 clinical features (i.e., gender and metastasis status). As *Figure 3A-3C* show, risk score and risk stratification obtained by X-tile were independent prognostic factors for OS.

To examine the predictive performance of the 3-gene signature, a nomogram was developed by integrating the expression of the 3 prognosis-related genes (i.e., *STAT4*, *HSPE1*, and *ARPC5*) to predict 3-, 5-, 10-, and 15-year survival (see *Figure 3D*). In the nomogram, the point of each gene was obtained easily based on its expression level. The total points of 1 patient could then be calculated by summing up all the gene points. According to the nomogram, *HSPE1* had the greatest effect on OS prognosis. In particular, the calibration curves for 3-, 5-, 10-, and 15-year survival had good consistency between the predicted and observed results, which indicated that the nomogram predicted OS survival accurately (see *Figure 3E*). All these results demonstrated the accurate predictive performance of the 3-gene signature for OS survival.

Validation of the 3-gene signature

An external data set GSE21257 comprising the gene expression and survival information of 53 OS samples was used to validate the prognostic signature. X-tile was used to divide the 53 samples into the following 2 groups based on risk score: (I) the high-risk group, containing 13 samples; and (II) the low-risk group, containing 40 samples. As *Figure 4A* shows, there were more deaths in the high-risk group than the low-risk group. The overall survival rate of the low-risk group was significantly higher than that of the high-risk group (P<0.05; see *Figure 4B*). Additionally, the AUCs were high (all >0.7) for the prediction of 1-, 3-, and 5-year survival, suggesting a good discriminative performance (see *Figure 4C*). The nomogram was developed to predict 1-, 3-, and 5-year survival (see *Figure 4D*). The high consistency of the observed and predicted results in the calibration curves indicated that the predictive performance of the 3-gene signature was good (see *Figure 4E*).

GSEA

To perform the biological annotation of the 3 genes, the GSEA method was used to explore the KEGG pathways enriched in the high- and low-risk groups in the TARGET database. A total of 34 signaling pathways were significantly enriched in the low-risk group (P<0.05; see Table S1). The top 3 pathways enriched with the most genes were the mitogen-activated protein kinase (MAPK) signaling pathway, the regulation of actin cytoskeleton pathway, and the calcium signaling pathway. Additionally, 1 signaling pathway was enriched in high-risk group; that is, the cytosolic DNA sensing pathway.

Construction of the 3-gene signature for recurrence prediction

Given the high rate of recurrence in OS, we next investigated whether the 3 genes could be used to predict OS recurrence in the TARGET database. In this database, the 76 OS samples with gene expression and complete recurrence data contained 32 cases with recurrence and 44 cases without recurrence. The 3 genes (i.e., *STAT4*, *HSPE1*, and *ARPC5*) were incorporated into a multivariate Cox regression model, and the coefficient for each gene is displayed in *Table 2*. The risk score for each patient was calculated, and the samples were then divided into high- (n=22) and low-risk (n=54) groups using X-tile. As *Figure 5A* shows, the number of patients with recurrence increased along with the risk score. As the KM curves in *Figure 5B* show, the high-risk patients had a significantly higher relapse rate than the low-risk patients (P<0.05). Additionally, the AUCs for predicting 3- and 10-year relapse were high (both >0.7), which shows the excellent predictive performance of our signature (see *Figure 5C*). Further, the univariate and multivariate Cox analyses revealed that risk stratification by X-tile was independently associated with the relapse of OS (P<0.05; see *Figure 5D,5E*). A nomogram was constructed to predict the 3- and 10-year relapse rate of OS patients (see *Figure 5F*). In the nomogram, *HSPE1* had the greatest effect on the OS relapse rate. The calibration curves showed the high predictive reliability of the 3-gene signature for the 3- and 10-year relapse rates of OS (see *Figure 5G*).

Construction of the 2-gene signature for OS diagnosis

The GSE42352 data set, which comprised 103 OS and 15 normal samples, was used to investigate whether the 3 genes could be used to develop a diagnostic model for OS. First, a logistic regression analysis was conducted to analyze the association between the 3 genes and a diagnosis of OS. *STAT4* and *HSPE1* were significantly associated with a diagnosis of OS (P<0.05), but no significant correlation was found between *ARPC5* and a diagnosis of OS (P>0.05; see *Table 3*). Additionally, the AIC value for the 3 genes (42.389) was larger than that for the 2 genes (42.329). Thus, the 2 genes (*STAT4* and *HSPE1*) were incorporated into the logistics model for OS diagnosis. The overdispersion occurred in the binomial logistic regression for the 2 genes. The quasibinomial logistic regression model of the 2 genes was thus constructed as the diagnosis signature for OS. The high AUC value (0.981) suggested that the 2-gene diagnosis signature for OS had a good discrimination (see *Figure 6A*).

