

Signature Based on Immune-Related LncRNA Can Predict Overall Survival of Osteosarcoma Patients

Longqing Li

Zhengzhou University First Affiliated Hospital

Lianghao Zhang

Zhengzhou University First Affiliated Hospital

Manhas Abdul Khader

Zhengzhou University First Affiliated Hospital

Yan Zhang

Zhengzhou University First Affiliated Hospital

Xinchang Lu

Zhengzhou University First Affiliated Hospital

Yi Zhang

Zhengzhou University First Affiliated Hospital

Yongkui Liu

Zhengzhou University First Affiliated Hospital

Jia Wen

Zhengzhou University First Affiliated Hospital

Tao Liu

Zhengzhou University First Affiliated Hospital

Yaobo Yuan

Zhengzhou University First Affiliated Hospital

Jiazhen Li (✉ jzhli6411@163.com)

Zhengzhou University First Affiliated Hospital <https://orcid.org/0000-0002-6416-4296>

Research article

Keywords: Osteosarcoma, TCGA, LncRNA, Tumor immunology, Prognosis

Posted Date: July 21st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-45370/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Osteosarcoma is a malignant bone tumor common in children and adolescents. Metastatic status remains the most important guideline for classifying patients and making clinical decisions. Despite many efforts, newly diagnosed patients receive the same therapy that patients have received over the last 4 decades. With the development of high-throughput sequencing technology and the rise of immunotherapy, it is necessary to deeply explore the immune molecular mechanism of osteosarcoma.

Methods: We obtained RNA-seq data and clinical information of osteosarcoma patients from TCGA database and TARGET database. With the help of co-expression analysis we identified immune-related lncRNA and then by means of univariate Cox regression analysis prognostic-related lncRNA was screened out. And also by using least absolute shrinkage and selection operator regression method a model based on immune-related lncRNA was constructed. The differences in overall survival, immune infiltration, immune checkpoint gene expression, and tumor microenvironmental immunity type between the two groups were evaluated.

Results: We constructed a signature consisting of 13 lncRNA. Our results show that signatures can reliably predict the overall survival of patients with osteosarcoma and can bring net clinical benefits. Further more, the signatures can be used for further risk stratification of the metastasis patients. Patients in the low-risk group had higher immune cell infiltration and immune checkpoint gene expression. The results from gene set variation analysis show that patients in low-risk group are closely related to immune-related pathways when compared with patients in high-risk group. Finally, patients in the low-risk group are more likely to be classified as TMIT I and hence more likely to benefit from immunotherapy.

Conclusion: Our signature may be a reliable marker for predicting the overall survival of patients with osteosarcoma.

1 Background

Osteosarcoma is a malignant bone tumor that commonly affects children and adolescents[1]. In the 1970s, chemotherapy was introduced and significantly improved patient-survival. Currently, patients with newly diagnosed osteosarcoma routinely receive neoadjuvant chemotherapy, surgical removal of the lesion, and undergo adjuvant chemotherapy. However, approximately 30% of patients die within 5 years of treatment due to being unresponsive to chemotherapy and metastasis[2]. Today, clinical characteristics such as metastatic status remain the most important criteria for stratifying patients and making clinical decisions. However, in clinical work, a notable difference in the prognosis of patients with similar clinical characteristics is often observed. In addition, perhaps due to the relatively low incidence of osteosarcoma, the formulation of new drugs and new treatment programs has reached a deadlock, and there has been no breakthrough in the past 40 years [3]. Hence, in order to find new biomarkers and therapeutic targets we need an even deeper understanding of the molecular mechanism of osteosarcoma, which will help us.

Nowadays, immunotherapy has been used as a new type of anti-tumor method, which has shown reliable efficacy in a variety of tumors including melanoma and hepatocellular carcinoma[4–6]. This new treatment method opens hope for patients with osteosarcoma[7, 8]. However, due to many factors, the effectiveness of these new therapies in osteosarcoma -patients is still unclear. Therefore, there is an urgent need to understand the immune molecular mechanisms of osteosarcoma. LncRNA is defined as a kind of non-coding RNA longer than 200 nucleotides[9]. Over a decade ago, little was known about this RNA. However, with the rapid development of technology, more and more evidence indicated that this non-coding RNA has a vital role in the development of many tumors, including osteosarcoma[10–13].

Therefore, with the help of co-expression analysis, this study identified the immune-related lncRNA in osteosarcoma and developed reliable prognostic signatures based on the lncRNA to stratify the patients more precisely. In addition, the differences in immune characteristics between high-risk and low-risk patients were identified. Finally, the relationship between our signature and the efficacy of immunotherapy was also explored based on marker or typing developed in the previous literature.

2 Methods

Data collection

Normalized RNA-Seq (FPKM format) data for 88 osteosarcoma patients was downloaded from the TCGA database (<https://cancergenome.nih.gov/>). At the same time, we downloaded the clinical data related to these patients from the TARGET database (<https://ocg.cancer.gov/programs/target>). Table 1 lists the clinical characteristics of these patients. All these data were obtained from a public database, so no additional informed consent was required.

Table 1
Summary of clinical characteristics of
Osteosarcoma patient data sets in the study.

Characteristic		TCGA dataset N = 88
Vital status, n (%)	Alive	57(64.8)
	Dead	29(33.0)
	Unknow	2(0.2)
Age, n (%)	> = 18	19(21.6)
	< 18	69(78.4)
Gender, n (%)	Male	51(58.0)
	Female	37(42.0)
Metastasis, n (%)	M0	66(75)
	M1	22(25)

Identification of immune-related lncRNA

A list of immune-related genes was downloaded from the immunology database and the analysis portal (ImmPort) database[14]. The list consisted of a total of 2498 unique immune-related genes. The lncRNA profile from RNA-seq data was extracted using R software. Correlation between lncRNA and immune-related genes was then calculated. lncRNA with a correlation coefficient of 0.4 and $P < 0.05$ are considered as immune-related lncRNA and were used for subsequent analysis.

Construction and evaluation of lncRNA model

Univariate Cox regression analysis was used to identify survival-related lncRNA from the above-mentioned immune-related lncRNA. The lncRNA with a P value from the univariate Cox proportional hazards analysis (Wald test for predictive potential) of less than 0.5 is considered as a prognostic-related lncRNA. The prognosis-related lncRNA expression data set was used as an input to the survival model. Subsequently, 1000 iterations were performed using the least absolute shrinkage and selection operator (LASSO), and the retained lncRNA in more than 50 iterations was considered as an important lncRNA. These important lncRNAs are incorporated into the proportional hazards model in turn, and the area under the receiver's operating characteristic curve (AUROC) was calculated. The model when AUROC reaches the peak is the optimal model. Subsequently, each patient's risk score based on the best model was calculated, and time-dependent receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off value for the risk score[15]. Risk score = $\beta_{lncRNA(1)} \times \text{expr}_{lncRNA(1)} + \beta_{lncRNA(2)} \times \text{expr}_{lncRNA(2)} + \dots + \beta_{lncRNA(n)} \times \text{expr}_{lncRNA(n)}$ [16]. The patients were then divided into high-risk group and low-risk group based on the best cut-off value. The log-rank test was used to achieve

the overall survival difference between the two groups, and the KM survival curve was drawn. Using the receiver operating characteristic (ROC) curve analysis the specificity and sensitivity of the risk score was assessed. Finally, multivariate Cox regression analysis was used to explore the independent prognostic value of risk scores.

Construction and evaluation of lncRNA model

The relationship between risk scores and clinical characteristics was further investigated. We divided patients into 6 subgroups based on metastatic status, gender and other clinical characteristics, and explored the prognostic value of risk scores in each subgroup. The forest plot of subgroup analysis was then drawn by means of R software. In addition, based on the metastatic status and risk status, the patients were divided into four groups, and the overall difference in the survival rate among patients in each group was calculated and a KM survival curve was drawn.

