Original Article

Identification of a 7-mRNA signature as a prognostic biomarker in pediatric osteosarcoma

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Background: The aim of the present study was to establish a prognostic model for the survival of children with osteosarcoma (OS).

Methods: The mRNA expression and clinical characteristics of pediatric patients with OS were extracted from the Therapeutically Available Research to Generate Effective Treatments (TARGET) database. After genes with differential mRNA expression were identified, univariate and multivariate Cox analyses were performed, and a prognostic model of pediatric OS was established. The prognostic values of a 7-mRNA signature were evaluated using the receiver-operating characteristic (ROC) curve in pediatric patients with OS.

Results: A total of 19,496 differentially expressed mRNAs were identified, including 267 upregulated mRNAs and 104 downregulated mRNAs. After univariate and multivariate Cox analyses, seven mRNA species (*SCGB3A1*, *MUC17*, *ADH1B*, *KRT83*, *RP1-37E16.12*, *FIGF*, and *SFTPD*) were found to be closely associated with survival. These mRNA species were mainly enriched in glycolysis/gluconeogenesis, arachidonic acid metabolism, cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction, tight junction, and complement and coagulation cascade pathways. A predictive model using the sum of independent prognostic values of the seven mRNA species as the risk score was proposed. The risk score was calculated as follows: risk score = 0.242257 × *SCGB3A1* + 0.168999 × *MUC17* + 0.415514 × *ADH1B* + 0.488864 × *KRT83* + 0.360864 × *RP1-37E16.12* – 0.2991 × *FIGF* – 0.39576 × *SFTPD*. Pediatric patients with OS were assigned to low- and high-risk groups based on the risk score. The ROC curve analysis showed that the 7-mRNA prediction model performed well [area under the curve (AUC): 0.858].

Conclusions: A 7-mRNA signature has the potential to predict the prognosis of pediatric patients with OS, and therefore warrants further validation.

Keywords: Biomarker; mRNAs; prognosis; osteosarcoma (OS); pediatric patients; 7-mRNA signature

Submitted Jun 25, 2020. Accepted for publication Oct 09, 2020.
doi: 10.21037/tcr-20-2407
View this article at: http://dx.doi.org/10.21037/tcr-20-2407

Introduction

Osteosarcoma (OS) is the most common primary bone cancer. The global incidence is 0.2–3/100,000 per year for the general population and is 0.8–11/100,000 for people aged 15–19 years (1,2). OS is common among children, and has a high malignancy, disability, and recurrence rate, along with a poor prognosis. The incidence of OS generally increases with age. Although chemotherapy has increased the 3-year survival rate from 20% to 60–70% (3), there has been no significant progress in targeted OS treatment in the past few decades. Therefore, a further understanding of the genetic etiology of OS is required for progress to be made in treating this cancer.

Currently, a number of molecules have become therapeutic targets for certain cancers. For example, transferrin receptor 1 (TFR1), a member of the TFR family, is a membrane protein that regulates iron input (4,5). The uptake of iron by TFRs is important for cancer cells to absorb iron. There is increasing evidence that TFR1 is involved in the development of tumors, and its expression is significantly dysregulated in many cancers (6,7). The relationship between TFR1 and cancer has been extensively studied, and TFR1 is considered a valuable drug target for cancer intervention (8-11). Angiogenesis are regulated by the vascular endothelial growth factor (VEGF)and its receptor (VEGFR) signaling pathway and play a key role in tumor growth and metastasis. The selective inhibition of VEGFR kinase has been explored as a successful clinical cancer treatment strategy. Many VEGFR inhibitors have already been approved for clinical use, and many more are in various stages of development (12). Cyclin-dependent protein kinase 9 (CDK9) has been shown to play an important role in the pathogenesis of malignant tumors. A recent study demonstrated that high CDK9 expression was associated with significantly shorter survival in patients with OS following immunohistochemistry. This suggests that a high expression of CDK9 is an independent predictor of poor prognosis in patients with OS. It also indicates that CDK9 is a new prognostic marker and a promising therapeutic target for OS (13).

At present, there are no well-established prognostic markers for pediatric patients with OS. Individual heterogeneity makes the tumor-node-metastasis (TNM) staging system clinically ineffective for the prognosis of OS. Although the alkaline phosphatase tumor biomarker has been used to predict OS (14), and gene modules have been found to be associated with OS (15-18), to the best of our knowledge, there have been no previously published studies specifically focused on the prognosis of OS in pediatric patients. Studies have shown that mRNA plays a very important role in the development of pediatric OS (19-22), which indicates that mRNAs may be used as a prognostic marker in this disease.