Next, a diagnosis nomogram model was established based on the expression of *STAT4* and *HSPE1* (see *Figure 6B*). The diagnostic rate was calculated using the points of the 2 genes. The calibration plot showed that the error between the predicted and actual value was 0.029, which showed the good predictive performance of our model (see *Figure 6C*). Subsequently, the confidence ellipse based on the PCA was plotted to evaluate the effectiveness of the diagnostic model. As *Figure 6D* shows, there was little overlap between the normal and tumor ellipses, and their centers were far

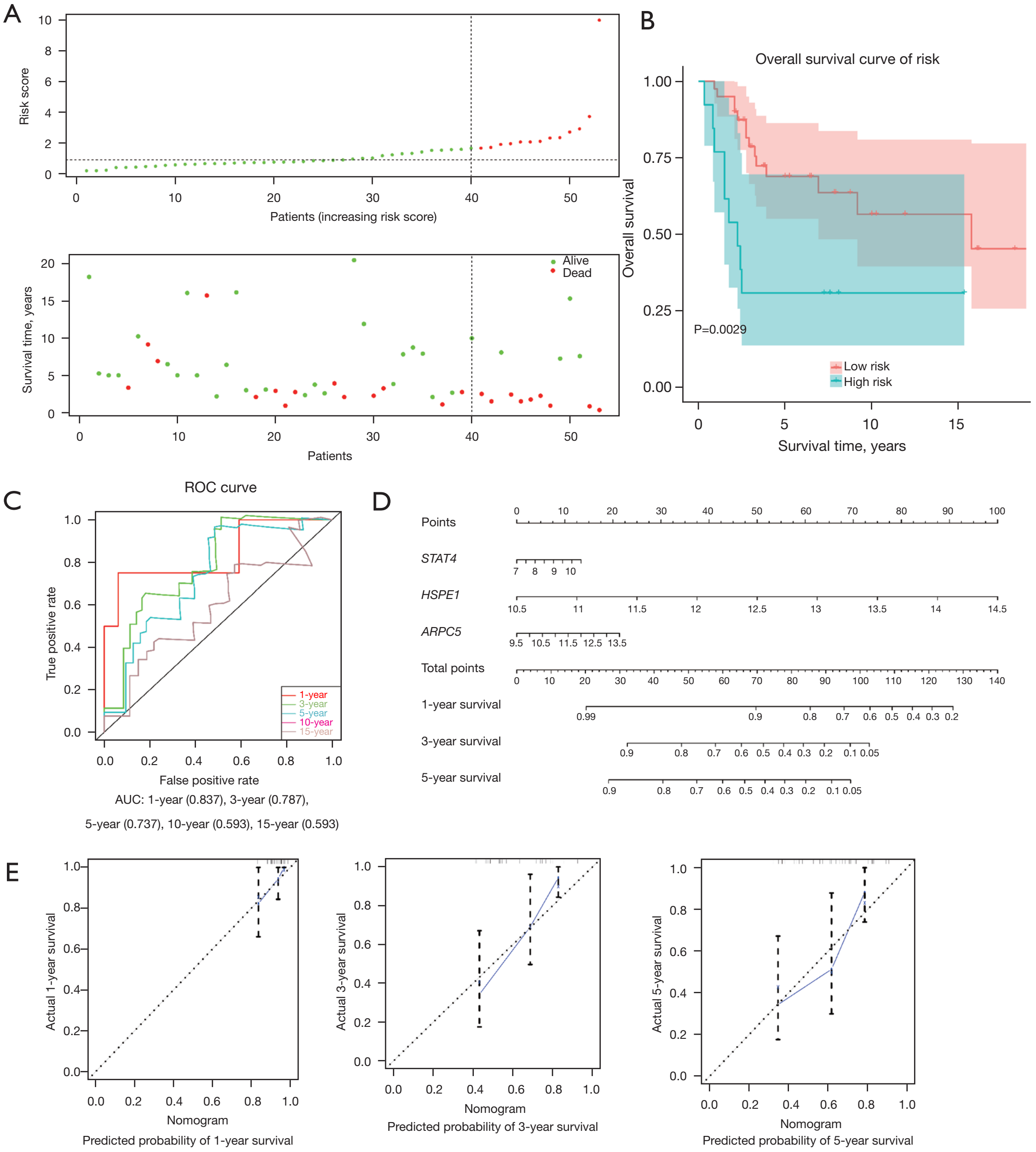

Figure 4 Validation of our 3-gene signature in the GSE21257 data set. (A) Risk score and survival time of OS patients. (B) Survival curves for high- and low-risk groups stratified based on risk score. (C) ROC curves of the 3-gene signature for the prediction of 1-, 3-, and 5-year survival. (D) The nomogram was developed to predict 1-, 3-, and 5-year survival. (E) Calibration curves of the 3-gene signature for the prediction of 1-, 3-, and 5-year survival. ROC, receiver operating characteristic; AUC, area under the ROC curve; *STAT4*, *signal transducer and activators of transcription factor 4*; *HSPE1*, *heat shock protein family E member 1*; *ARPC5*, *actin-related protein 2/3 complex subunit 5*; OS, osteosarcoma.