Estimation of immune infiltration

With the help of 'Microenvironmental cell counter (MCP-counter)' method the immune infiltration was assessed. This method can reliably quantify the absolute abundance of 8 immune and 2 stromal cell population[17]. Then by means of the 'ESTIMATE' R package the stromal score, immune score and estimate score were estimated ([https://sourceforge.net/projects/ estimateproject /](https://sourceforge.net/projects/estimateproject/))[18]. Finally, the single sample GSEA was used to evaluate the enrichment of 29 immune-related gene sets in each sample[19]. The difference in immune infiltration between the two groups of patients was evaluated. 'Tumor microenvironment immune type (TMIT)' was used to speculate the efficacy of anti-PD-1 / PD-L1 treatment. TMIT divides patients into four types based on PD-L1 and CD8A mRNA expression, which has been shown to predict patients' response to immune checkpoint inhibitors in pan-cancer analysis[20]. Using SubMap analysis (Gene Pattern) to compare gene expression profiles of osteosarcoma patients with melanoma patients treated with immunotherapy to indirectly predict the efficacy of immunotherapy in osteosarcoma patients[21, 22].

Gene set variation analysis

Genome Variation Analysis (GSVA) is an unsupervised gene set enrichment method that can estimate the scores of certain pathways or markers over a sample population[23]. We downloaded the 'c2.cp.kegg.v7.1.symbols' and 'c5.all.v7.1.symbols' gene sets from the 'Molecular Signatures Database' for GSVA. Subsequently, the differential analysis of these gene sets was performed using the LIMMA package of R software, and the gene set with adjusted $P < 0.05$ was regarded as the differentially expressed gene set.

Construction of the nomogram

The rms package of R software was used to build a nomogram based on clinical factors and immune-related lncRNA risk scores. We then assessed the predictive capability using the concordance index (C-

index). In addition, calibration plots were drawn to verify the accuracy of the nomogram. Finally, by means of decision curve analysis, the clinical utility of the nomogram was assessed.

Statistical analysis

All analyses in this study were performed with R software (version 3.6.3) and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Identification of prognostic-related immune-related lncRNA in osteosarcoma

Firstly, we isolated lncRNA expression data from the RNA-SEQ data of 88 patients downloaded from the TCGA database. Subsequently, a total of 2498 immune-related genes were extracted from the ImmPort database. Supplementary Table 1 provides detailed information on these immune-related genes. As shown in Supplementary Table 2, 1986 immune-associated lncRNA were identified by constructing immune-lncRNA co-expression networks. We analyzed the relationship between these immune-related lncRNA and the survival of 85 patients using univariate Cox regression analysis (3 patients lack valid clinical data). Finally, 240 lncRNAs were identified as survival-related immune-related lncRNA and used for further analysis. Supplementary Fig. 1 and Supplementary Table 3 shows the results of univariate cox analysis of these lncRNAs.

3.2 Establishing a risk score and testing its prognostic value in osteosarcoma

As mentioned above, these lncRNAs related to prognosis were subjected to 1000 iterations using LASSO analysis. Subsequently, the 29 lncRNA retained in more than 50 iterations were considered important lncRNA for further analysis. Supplementary Table 1 shows the details of these lncRNA. Incorporating these important lncRNA into the COX model, the optimal model consisting of 13 lncRNA was determined based on the 5-year survival AUROC. The risk score of each patient was calculated, and the cut-off value of the risk score was determined to be 0.186 using the time-dependent receiver operating characteristic (ROC) curve analysis. The results of the survival analysis showed that patients in the low-risk group had longer survival rate than patients in the high-risk group (Fig. 1D, $P < 0.001$). Figure 1 shows the process of building this model. Supplementary Fig. 2 shows the relationship between 13 lncRNA and immune genes.

To verify the independent prognostic value of the risk score, we performed a multivariate regression analysis. As shown in Fig. 2B, after adjusting for other variables (including age, gender, and metastatic information), we found that the risk score may be an independent predictor. The results of the forest plot show that the higher the risk score, the shorter the overall survival of the patient (hazard ratios: 2.974, 95% of confidence intervals: 2.164–4.088, $P = 1.89e - 11$). The results of the time-dependent ROC analysis show that the risk score has good discriminative ability. As shown in Fig. 2A, no matter how the

patient's survival time changes, the risk score always has an excellent discriminating ability. On the contrary, as the patient's survival time prolongs, the discriminating ability of metastatic status continues to decline.

3.3 Relationship between risk score and clinical characteristics

To further explore the strength of the risk score, we divided patients into 6 subgroups based on their age, gender and metastatic status, and explored the prognostic value of risk scores in each subgroup. As shown in Fig. 3A, the risk score shows a good prognostic value in each subgroup. Metastatic status is an important basis for formulating treatment plans for patients with osteosarcoma. Therefore, we further divided patients into 4 groups according to their metastatic status and risk status. As shown in Fig. 3B, there was no significant difference in overall survival rate between metastatic patients and non-metastatic patients in the low-risk group. Among patients in the metastasis group, patients in the low-risk group had a longer overall survival than those in the high-risk group.

3.4 Evaluation of differences in immune infiltration between high- and low-risk groups

We further evaluated the differences in immunological characteristics between the two groups of patients. As mentioned above, the abundance of 10 immune-related cells was calculated using the MCP-counter method. As shown in Fig. 4A, a significant difference was observed between the two groups of patients. Compared with patients in the high-risk group, the abundance of the 8 cell populations in the patients in the low-risk group was higher (B-cell lineage, CD8 + T cells, Cytotoxic lymphocytes, Endothelial cells, Monocytic lineage cells, Neutrophils, NK cells, T cells). Then, the estimate, immune and stromal scores were calculated using the ESTIMATE algorithm. Similarly, patients in the low-risk group had higher estimate scores, immune scores, and stromal scores than those in the high-risk group ($p < 0.001$, Fig. 4B). We further used ssGSEA to evaluate the difference of 29 immune-related gene sets or immune cells between the two groups of patients as a supplement. The results of ssGSEA have reached similar conclusions. Most immune-related gene sets or immune cells are significantly enriched in the low-risk group (Fig. 4C). Finally, we explored the relationship between abundance of 10 immune-related cells and risk score. As shown in Fig. 5, with the increasing risk score, the abundance of immune cells kept decreasing, especially CD8 + T cells.

The differences in gene expression of some immune-related pathways were further evaluated and heatmaps were drawn to show the results. We found that genes related to activated T effector and IFN γ pathway such as STAT1, CCL4, CXCL9, CXCL10 were significantly up-regulated in the low-risk group (Fig. 6B).

3.5 The relationship between signature and the expression of immune checkpoint gene, tumor microenvironment type

As mentioned above, the association between the expression of 7 potentially targetable immune checkpoint genes between the two groups was assessed. As shown in the figure, all immune checkpoint genes in the low-risk group were more highly expressed, although PDCD1 did not show statistical significance (Fig. 6A). Further analysis showed that the CD8A gene is also highly expressed in the low-risk group. Similarly, tumor lymphocyte infiltration was higher in the low-risk group. However, the optimal cut-off values for PD-L1 and CD8A mRNA expression have not been determined. Therefore, we analyzed the relationship between the risk score value and PD-L1 gene expression, CD8A gene expression, and TIL. The results showed that as the risk score increased, the expression of PD-L1 gene and CD8A gene decreased (Fig. 6C-D). Unfortunately, although TIL also showed a similar trend, it did not reach statistical significance (Fig. 6E). However, the results of the box plot show that the TIL of the low-risk group is higher than that of the high-risk group (Fig. 4C).

In addition, we use SubMap analysis to further study the relationship between MRGP signature and immunotherapy efficiency. Using subclass mapping, the expression profiles of the two groups of patients (high-risk group and low-risk group) were compared with a published immunotherapy data set. This data set records the expression data of 47 melanoma patients treated with programmed cell death protein 1 (PD-1) immune checkpoint inhibitors or cytotoxic T lymphocyte associated protein 4 (CTLA-4) immune checkpoint inhibitors. The results showed that the expression profiles of patients in the low-risk group were correlated with those in the PD-L1 response group (Fig. 6F). This indicates that patients in the low-risk group are more likely to benefit from PD-L1 therapy.