The purpose of the present study was to evaluate the use of mRNAs as a prognostic marker of OS in pediatric patients by analyzing the expression of mRNAs available from the Therapeutically Available Research to Generate Effective Treatments (TARGET) OS database.

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/tcr-20-2407).

Methods

Extraction of TARGET pediatric OS data

mRNA sequencing data and corresponding clinical target data were downloaded from the TARGET database (portal.gdc.cancer.gov/). Because the data are standardized, no further processing was required, and no data were deleted. There were 101 cases of gating in the database, including 0 normal samples and 101 OS samples. There were 39 females and 62 males, ranging in age from 4 to 23 years, with an average age of 16 years. Differential expression analysis was performed on level 3RNA sequencing data of OS tissues using the edgeR package based on R language. Genes with absolute log2 fold change >1 and $P<0.05$ in mRNA expression levels were considered to be differentially expressed. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Because the data are from a public database, no ethics committee approval was required.

Survival analysis

The mRNA expression data of pediatric patients with OS combined with clinical data from the TARGET database were used to determine the differential expression of mRNA signals that affect prognosis. The survival curve of the samples with differential mRNA expression was drawn with the Kaplan-Meier plot, and the total survival rate was determined. Univariate and multivariate Cox analyses were performed to calculate the risk ratio and P values of all differentially expressed mRNAs. The sensitivity and specificity of the risk score in predicting the overall survival

rate of pediatric patients with OS were assessed based on the area under the curve (AUC) of the receiver-operating characteristic (ROC) analysis.

Pathway analysis

Pathway analyses were performed among the survival-related mRNAs from the univariate Cox analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/pathway.html) pathway databases.

Statistical analysis

Pediatric patients with OS tumors were allocated to high- and low-expression groups according to the median expression level of each differentially expressed mRNA. Overall survival was determined using the Kaplan-Meier survival analysis. The difference in survival of the pediatric patients was determined using the log-rank test with the survival analysis in R package. A P value <0.05 was considered to be statistically significant.

Results

Single mRNA species were related to survival in pediatric patients with OS

A total of 19,496 mRNA species were found to be differentially expressed, including 104 downregulated mRNAs and 267 upregulated mRNAs. Kaplan-Meier and Cox P value survival analyses of the deferentially expressed mRNA species indicated that 33 genes with differential mRNA expression were significantly related to survival (P<0.05) (*Table 1*).

A group of mRNA species was closely related to survival in pediatric patients with OS

Univariate Cox analysis was performed on all differentially expressed mRNAs, and 22 potential candidates were selected (*Table 2*). Multivariate Cox analysis was then performed on the candidate mRNA species to determine the mRNA species closely related to survival, with a cutoff threshold of significance set at 0.01 (P<0.01). Seven mRNA species were identified (*SCGB3A1*, *MUC17*, *ADH1B*, *KRT83*, *RP1-37E16.12*, *FIGF*, and *SFTPD*). The independent prognostic values of these seven hub mRNAs were generated by multivariate Cox analysis (*Table 3*). Five mRNAs were related to high mortality among pediatric patients with OS, including *SCGB3A1*, *MUC17*, *ADH1B*, *KRT83*, and *RP1-37E16.12*. The risk mortality was 63% higher in patients with a high expression of *KRT83* than in patients with a low *KRT83* expression; the risk of mortality was 52% higher in patients with a high expression of *ADH1B* than in patients with a low *ADH1B* expression. *FIGF* and *SFTPD* were associated with a low risk of mortality in pediatric patients with OS.

Pathways involved in survival

Through Reactome pathway analysis, six enriched pathways that were most likely involved in the survival of pediatric patients with OS were identified (*Table 4*): glycolysis/gluconeogenesis, arachidonic acid metabolism, cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction, tight junction, and complement and coagulation cascades.