Table 2 Information for our 3-gene signature analyzed by a multivariate Cox regression

ID	Coef	P value	HR	Low 95% CI	High 95% CI
STAT4	–0.005299372	0.117497645	0.994714644	0.988136313	1.00133677
HSPE1	0.135647621	0.187789355	1.145278251	0.935942617	1.401434498
ARPC5	–0.000277364	0.888932242	0.999722675	0.99583872	1.003621778

STAT4, *signal transducer and activators of transcription factor 4*; *HSPE1*, *heat shock protein family E member 1*; *ARPC5*, *actin-related protein 2/3 complex subunit 5*; Coef, coefficient; HR, hazard ratio; CI, confidence interval.

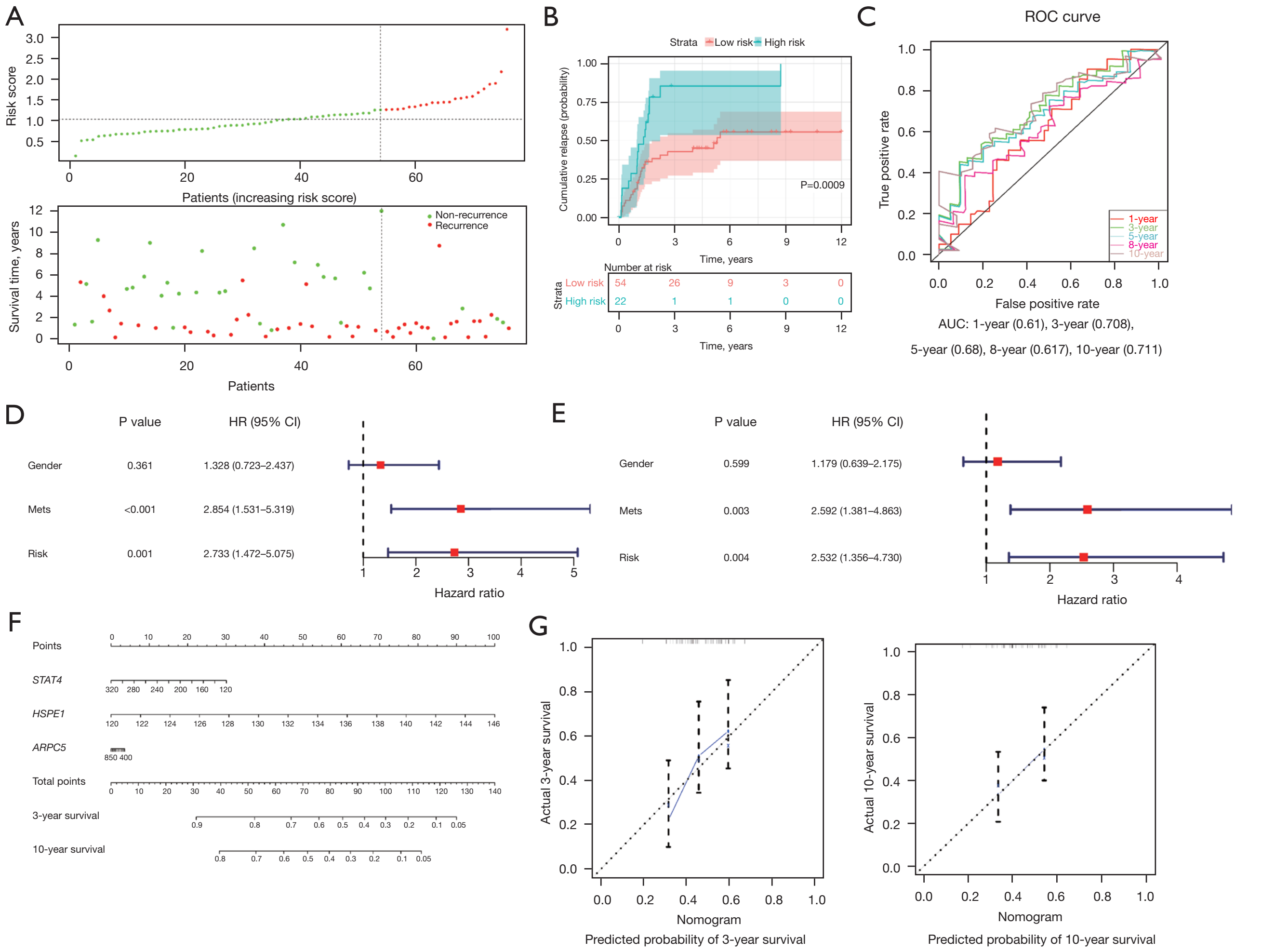

Figure 5 Construction of the 3-gene signature for recurrence prediction. (A) Risk score and relapse rate of OS patients in the TARGET database. (B) Relapse curves for high- and low-risk groups stratified based on risk score. (C) ROC curves of our 3-gene signature for the prediction of 1-, 3-, 5-, 8-, and 10-year relapse. (D) Univariate Cox analysis of the 3 factors of gender, metastasis status and risk stratification by X-tile. (E) Multivariate Cox analysis of the 3 factors of gender, metastasis status, and risk stratification by X-tile. (F) A nomogram was developed to predict 3- and 10-year relapse. (G) Calibration curves of the 3-gene signature for the prediction of 3- and 10-year relapse. ROC, receiver operating characteristic; AUC, area under the ROC curve; HR, hazard ratio; CI, confidence interval; *STAT4*, *signal transducer and activators of transcription factor 4*; *HSPE1*, *heat shock protein family E member 1*; *ARPC5*, *actin-related protein 2/3 complex subunit 5*; OS, osteosarcoma; TARGET, Therapeutically Applicable Research to Generate Effective Treatments.