3.6 Identifying the differences in biological pathways and processes between the two groups of patients

We used GSVA to study the differences in biological pathways between the two groups of patients. As shown in the Fig. 7, significant differences were observed in the biological pathways between the two groups of patients. We found that in the low-risk group, most immunization-related pathways had higher GSVA scores. It is worth noting that patients in the high-risk group had higher GSVA scores for certain metabolic-related pathways. Supplemental Table 4 shows detailed information on GSVA results.

3.7 Construction of Nomogram Based on Risk Score

We developed a nomogram that combined risk scores with traditional clinical factors (Fig. 8), and tested the accuracy of the nomogram using a calibration curve. As shown in Fig. 8B, the 3-year and 5-year overall survival predictions show that the nomograms have good accuracy. The C index of the nomogram is 0.924, which indicates that our nomogram has a good degree of discrimination. These results suggest that the new nomogram shows reliable performance in predicting patient prognosis. Finally, the results of the DCA analysis show that the model combined with the risk score can bring clinical net benefits.

4 Discussion

Although with the increasing development of limb salvage surgery techniques for osteosarcoma, the patient's postoperative limb function has been greatly improved, but the patient's overall survival is still similar to that of decades ago[24–26]. In recent years, with the continuous development of high-throughput sequencing technology, exploring specific mRNA expressions, such as tumor microenvironment genes and metabolic genes, can help us better identify tumor diversity and develop personalized treatment strategies[27, 28]. This study identified immune-related lncRNA in osteosarcoma patients based on IRG in the Immport database. These lncRNA were then used to construct a signature consisting of 13 lncRNA. Our signature can reliably identify high-risk patients. The results of subgroup analysis and nomogram further support our conclusion. Considering that our signature is based on immune-related lncRNA, we used three methods to study the differences in immune characteristics between the two groups of patients. The results showed that the low-risk group was closely related to immune cell infiltration and immune-related gene expression. In addition, compared with the high-risk group, the immune checkpoint gene expression was higher in the low-risk group. These results indicate that our signature can effectively identify high-risk patients and help to understand the immune microenvironment of osteosarcoma.

Presently, the presence or absence of metastases at diagnosis is still a key indicator for identifying patients with high-risk osteosarcoma[29]. However, some patients have a good prognosis, despite the diagnosis of metastasis. Therefore, effective identification of this part of patients helps to formulate more accurate treatment plans. By means of further analysis of the patient's risk score and metastatic status, we found that the risk score can effectively screen this part of the patient. Compared with metastatic patients in the high-risk group, metastatic patients in the low-risk group had significantly higher overall survival. However, due to the limitation of sample size, further prospective studies are needed to verify our results.

Recently, immunotherapy, especially immune checkpoint inhibitors, have shown great value in the treatment of various tumors[30–32]. But selecting patients who may benefit from this treatment is crucial. Recently, various markers or types have been developed to predict the efficacy of immunotherapy. Among them, the expression of PD-L1 gene has been proved to be an effective biomarker for anti-PD-1 / PD-L1 treatment in various tumor[33–35]. Therefore, we explored the expression differences of 7 immune checkpoint genes including PD-L1 gene between the two groups. The results showed that although the PDCD1 gene did not reach statistical significance, all immune checkpoint genes tended to be highly expressed in the low-risk group. In addition, studies have shown that combining PD-L1 gene expression with tumor lymphocyte infiltration can be very helpful in dividing the patients into four types : type I or adaptive immune resistance (PDL1 (+), TIL (+)), II Type or immunological ignorance (PD-L1) (-), TIL (-), type III (PD-L1 (+), TIL (-)) and type IV or immune tolerance (PD-L1 (-), TIL (+)).Among patients with melanoma, patients with type I are most likely to benefit from immunotherapy[36]. In our study, compared with patients in the high-risk group, patients in the low-risk group were more likely to be classified as type I because of the high expression of the PD-L1 gene and TIL. A recent pan-cancer based study showed

that combining PD-L1 gene and CD8A gene expression can also divide patients into four types: TMIT I (PDL1 (+), CD8A (+)), TMIT II (PDL1 (-), CD8A (-)), TMIT III (PDL1 (+), CD8A (-)), TMIT IV (PDL1 (-), CD8A (+)). From the perspective of pan-cancer genome, TMIT I (PD-L1 + CD8A +) is associated with high PD-L1 expression, high mutation load / new antigen, high MSI, PD-L1 amplification and the presence of oncogenic viruses[20]. In addition, Yu-Pei Chen et al further proved that in some solid tumors, patients with TMIT type I are most likely to benefit from immunotherapy and calculate the optimal cut-off value[37]. Similarly, our results indicate that patients with lower risk scores are more likely to be classified as TMIT I patients. In addition, we compared the expression profiles of osteosarcoma patients with melanoma patients treated with immune checkpoint inhibitors by submap analysis. There is a certain correlation between the expression profiles of the low-risk group and the PD-L1 response group. In conclusion, it is reasonable to speculate that immunotherapy may be an effective treatment for low-risk groups. However, due to the lack of data on immunotherapy in patients with osteosarcoma, we were unable to calculate the cut-off value of the risk score to predict the patient's response to immunotherapy. In addition, further research is also needed to verify the application of risk score in predicting the efficacy of osteosarcoma-immunotherapy.

It must be admitted that our research has some limitations. Firstly, the sample size is relatively small. Although osteosarcoma is a rare tumor with a low incidence, we believe that only a larger sample size will make the conclusion more convincing. Secondly, due to the scarcity of lncRNA research in osteosarcoma, we were unable to find other lncRNA data with clinical information, so we failed to set up external validation. Finally, due to the lack of immunotherapy data of osteosarcoma patients, we could only combine the conclusions of previous studies to speculate the value of risk scores in predicting patients' response to immunotherapy. Further research is needed to prove our conclusion.

5 Conclusion

In conclusion, our signature can effectively predict the overall survival of osteosarcoma patients and can be used as a reliable supplement to the metastatic state to formulate more accurate treatment plans. Finally, our signature is expected to provide some guidance for identifying patients who may benefit from immunotherapy.

Declarations

Ethics approval and consent to participate

No ethical approval nor informed consent was required in this study due to the public-availability of the data used.

Consent for publication

All authors consent to publication.

Competing interests

The authors declare that there are no conflicts of interest.

Author Contributions

L-LQ collected and analyzed the data and wrote the paper. Z-LH and Manhas assisted in collecting the data and participated in the writing. Z-Y, L-XC, Z-Y, W-J, L-T, Y-YB and L-YK assisted in the design of this study. L-JZ is responsible for all the integrity of data and the accuracy of data analysis. All authors have thoroughly revised the manuscript.

Funding

This work was supported by grants from the Department of Henan University of Science and Technology (No.20B320027) to L-JZ and the Science and Technology Department, Henan Province (192102310389 and 182102310370) to ZY.

Acknowledgments

The authors would like to thank the TCGA and TARGET databases for the availability of the data.

Availability of data and materials

RNA-seq data of the TCGA cohort can be obtained from the TCGA database (<https://portal.gdc.cancer.gov>). Clinical data of these patients can be obtained from the TARGET database (<https://ocg.cancer.gov/programs/target>).

References

1. BS M, GM O, EP R, SK R, NK W, MT W, LA M, RS L, NA T, SD M *et al*: **A Sleeping Beauty forward genetic screen identifies new genes and pathways driving osteosarcoma development and metastasis.** *Nature genetics* 2015, **47**(6):615-624.
2. Lindsey BA, Markel JE, Kleinerman ES: **Osteosarcoma Overview.** *Rheumatol Ther* 2017, **4**(1):25-43.
3. Isakoff MS, Bielack SS, Meltzer P, Gorlick R: **Osteosarcoma: Current Treatment and a Collaborative Pathway to Success.** *J Clin Oncol* 2015, **33**(27):3029-3035.