Seven-mRNA prognostic model

For each patient, a risk score was generated from the independent prognostic values of the seven mRNAs using the following formula: risk score = 0.242257 × *SCGB3A1* + 0.168999 × *MUC17* + 0.415514 × *ADH1B* + 0.488864 × *KRT83* + 0.360864 × *RP1-37E16.12* – 0.2991 × *FIGF* – 0.39576 × *SFTPD*. The distribution of patients with different survival risk scores of these seven mRNAs and mRNA-related survival time are shown in *Figure 1A*,*B*, respectively. The expression heatmap of the 7-mRNA signature is shown in *Figure 1C*. Pediatric patients with OS were assigned to low- and high-risk groups according to the risk scores (*Figure 1D*). Log-rank test indicated that pediatric patients with OS in the low-risk group had significantly longer survival (*P*<0.05) than patients in the high-risk group (*Figure 1D*). The area under the ROC curve was 0.858 (*Figure 2*). These findings indicate that the 7-mRNA prognostic model is promising, sensitive, and specific in predicting the survival outcomes of pediatric patients with OS.

Discussion

In the present study, a 7-mRNA model was proposed to predict the prognosis of pediatric patients with OS. Of the genes with differential mRNA expression in the TARGET

Table 1 mRNA species related to survival resulted from Kaplan-Meier and Cox P value method analysis

Gene	Kaplan-Meier value	HR	HR.95L	HR.95H	Cox P value
MUC17	0.024729	1.000261	1.00006	1.000463	0.011048
SCGB1A1	0.002324	1.000069	1.000009	1.000129	0.023514
KRT83	0.005254	1.007786	1.00261	1.012988	0.003153
CFAP57	0.043358	1.007527	1.00099	1.014106	0.023945
SFTA3	0.014252	1.001505	1.000187	1.002826	0.025206
ADRB3	0.028427	1.003499	1.000898	1.006108	0.008347
AGR3	0.006579	1.002917	1.000391	1.005449	0.023561
RP11-598P20.5	0.034737	1.001679	1.000369	1.002991	0.011985
CYP4F8	0.037341	1.000538	1.000086	1.00099	0.019618
ALDH1A1	0.004767	1.000117	1.000041	1.000194	0.002573
TNXB	0.03894	1.000203	1.000056	1.000351	0.006823
GABRB3	0.039065	1.000312	1.000038	1.000587	0.025833
CFAP73	0.006869	1.003911	1.000594	1.007239	0.020798
MYOC	0.011729	1.005669	1.001098	1.01026	0.015002
RAB25	0.021731	1.004769	1.000384	1.009173	0.033002
C19orf33	0.039076	1.003781	1.000497	1.007076	0.023996
IGF2	0.00266	0.999955	0.999915	0.999996	0.029667
CLEC4M	0.016707	1.004171	1.000466	1.00789	0.027301
HP	0.038545	1.002115	1.000463	1.003771	0.012093
GABRA5	0.047178	1.001137	1.000161	1.002113	0.022432
TRIM49C	0.017894	1.002371	1.000414	1.004332	0.017556
MEIS3	0.019896	0.997913	0.99598	0.999849	0.034657
MYC	0.024538	1.000058	1.000026	1.000089	0.000315
TNFRSF21	0.004399	0.999503	0.999153	0.999853	0.00539
ACKR4	0.024598	1.007749	1.001252	1.014288	0.019331
HERC5	0.000518	0.997795	0.996052	0.999542	0.013366
TRIM16L	0.007439	0.996127	0.993405	0.998857	0.00545
SYT2	0.042662	0.994201	0.988901	0.999528	0.032928
ST8SIA6	0.007765	0.980774	0.964442	0.997383	0.023464
HSD11B1	0.021879	1.012085	1.00466	1.019566	0.001387
DRD2	0.026452	0.983503	0.967999	0.999256	0.040187
NDST3	0.009417	0.988577	0.979798	0.997434	0.011583
UNC5A	0.032069	1.001864	1.000307	1.003423	0.018925
MUC4	0.023129	1.00491	1.001114	1.00872	0.011196
TRABD2A	0.019783	0.99763	0.995713	0.999551	0.015626
EFHC2	0.001406	0.991072	0.984883	0.997299	0.005012
ZNF488	0.008545	0.975283	0.95449	0.996528	0.022827
GDNF	0.013354	1.00078	1.000016	1.001544	0.045517

Table 2 mRNA species related to survival resulted from univariate and multivariate Cox analysis of overall survival