apart. This demonstrated that the distributions of the principal components in the normal and tumor groups were respectively concentrated and differed from each other. The PCA results indicated that our diagnostic model could discriminate between tumor and normal patients effectively.

Next, the gene expression profiles from the GSE19276 (which comprised 5 normal and 44 tumor cases) and GSE36001 (which comprised 6 normal and 19 tumor cases) data sets were downloaded to validate our diagnostic model. A logistics regression model was used based on the expression of *HSPE1* and *STAT4*. The ROC curves showed that our model had great discriminative power with high AUCs (0.905 in GSE19276 and 0.825 in GSE36001; see *Figure 6E,6F*). All the results showed the excellent predictive performance of the 2-gene diagnostic signature.

Table 3 Information for our 3-gene signature analyzed by a logistic regression

ID	Estimate	Standard error	Z value	Pr (>|z|)
ARPC5	0.7079	0.5551	1.275	0.2022
HSPE1	1.3956	0.6449	2.164	0.0304*
STAT4	−3.6393	0.8501	−4.281	1.86e-05***

*P<0.05; ***P<0.01. *ARPC5*, *actin-related protein 2/3 complex subunit 5*; *HSPE1*, *heat shock protein family E member 1*; *STAT4*, *signal transducer and activators of transcription factor 4*.

Discussion

OS is a malignant bone tumor that often occurs in children and young adults aged 10–19 years. Recurrence and metastasis severely decrease the survival time and the quality of life of OS patients. Given the importance of early detection and recurrence in OS prognosis, it is imperative that gene biomarkers for OS prognosis, diagnosis, and