4. Guerra AD, Yeung OWH, Qi X, Kao WJ, Man K: **The Anti-Tumor Effects of M1 Macrophage-Loaded Poly (ethylene glycol) and Gelatin-Based Hydrogels on Hepatocellular Carcinoma.** *Theranostics* 2017, **7**(15):3732-3744.
5. Wan MT, Ming ME: **Nivolumab versus ipilimumab in the treatment of advanced melanoma: a critical appraisal: ORIGINAL ARTICLE: Wolchok JD, Chiarion-Sileni V, Gonzalez R et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma.** *N Engl J Med* 2017; **377**:1345-56. *Br J Dermatol* 2018, **179**(2):296-300.
6. Long GV, Atkinson V, Lo S, Sandhu S, Guminski AD, Brown MP, Wilmott JS, Edwards J, Gonzalez M, Scolyer RA *et al.* **Combination nivolumab and ipilimumab or nivolumab alone in melanoma brain metastases: a multicentre randomised phase 2 study.** *Lancet Oncol* 2018, **19**(5):672-681.
7. A LC, P M-B, JY B, N G, F B, N P, E B, S C, M T, A B *et al.* **Programmed cell death 1 (PD-1) targeting in patients with advanced osteosarcomas: results from the PEMBROSARC study.** *European journal of cancer (Oxford, England : 1990)* 2019, **119**:151-157.
8. M K, MW T, MJ S, DM T: **Translational biology of osteosarcoma.** *Nature reviews Cancer* 2014, **14**(11):722-735.
9. Engreitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, McDonel PE, Guttman M, Lander ES: **Local regulation of gene expression by lncRNA promoters, transcription and splicing.** *Nature* 2016, **539**(7629):452-455.
10. Zhan Y, Chen Z, He S, Gong Y, He A, Li Y, Zhang L, Zhang X, Fang D, Li X *et al.* **Long non-coding RNA SOX2OT promotes the stemness phenotype of bladder cancer cells by modulating SOX2.** *Mol Cancer* 2020, **19**(1):25.
11. Shi X, Sun M, Liu H, Yao Y, Song Y: **Long non-coding RNAs: a new frontier in the study of human diseases.** *Cancer Lett* 2013, **339**(2):159-166.
12. Ba Z, Gu L, Hao S, Wang X, Cheng Z, Nie G: **Downregulation of lncRNA CASC2 facilitates osteosarcoma growth and invasion through miR-181a.** *Cell Prolif* 2018, **51**(1).
13. Ye H, Lin J, Yao X, Li Y, Lin X, Lu H: **Overexpression of Long Non-Coding RNA NNT-AS1 Correlates with Tumor Progression and Poor Prognosis in Osteosarcoma.** *Cell Physiol Biochem* 2018, **45**(5):1904-1914.
14. S B, S A, L G, P D, H S, J P, P B, V D, T S, J C *et al.* **ImmPort: disseminating data to the public for the future of immunology.** *Immunologic research* 2014, **58**:234-239.
15. PJ H, T L, MS P: **Time-dependent ROC curves for censored survival data and a diagnostic marker.** *Biometrics* 2000, **56**(2):337-344.
16. Zhang CB, Zhu P, Yang P, Cai JQ, Wang ZL, Li QB, Bao ZS, Zhang W, Jiang T: **Identification of high risk anaplastic gliomas by a diagnostic and prognostic signature derived from mRNA expression profiling.** *Oncotarget* 2015, **6**(34):36643-36651.
17. E B, NA G, L L, B B, N E, F P, J S, P L-P, C S-F, WH F *et al.* **Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression.** *Genome biology* 2016, **17**(1):218.

18. K Y, M S, E M, R V, H K, W T-G, V T, H S, P W L, D A L *et al*: **Inferring tumour purity and stromal and immune cell admixture from expression data.** *Nature communications* 2013, **4**:2612.
19. D A B, P T, J S B, S Y K, S E M, I F D, A C S, P S, E M, C S *et al*: **Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1.** *Nature* 2009, **462**(7269):108-112.
20. C Y O, B K, S K, J S L, M K, T M K, Y K J, D W K, D H C, D S H: **Pan-Cancer Immunogenomic Perspective on the Tumor Microenvironment Based on PD-L1 and CD8 T-Cell Infiltration.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 2016, **22**(9):2261-2270.
21. Y H, J P B, P T, T R G, J P M: **Subclass mapping: identifying common subtypes in independent disease data sets.** *PLoS one* 2007, **2**(11):e1195.
22. W R, P L C, A R, C N S, P A P, J P M, V G, F W, Z A C, S M R *et al*: **Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance.** *Science translational medicine* 2017, **9**(379).
23. S H, R C, J G: **GSVA: gene set variation analysis for microarray and RNA-seq data.** *BMC bioinformatics* 2013, **14**:7.
24. A T, N Y, K H, H M, S M, K I, H T: **Joint-preservation surgery for pediatric osteosarcoma of the knee joint.** *Cancer metastasis reviews* 2019, **38**(4):709-722.
25. N M M, S S, S S B, M B, G J, M D K, J M H, C A, H v d B, B B *et al*: **Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial.** *The Lancet Oncology* 2016, **17**(10):1396-1408.
26. E K: **Maximum benefit of chemotherapy for osteosarcoma achieved-what are the next steps?** *The Lancet Oncology* 2016, **17**(10):1340-1342.
27. R D R, M M L, D R R, P H, J G, W A-R, T F, C K, E A S-C, T C *et al*: **Provocative questions in osteosarcoma basic and translational biology: A report from the Children's Oncology Group.** *Cancer* 2019, **125**(20):3514-3525.
28. R Z, D Z, J Z, H S, J W, N L, L L, M S, J B, Y L *et al*: **A robust panel based on tumour microenvironment genes for prognostic prediction and tailoring therapies in stage III colon cancer.** *EBioMedicine* 2019, **42**:420-430.
29. J S W, L E D: **Osteosarcoma, Chondrosarcoma, and Chordoma.** *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2018, **36**(2):188-193.
30. S L T, F S H, J R B, S N G, D C S, D F M, J D P, R D C, J A S, M B A *et al*: **Safety, activity, and immune correlates of anti-PD-1 antibody in cancer.** *The New England journal of medicine* 2012, **366**(26):2443-2454.
31. O H, C R, A D, F S H, W J H, R K, J D W, P H, R W J, J S W *et al*: **Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma.** *The New England journal of medicine* 2013, **369**(2):134-144.
32. J B, K L R, P B, L C, W E E, E P, S A, A P, E E V, E H *et al*: **Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer.** *The New England journal of medicine* 2015, **373**(2):123-

135.

33. EB G, NA R, R H, N L, AS B, JP E, A P, C A, M G, L H *et al*: **Pembrolizumab for the treatment of non-small-cell lung cancer**. *The New England journal of medicine* 2015, **372**(21):2018-2028.
34. G R, SP C, G N, E M: **Results from a meta-analysis of immune checkpoint inhibitors in first-line renal cancer patients: does PD-L1 matter?** *Therapeutic advances in medical oncology* 2019, **11**:1758835919861905.
35. K G, E A, M K, D H-B, L R-R, CM B, FE V-B: **PD-L1 expression and clinical outcomes in patients with advanced urothelial carcinoma treated with checkpoint inhibitors: A meta-analysis**. *Cancer treatment reviews* 2019, **76**:51-56.
36. MW T, SF N, A R, MJ S: **Classifying Cancers Based on T-cell Infiltration and PD-L1**. *Cancer research* 2015, **75**(11):2139-2145.
37. YP C, Y Z, JW L, YQ L, YQ W, QM H, XJ Y, Y S, YP M, JP Y *et al*: **Genomic Analysis of Tumor Microenvironment Immune Types across 14 Solid Cancer Types: Immunotherapeutic Implications**. *Theranostics* 2017, **7**(14):3585-3594.