Gene	HR	Z	P value
SCGB3A1	1.060302	0.547207	0.000424
FIGF	0.982092	–0.1604	0.000785
INMT	1.236569	2.016269	0.001369
SLC34A2	1.124608	1.184404	0.001438
PGC	1.16949	1.520409	0.001506
MUC17	1.189722	2.081223	0.002025
SFTPB	1.115756	1.345639	0.00209
SCGB1A1	1.188812	2.662658	0.002559
TCF21	1.116229	1.238056	0.002667
NAPSA	1.024285	0.209626	0.003016
ADAMTS8	1.07829	0.748342	0.003384
SFTPA2	1.0807	1.009918	0.005021
ADH1B	1.213052	2.400327	0.005168
KRT83	1.453494	3.173515	0.005172
RP1-37E16.12	1.282076	2.675284	0.005424
HPSE2	1.187637	1.876842	0.006284
SFTPD	0.853388	–1.2376	0.006428
MAP1LC3C	1.16887	1.191472	0.007302
PTGER1	1.222665	2.059195	0.007467
SCGB3A2	1.284029	2.292198	0.007753
SFTPC	1.08178	1.140528	0.008273
C4BPA	1.142128	1.43618	0.008797
CXCL17	1.045608	0.354362	0.009551

database, seven mRNA species (*SCGB3A1*, *MUC17*, *ADH1B*, *KRT83*, *RP1-37E16.12*, *FIGF*, and *SFTPD*) were found to be closely related to the survival of pediatric patients with OS. The combination of the seven mRNA species could sensitively and specifically predict survival outcomes in pediatric patients with OS. Further study and confirmation of this 7-mRNA model is required in the future to predict the prognosis of pediatric patients with OS.

The abnormal transcription process is one of the factors influencing the development of OS. Activated protein 1 complex (AP-1) is composed of fos and jun proteins, which are the products of c-fos and c-jun proto oncogenes, respectively. AP-1controls cell proliferation, differentiation, and bone metabolism (23-26). Leaner *et al.* recently found that AP-1-mediated transcriptional inhibition leads to a reduction of migration, invasion, and metastasis in OS mouse models (27). When delivered via nanoparticles, the enzyme dz13 cleaves human c-jun mRNA and inhibits the growth and progression of OS in mouse models (28). Myc is a transcription factor, and its amplification is related to the pathogenesis of OS and chemotherapy resistance (29). The overexpression of myc in bone marrow stromal cells leads to OS and fat loss. Myc has been found to be amplified in the U2OS cell line; it has high resistance to adriamycin, and is increased in the Saos-2 methotrexate-resistant cell line (30). In addition, myc is considered to be the target treatment of OS. The downregulation of myc enhances the therapeutic effect of methotrexate on OS cells (31). These findings suggest that mRNAs, especially mRNAs resulting from abnormal transcription processes, may be good markers for the prognosis of OS.

In the present study, *SCGB3A1*, *MUC17*, *ADH1B*, *KRT83*, *RP1-37E16.12*, *FIGF*, and *SFTPD* mRNA species were found to be highly related to the survival of pediatric patients with OS. *MUC17* encodes mucin17, which functions in epithelial cells to provide cytoprotection and signal transduction, maintain luminal structure and homeostasis of the mucosal surface, and confer anti-adhesive properties to cancer cells that lose their apical/basal polarization (32). *ADH1B* encodes alcohol dehydrogenase 1B (class I), β polypeptide [which metabolizes a wide variety of substrates, including ethanol (alcohol beverage)], retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products (33). *SFTPD* encodes pulmonary surfactant protein D, which is an innate immune system collect in (34). *SCGB3A1* is a type of secretory immunoglobulin, and its biologic functions are mainly unknown (35). *KRT83* is a hair keratin (36). *FIGF* or c-fos-induced growth factor is a VEGF that promotes the metastasis of cancers to lymph nodes (37). *RP1-37e16.12* is a filamentous actin-binding protein that is associated with the guanine nucleotide exchange factor and regulates actin cytoskeleton organization (38). Although the roles of these genes in OS progression are not well understood, the KEGG pathway databases indicate that they are related to neuroactive ligand-receptor interaction, arachidonic acid metabolism, cytokine-cytokine receptor interaction, tight junction, and complement and coagulation cascade signaling pathways. The neuroactive ligand-receptor interaction pathway is related to OS tumorigenesis and poor prognosis