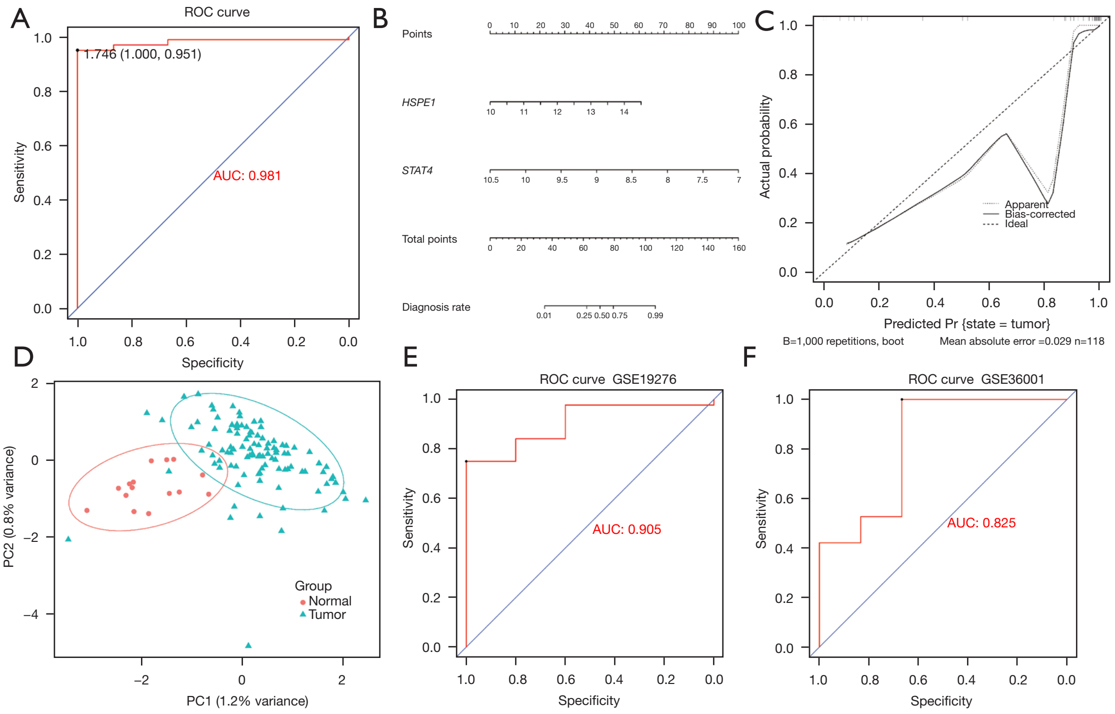

Figure 6 Construction of our 2-gene signature for OS diagnosis. (A) ROC curve of our 2-gene signature for OS diagnosis in the GSE42352 data set. (B) A diagnosis nomogram model was established based on the expression of *STAT4* and *HSPE1*. (C) The calibration plot for the nomogram showed that the error between the predicted and actual value was 0.029. (D) The confidence ellipse based on the PCA was plotted to evaluate the effectiveness of the diagnosis model. (E,F) ROC curve of the 2-gene signature for OS diagnosis in validation sets, including the GSE19276 (E) and GSE36001 (F) data sets. ROC, receiver operating characteristic; AUC, area under the ROC curve; *HSPE1*, *heat shock protein family E member 1*; *STAT4*, *signal transducer and activators of transcription factor 4*; PC, principal component; OS, osteosarcoma.

recurrence be identified.

In this study, the HMDD database and 3 microarray data sets were downloaded to screen OS-associated genes. After comparing the targets of the OS-associated miRNAs and DEGs related to OS, a total of 267 candidate genes associated with OS were identified. Subsequently, the univariate Cox and KM analyses revealed that the expression of 14 of the genes were significantly associated with the outcomes of OS patients. Finally, 3 genes (i.e., *STAT4*, *HSPE1*, and *ARPC5*) were selected based on the PH assumption and stepwise multivariate Cox regression, and we established a 3-gene prognostic signature for the survival and recurrence of OS. The reliability of the prognostic signature was then evaluated and validated by risk stratification and nomogram construction. Additionally, a logistic regression model integrating *STAT4* and *HSPE1* was analyzed and validated and found to be effective for OS diagnosis.

The 3 genes in the signature have been reported to be involved in the progression of various tumors in many studies. Some of the 7 members of the *STAT* family have been identified as biomarkers and targets for prognosis and immunotherapy for some cancers; for example, *STAT3* and *STAT5A* in breast, lung, and prostate cancer, and *STAT6* in lung cancer (21,22). Many studies have investigated the role of *STAT* members in OS but have focused on *STAT3* and *STAT5* (23-25). The role of *STAT4* in cancer is controversial. Previous studies have reported that *STAT4* promotes the metastasis of gastric and ovarian cancer (26,27). However, in these 2 cancers and breast cancer, the expression of *STAT4* was shown to be positively associated with prognosis (28-30).

As critical chaperonins, members of the heat shock protein family play important roles in inhibiting the accumulation of misfolded proteins by promoting the refolding and degradation of these proteins (31). *HSPE1* is one of the main constituents of chaperonin in mitochondria and has been proven to be involved in tumor development and progression (32-34). *HSPE1* has been reported to be upregulated and inhibit the activation of T cells in ovarian cancer, which would support the immune escape of this cancer (34). Additionally, the plasma concentration of *HSPE1* has been found to be higher in breast cancer patients than in controls (35).

The oncogenic role of *ARPC5* has been investigated in many cancers, including head and neck squamous cell carcinoma, prostate cancer and multiple myeloma (36-38). *ARPC5L*, an important paralog of *ARPC5*, has been proven to directly regulate the antimetastatic effect of SI-83, a promising OS drug, by dephosphorylation (39).

To the best of our knowledge, no previous study has sought to uncover the associations of *STAT4*, *HSPE1*, and *ARPC5* with the prognosis of OS. In our study, *STAT4* and *ARPC5* were found to be positive prognostic predictors for OS. However, we detected a negative association between *HSPE1* expression and the survival outcome of OS patients. The clinical significance of these genes and their association in OS needs further investigations.