Figures

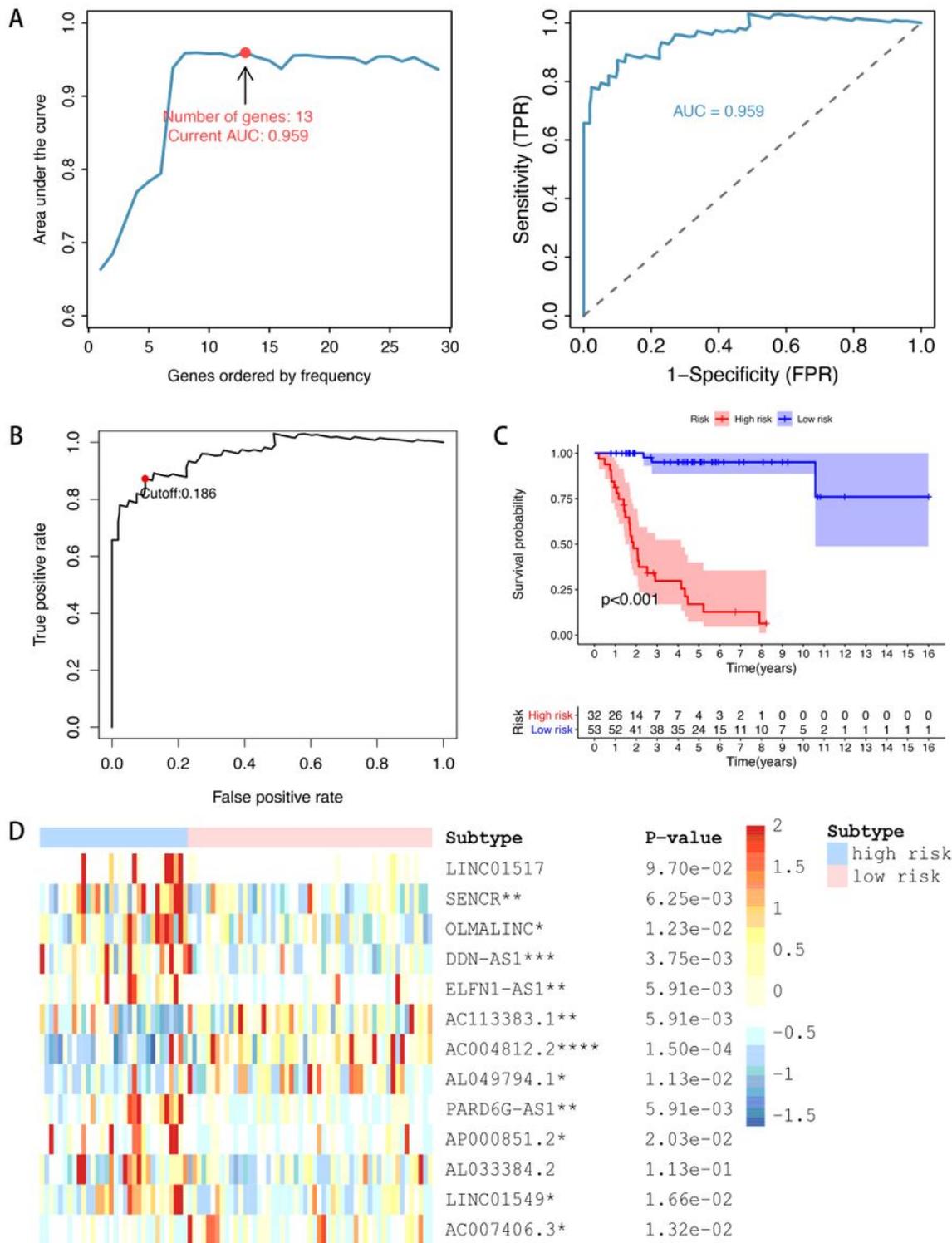


Figure 1

Construction of lncRNA-based signature. (A) Calculate the area under the receiver operating characteristic curve (AUROC) and identify its peak value. (B) Time-dependent ROC curve of lncRNA signature. The optimal cut-off value of lncRNA signature is 0.186, and patients are divided into high-risk group and low-risk group according to the cut-off value (C) Kaplan–Meier curves of overall survival according to lncRNA

signature groups in the TCGA cohort. (D) Heat map of the expression of lncRNA constituting the signature in two groups of patients.

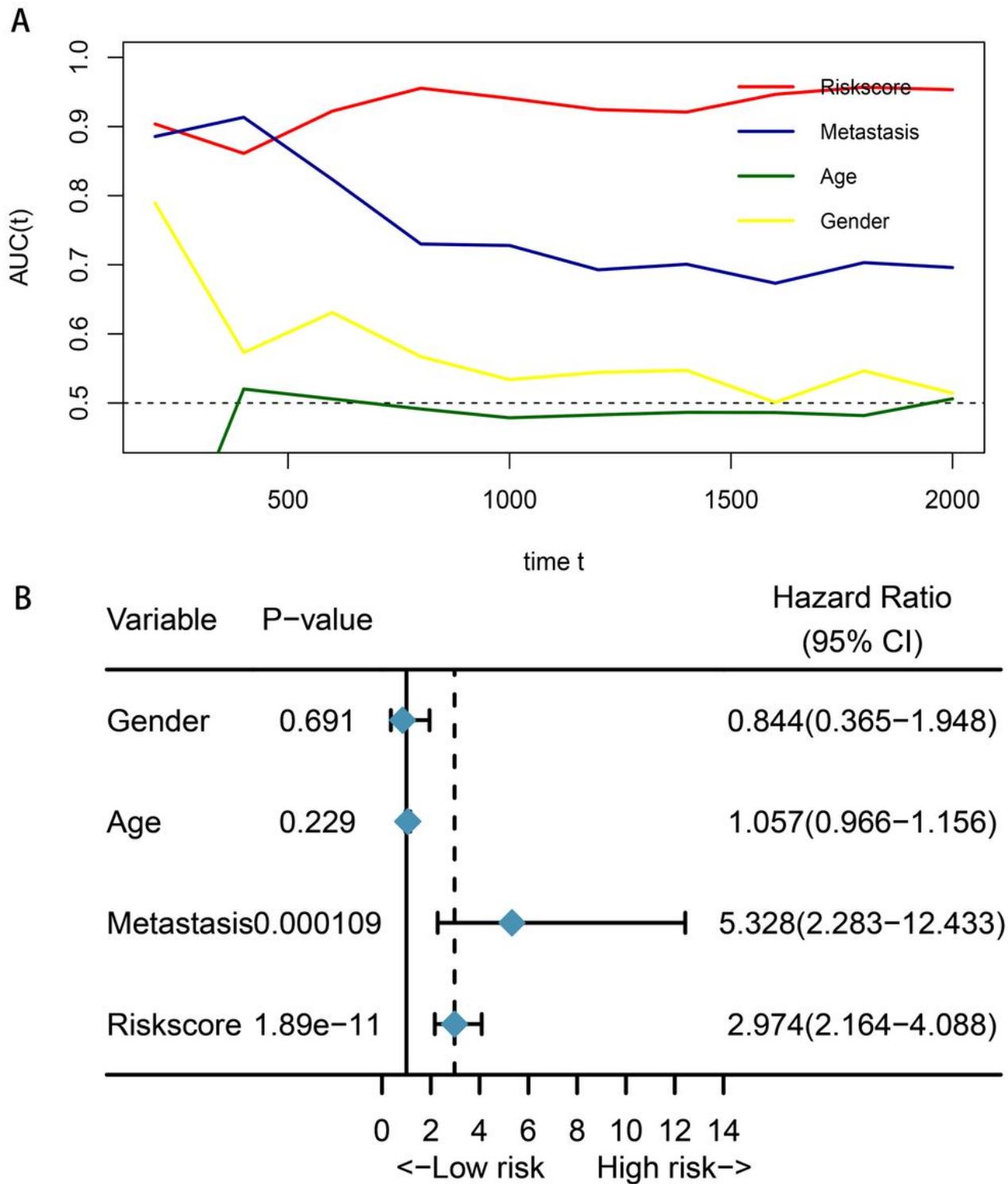


Figure 2

Evaluate whether lncRNA signature is an independent prognostic factor. (A) ROC curve of clinical characteristics and lncRNA signature. (B) Forest plot of multivariate Cox regression results of lncRNA signature and clinical characteristics.