Table 3 Seven mRNA species which were most closely related to survival

ID	Coef.	Exp(coef.)	Se(coef.)	Z	Pr(>|z|)
SCGB3A1	0.242257	1.274122	0.136497	1.774825	0.075927
FIGF	–0.2991	0.741483	0.15569	–1.92115	0.054713
MUC17	0.168999	1.184118	0.086462	1.954604	0.05063
ADH1B	0.415514	1.515149	0.128483	3.234008	0.001221
KRT83	0.488864	1.630463	0.134531	3.633843	0.000279
RP1-37E16.12	0.360864	1.434568	0.113838	3.169984	0.001524
SFTPD	–0.39576	0.673166	0.163477	–2.42091	0.015482

Table 4 The seven mRNA species related pathways

Category	Term	Count	P value
KEGG_PATHWAY	Glycolysis/Gluconeogenesis	5	3.3E–2
KEGG_PATHWAY	Arachidonic acid metabolism	5	2.4E–2
KEGG_PATHWAY	Cytokine-cytokine receptor interaction	11	1.3E–2
KEGG_PATHWAY	Neuroactive ligand-receptor interaction	13	4.3E–3
KEGG_PATHWAY	Tight junction	5	7.3E–2
KEGG_PATHWAY	Complement and coagulation cascades	5	3.6E–2

(39,40). Although arachidonic acid is not carcinogenic (40-43), arachidonic acid can be converted into prostaglandin E2 and other prostaglandins by cyclooxygenase-2 (44), which plays a role in regulating the migratory and invasive behavior of cells during the development and progression of cancer (16,45). Abnormality in cytokine-cytokine receptor interaction is related to OS genesis and poor prognosis (46,47). Abnormality in tight junction promotes a malignant phenotype of OS cells and causes poor prognosis (48-50). Imbalanced complement and coagulation interaction promote tumor growth (51,52). These findings indicate that the seven identified mRNA species have the potential to be used as prognostic markers in pediatric patients with OS. Multivariate Cox analysis-generated independent prognostic values indicated that *SCGB3A1*, *MUC17*, *ADH1B*, *KRT83*, and *RP1-37E16.12* are related to high mortality in pediatric patients with OS, and *FIGF* and *SFTPD* are associated with a low mortality rate. In particular, the high expression of *KRT83* and *ADH1B* led to higher risk of mortality in these patients. These findings further suggest that these mRNA species might be able to serve as prognostic markers. The prognostic model using the sum of the independent prognostic values of the seven mRNA species could be used as prognostic markers among pediatric patients with OS, with high sensitivity and specificity.

Many approaches have the potential to improve the prognosis of OS patients, including immunotherapy in activating monocytes and macrophages against OS cells (53-56), the inhibition of the mammalian target of rapamycin-mediated signal transduction pathway (57-60), the inhibition of tyrosine kinases (61-63), and the use of novel antifolates. Combination target therapy may have the greatest potential for improvement in outcomes. However, whether this improvement in outcomes among OS patients is achieved by these approaches correlates with the 7-mRNA species predictive value requires further investigation.

The predictive value of the 7-mRNA model was not validated in the present study. Therefore, a validation study using OS tissues from pediatric patients is warranted.

Conclusions

Seven mRNA species were found to be closely related to overall survival in pediatric patients with OS. A prognostic model using a combination of these 7-mRNA species has