The significance of the signaling pathways has been investigated in OS. Takahashi *et al.* reported that actin cytoskeleton stimulated the differentiation of adipocytes and repressed the initiation of OS via depolymerization (40). The MAPK signaling pathway has been revealed to be involved in the autophagy and apoptosis of OS cells (41). Yang *et al.* found that calcium signaling positively regulates the transcription of caveolin-1, a transformation suppressor protein in OS cells (42). In our study, the GSEA results revealed that the MAPK signaling pathway, the regulation of actin cytoskeleton pathway, and the calcium signaling pathway were most enriched in the low-risk group, and the cytosolic DNA sensing pathway was significantly enriched in the high-risk group.

There are multiple studies on prognostic models for OS. For example, Zhang *et al.* constructed a prognostic model incorporating gene signature and clinical factors for OS patients with recurrence (43). Zhu *et al.* identified 7 energy metabolism-associated genes to predict the prognosis of OS patients (44). Another 4 immune-related gene prognostic signature was established to predict the survival of OS patients (45). All of these studies only identified gene signatures for survival prediction. Conversely, in the present study, in addition to survival prediction, we first established a gene prognostic signature for recurrence and diagnosis that had excellent predictive performance.

However, the limitations of our study should be noted. First, our signature was based on the retrospective analysis of cases in the TARGET and GEO databases. Thus, further clinical verifications are needed. Second, due to the limitation of the databases, some important clinical prognostic factors, such as surgery, age and grade, were not integrated to analyze the prognostic value of our signature. Third, due to the low incidence of OS, the number of the samples in the databases was relatively small, which may have led to selection bias in this study. Fourth, cell and animal experiments need to be conducted to verify the effects of the genes in our signature on OS.

Conclusions

In summary, 3 genes (*STAT4*, *HSPE1*, and *ARPC5*) were identified and incorporated in an accurate and reliable prognostic signature for the survival, recurrence, and diagnosis of OS by conducting a comprehensive bioinformatics analysis. Our signature is favorable for the individual management of OS patients and could be a promising therapeutic target for OS.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1706/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1706/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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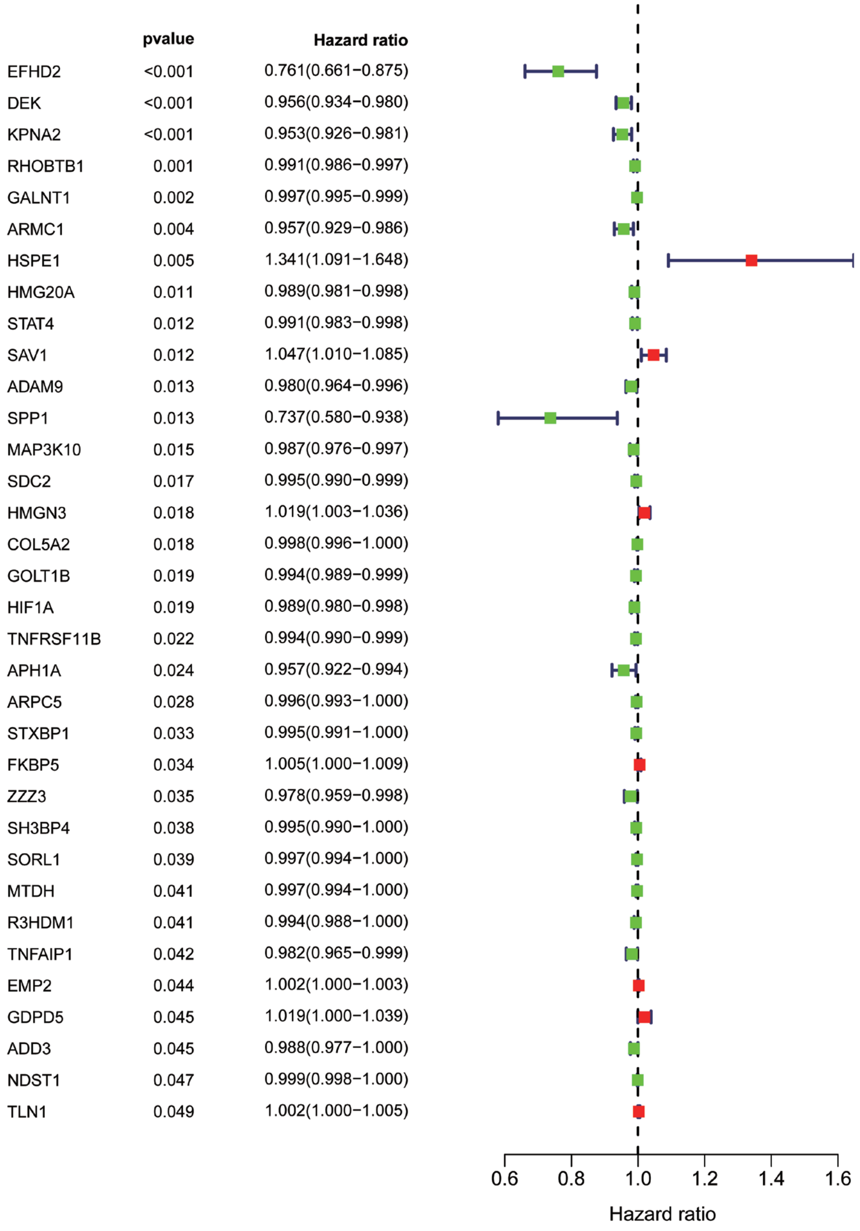