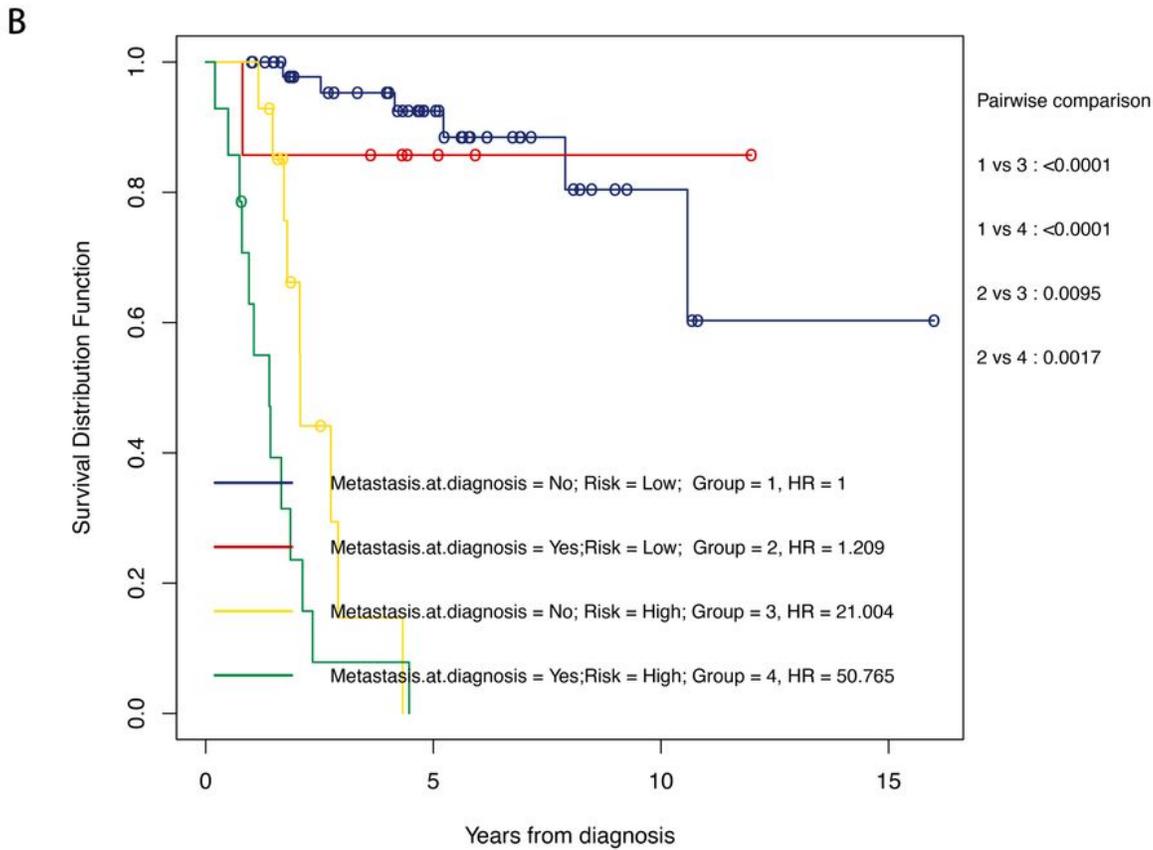
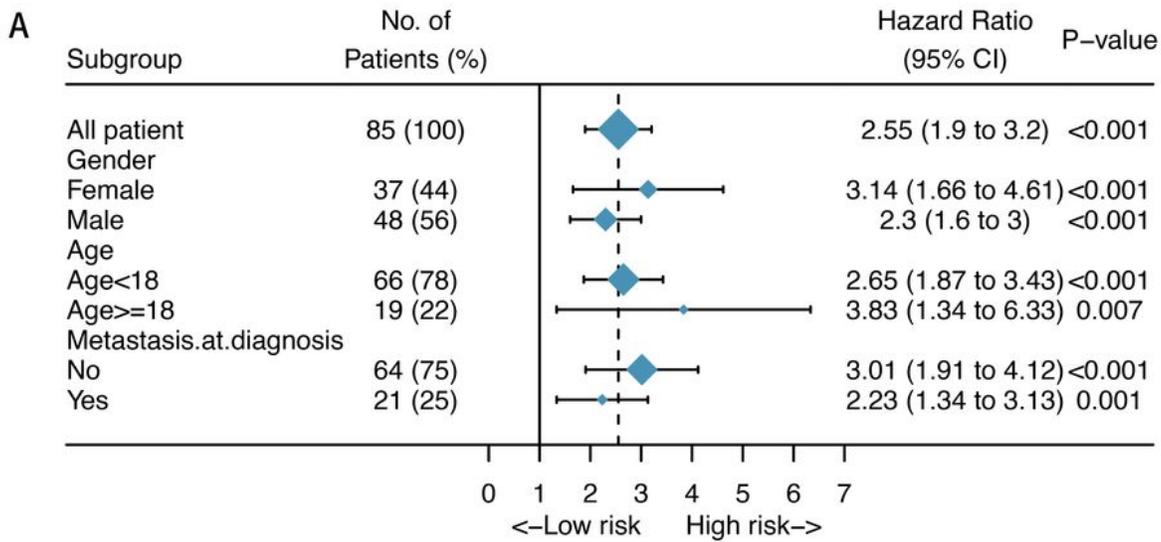


Figure 3

Subgroup analysis based on clinical characteristics. (A) Forest plot to assess the prognostic value of lncRNA signature in subgroups. (B) KM survival curve of four groups of patients classified by signature and metastatic status.

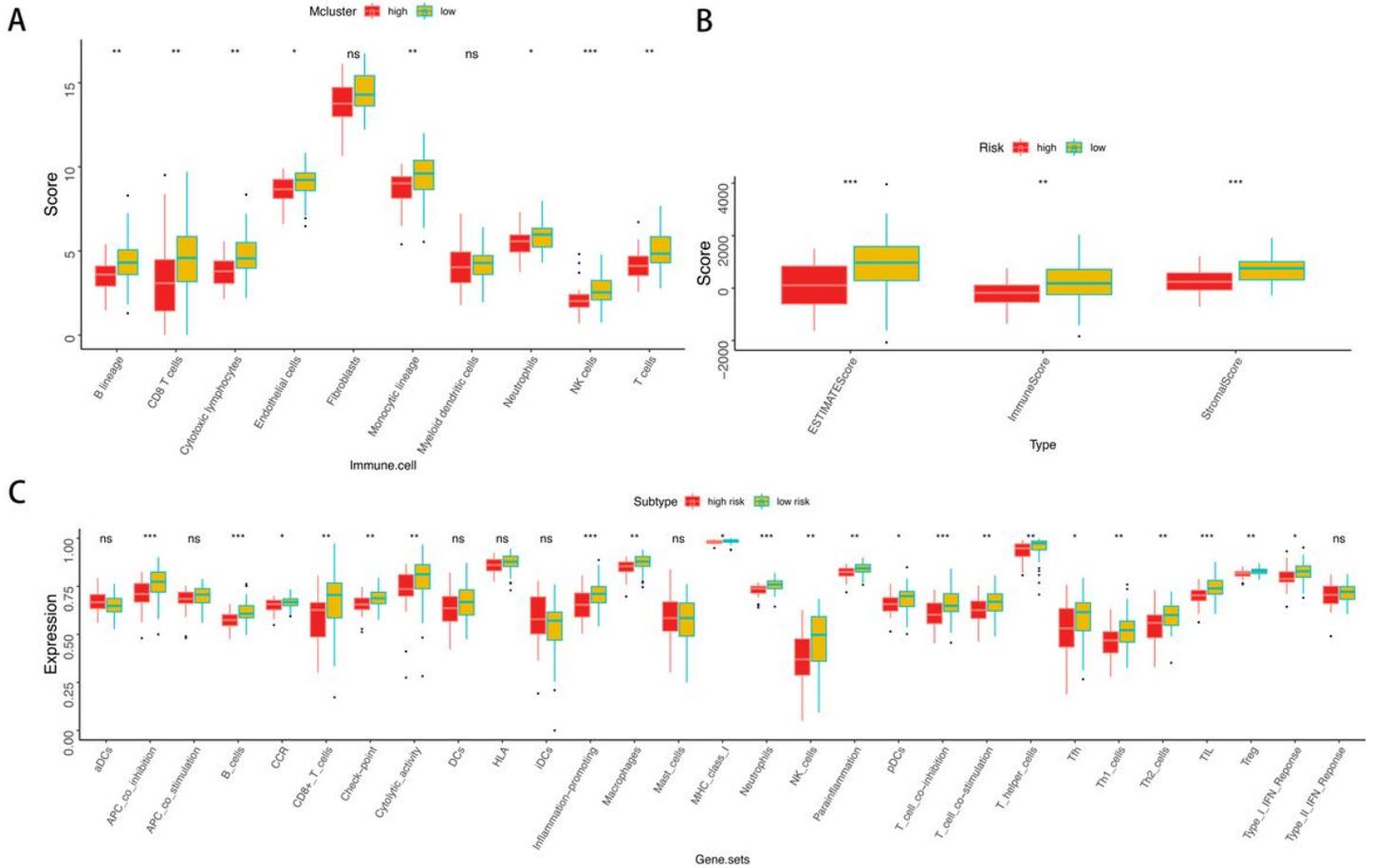


Figure 4

Assess the difference in immune infiltration between the two groups. (A) Differences of 10 cell abundances calculated by MCP-counter method between two groups of patients. (B) Box plot of stromal score, immune score and estimate score calculated by ESTIMATE method. (C) Box plot of 29 gene sets calculated by ssGSEA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