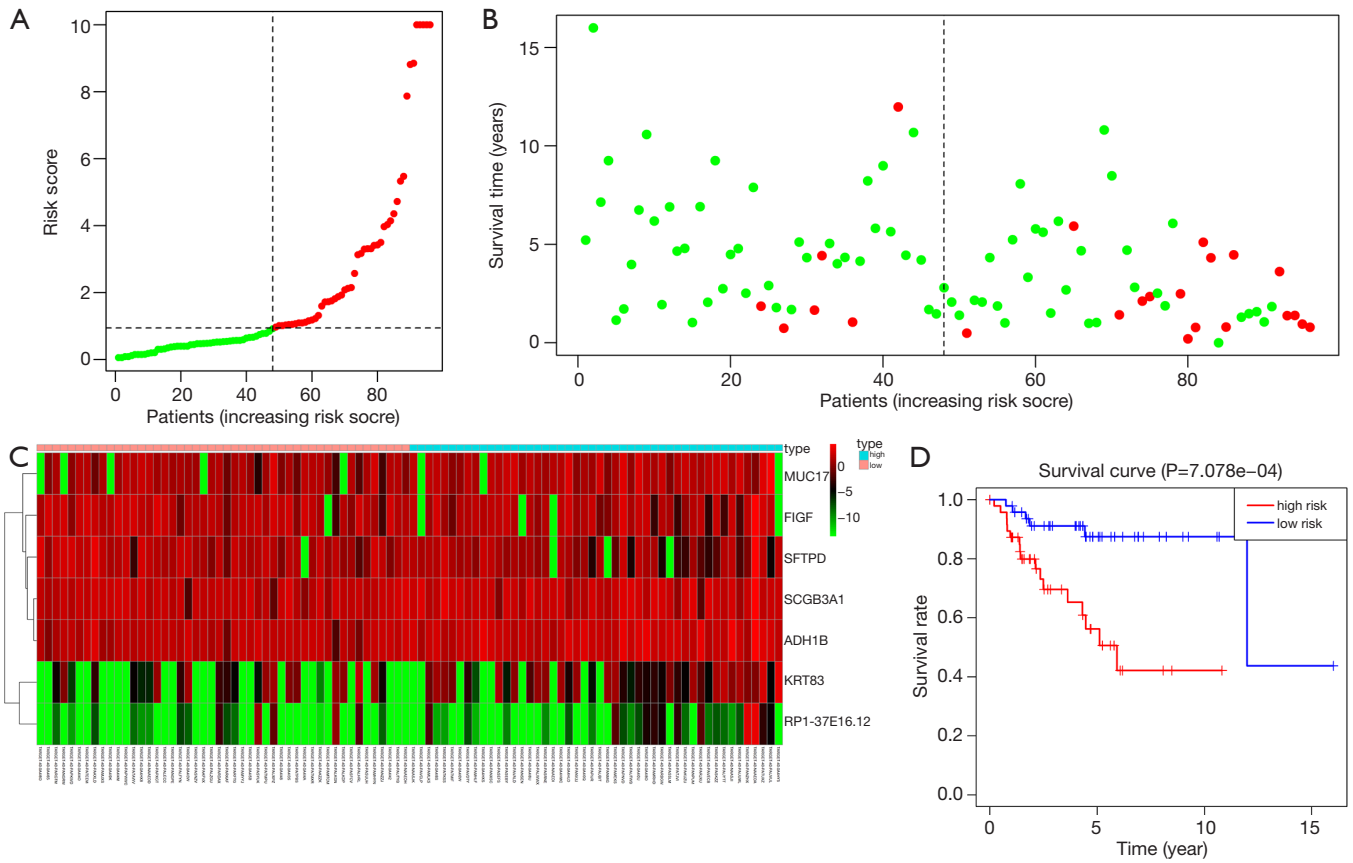

Figure 1 Evaluation of the predictive value of the 7-mRNA prognostic model. Distribution of the patients with different (A) mRNA-related risk scores and (B) mRNA-related survival time; (C) expression heatmap of the seven identified genes in the high- and low-risk groups; (D) overall survival curve of pediatric OS patients generated through Kaplan-Meier survival curve analysis between low-and high-risk groups. OS, osteosarcoma.

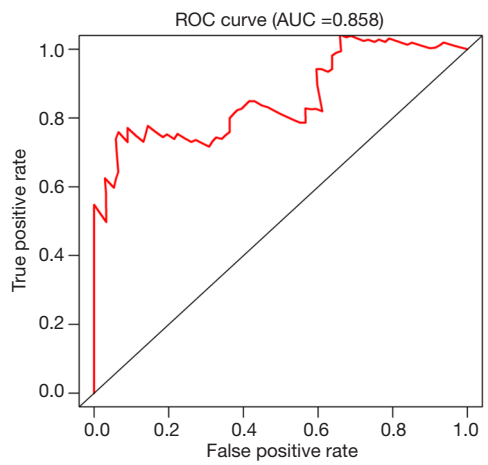

Figure 2 ROC curve of the 7-mRNA prognostic model. ROC, receiver-operating characteristic; AUC, area under the curve.

the potential to predict the prognosis of pediatric patients with OS; however, this requires further verification.

Acknowledgments

The authors would like to thank Profs. Hui Wang and Zhixia Sun for helpful discussions and kind support.
Funding: None.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at http://dx.doi.org/10.21037/tcr-20-2407

Conflicts of Interest: All authors have completed the

ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-2407). The authors have no conflicts of interest to declare.

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

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Cite this article as: Cui T, Guo J, Jin C. Identification of a 7-mRNA signature as a prognostic biomarker in pediatric osteosarcoma. Transl Cancer Res 2020;9(11):6733-6742. doi: 10.21037/tcr-20-2407