	pvalue	Hazard ratio
EFHD2	<0.001	0.761(0.661−0.875)
DEK	<0.001	0.956(0.934−0.980)
KPNA2	<0.001	0.953(0.926−0.981)
RHOBTB1	0.001	0.991(0.986−0.997)
GALNT1	0.002	0.997(0.995−0.999)
ARMC1	0.004	0.957(0.929−0.986)
HSPE1	0.005	1.341(1.091−1.648)
HMG20A	0.011	0.989(0.981−0.998)
STAT4	0.012	0.991(0.983−0.998)
SAV1	0.012	1.047(1.010−1.085)
ADAM9	0.013	0.980(0.964−0.996)
SPP1	0.013	0.737(0.580−0.938)
MAP3K10	0.015	0.987(0.976−0.997)
SDC2	0.017	0.995(0.990−0.999)
HMGN3	0.018	1.019(1.003−1.036)
COL5A2	0.018	0.998(0.996−1.000)
GOLT1B	0.019	0.994(0.989−0.999)
HIF1A	0.019	0.989(0.980−0.998)
TNFRSF11B	0.022	0.994(0.990−0.999)
APH1A	0.024	0.957(0.922−0.994)
ARPC5	0.028	0.996(0.993−1.000)
STXBP1	0.033	0.995(0.991−1.000)
FKBP5	0.034	1.005(1.000−1.009)
ZZZ3	0.035	0.978(0.959−0.998)
SH3BP4	0.038	0.995(0.990−1.000)
SORL1	0.039	0.997(0.994−1.000)
MTDH	0.041	0.997(0.994−1.000)
R3HDM1	0.041	0.994(0.988−1.000)
TNFAIP1	0.042	0.982(0.965−0.999)
EMP2	0.044	1.002(1.000−1.003)
GDPD5	0.045	1.019(1.000−1.039)
ADD3	0.045	0.988(0.977−1.000)
NDST1	0.047	0.999(0.998−1.000)
TLN1	0.049	1.002(1.000−1.005)

Figure S1 Forest plot of the relationship of the genes with the survival of OS patients. P<0.05. OS, osteosarcoma.