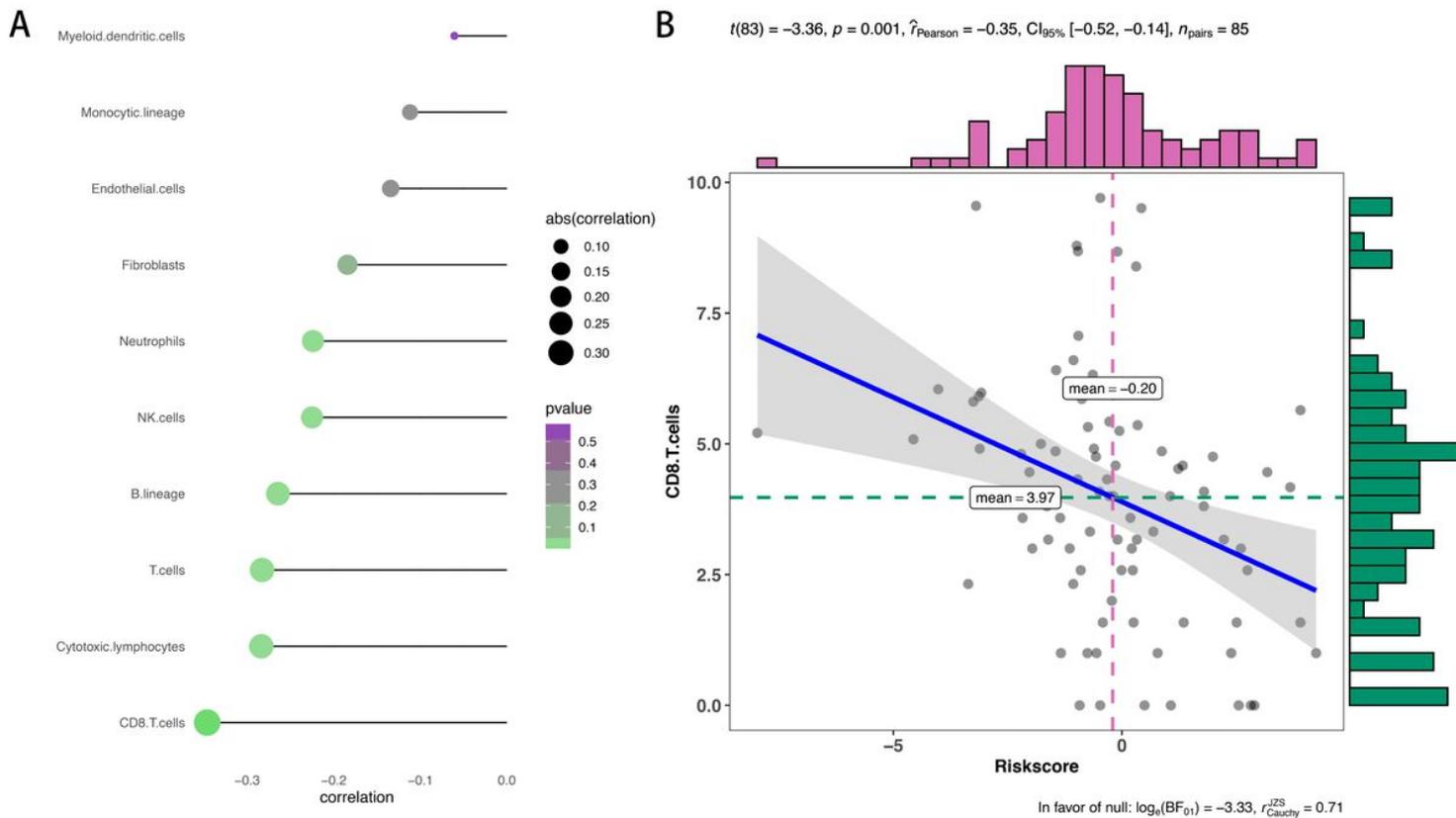


Figure 5

Assess the relationship between immune infiltration and risk score. (A) Assess the relationship between abundance of 10 immune-related cells and risk score. (B) Assess the relationship between CD8.T cell abundance and risk score.

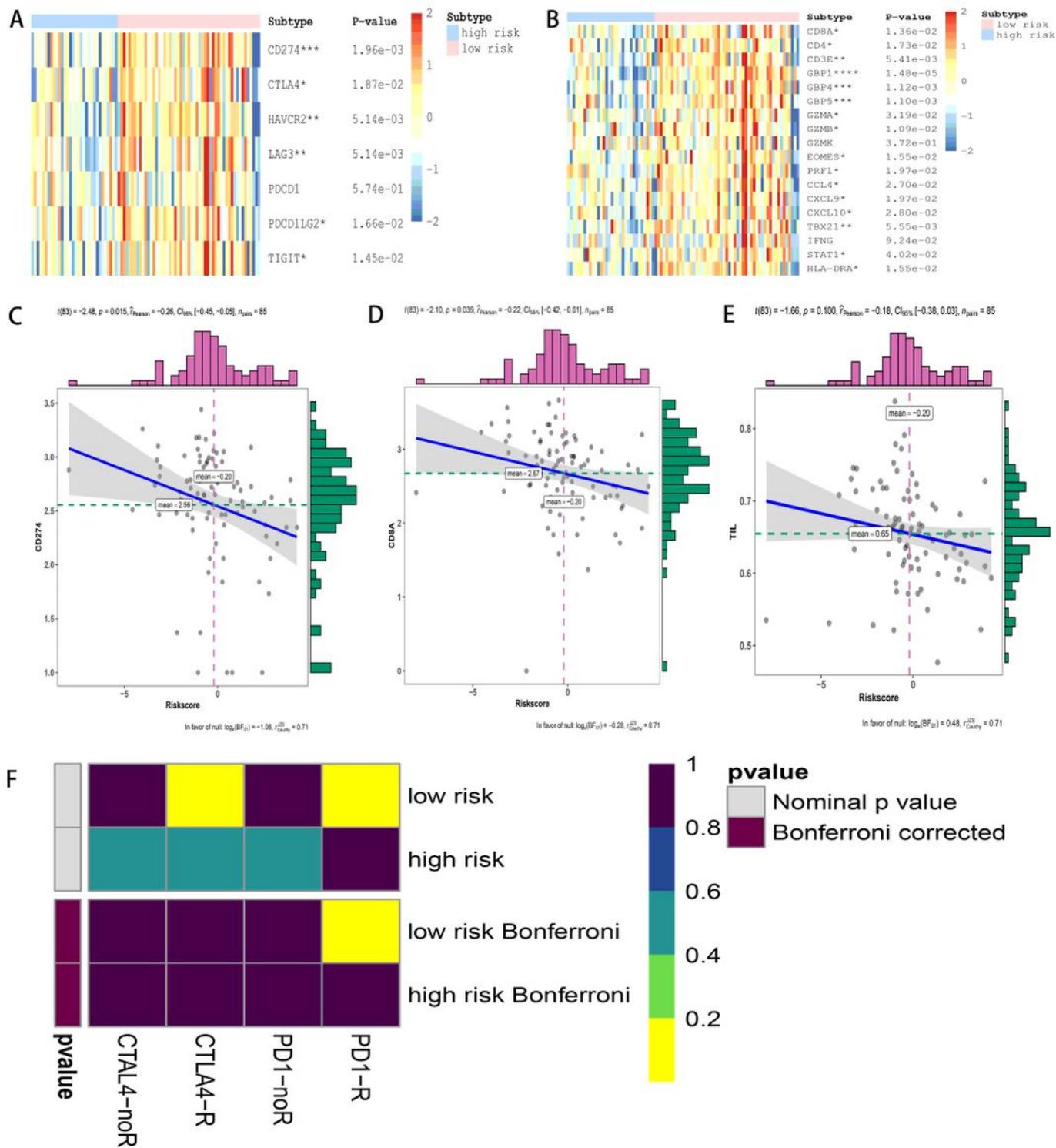


Figure 6

Assess the patient's Tumor microenvironment immune type. (A) Heat map of 7 immune checkpoint gene expressions in two groups of patients. (B) Heat map of expression of immune-related genes in two groups of patients. (C) Relationship between CD274 expression and risk score. (D) Relationship between CD8A expression and risk score. (E) Relationship between TIL and risk score. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

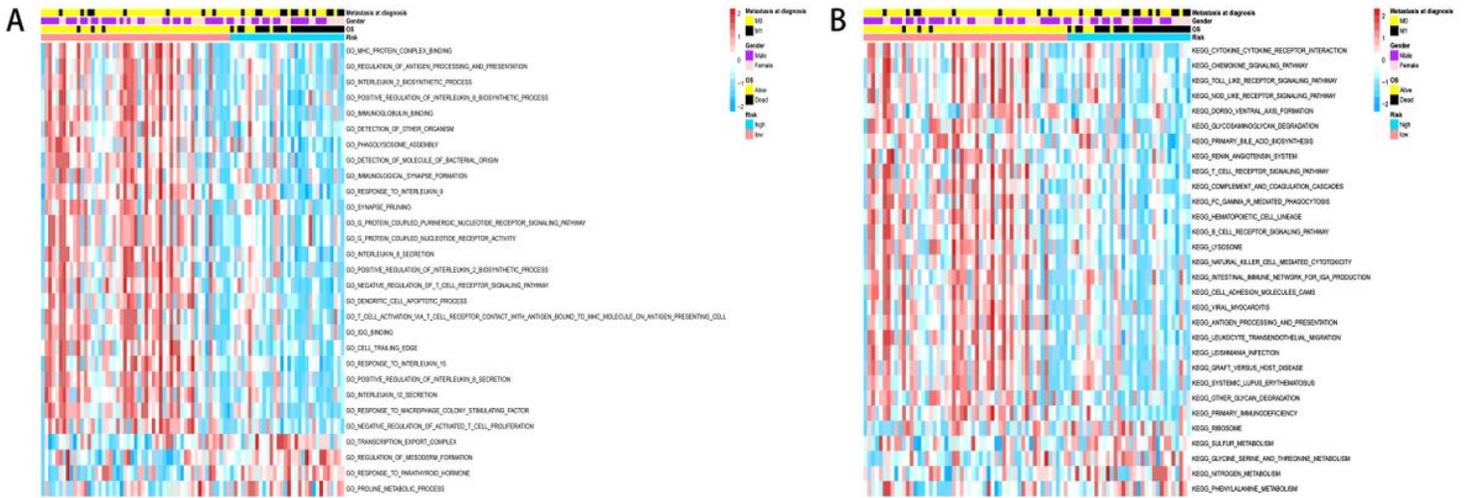


Figure 7

Biological function of two groups of patients. (A) Heat map of KEGG pathway score calculated by GSVA for two groups of patients. (B) Heat map of GO score calculated by GSVA for two groups of patients.

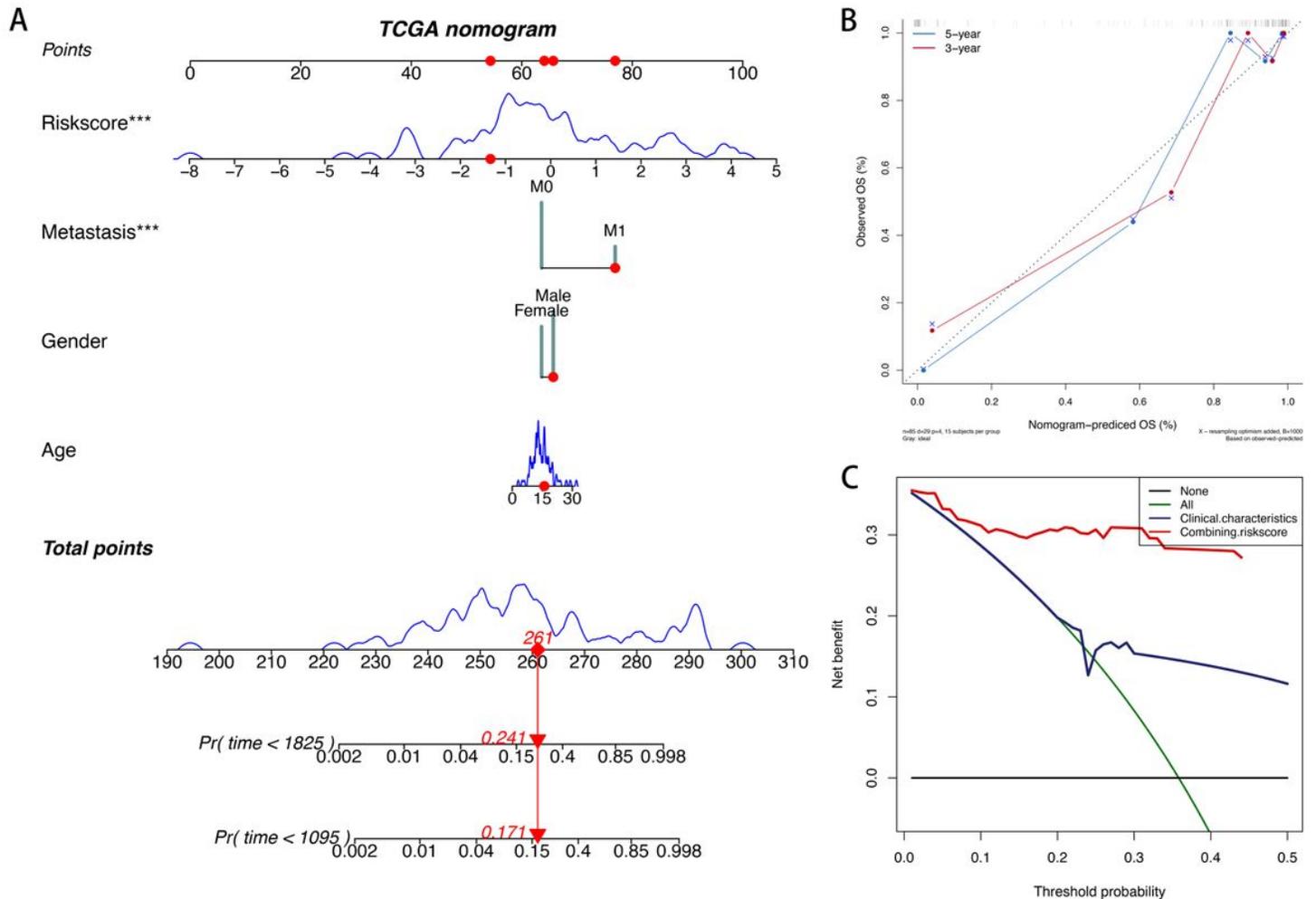


Figure 8

Construct and evaluate nomogram. (A) Nomograms for predicting the probability of patient mortality based on lncRNA signature and clinical variables. (B) The calibration plot for internal validation of the nomogram. (C) Decision curve analyses of the nomograms based on lncRNA signature for 3-year overall survival. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarytable.xlsx](#)
- [SupplementaryFigure2.tif](#)
- [SupplementaryFigure1.tif](#)