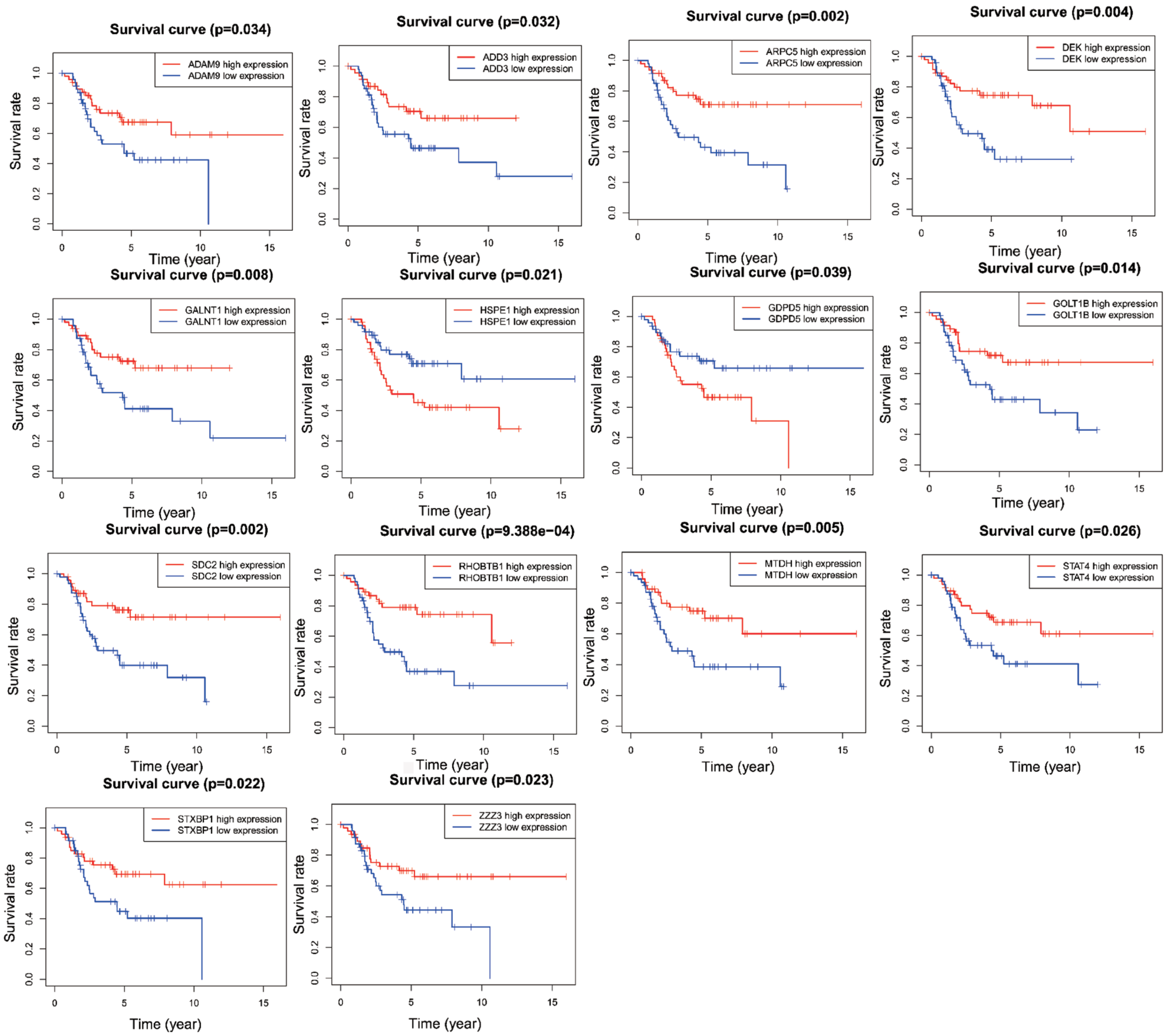

Figure S2 Survival curves of the high- and low-expression groups of 14 genes. P<0.05.

Table S1 The signaling pathways enriched in the high- and low-risk groups

Group	Number	Pathway	Size	P value
Low-risk group	1	KEGG_MAPK_SIGNALING_PATHWAY	265	0.0315
	2	KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	212	0.0301
	3	KEGG_CALCIUM_SIGNALING_PATHWAY	176	0.0386
	4	KEGG_ALZHEIMERS_DISEASE	156	0.0258
	5	KEGG_JAK_STAT_SIGNALING_PATHWAY	151	0.0216
	6	KEGG_WNT_SIGNALING_PATHWAY	149	0.0156
	7	KEGG_INSULIN_SIGNALING_PATHWAY	137	0.0037
	8	KEGG_TIGHT_JUNCTION	130	0.0344
	9	KEGG_AXON_GUIDANCE	127	0.0020
	10	KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	126	0.0019
	11	KEGG_GNRH_SIGNALING_PATHWAY	101	0.0075
	12	KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	101	0.0263
	13	KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	92	0.0132
	14	KEGG_GAP_JUNCTION	89	0.0399
	15	KEGG_ERBB_SIGNALING_PATHWAY	87	0
	16	KEGG_APOPTOSIS	86	0
	17	KEGG_TGF_BETA_SIGNALING_PATHWAY	85	0.0101
	18	KEGG_VEGF_SIGNALING_PATHWAY	75	0.0097
	19	KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	74	0.0165
	20	KEGG_ADHERENS_JUNCTION	73	0.0019
	21	KEGG_CHRONIC_MYELOID_LEUKEMIA	73	0.0220
	22	KEGG_PANCREATIC_CANCER	70	0.0057
	23	KEGG_RENAL_CELL_CARCINOMA	70	0.0234
	24	KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	67	0.0461
	25	KEGG_COLORECTAL_CANCER	62	0.0175
	26	KEGG_GLYCOLYSIS_GLUCONEOGENESIS	61	0.0018
	27	KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	55	0.0298
	28	KEGG_NON_SMALL_CELL_LUNG_CANCER	54	0.0447
	29	KEGG_MTOR_SIGNALING_PATHWAY	52	0.0040
	30	KEGG_ENDOMETRIAL_CANCER	52	0.0301
	31	KEGG_NOTCH_SIGNALING_PATHWAY	47	0.0283
	32	KEGG_TYPE_II_DIABETES_MELLITUS	46	0.0153
	33	KEGG_GLYCEROLIPID_METABOLISM	42	0.0429
	34	KEGG_PROPANOATE_METABOLISM	32	0.0176
High-risk group	1	KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	54	0.0214

Size represented the number of genes enriched in the pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes.