The Signature of Glycometabolism-Related Genes in Predicting the Prognosis of Patients with Osteosarcoma

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Abstract

Background

Osteosarcoma is a primary malignant bone tumor with high metastatic potential and an inferior prognosis. Glycometabolism also plays a role in the disease. However, the clinical significance of glycometabolism-related genes in patients with osteosarcoma has been unclear.

Methods

We downloaded the expression profile and corresponding clinical data of osteosarcoma samples from database. Glycometabolism-related gene sets were obtained. Regression analyses were performed to construct a glycometabolism-related prognostic gene signature. The independent prognostic value of the signature was further assessed by univariate and multivariate Cox regression analysis, and the correlation between immune cells and the signature was investigated. The regulatory mechanism of the prognostic genes was explored by constructing a ceRNA network.

Results

A glycometabolism-related prognostic gene signature based on *PRKACB*, *SEPHS2*, *GPX7*, and *PFKFB3* was constructed, and the survival and receiver operating characteristic curves showed that the glycometabolism-related gene signature had a good performance in predicting the overall survival of patients with osteosarcoma. Univariate and multivariate Cox regression analyses confirmed that the glycometabolism-related gene signature was an independent prognostic factor among patients. Correlation analysis revealed that *SEPHS2*, *PRKACB*, and *GPX7* were correlated with immune cells. A ceRNA network comprised of four genes, 148 miRNAs, and 91 lncRNAs was constructed.

Conclusions

A novel signature based on four glycometabolism-related genes, was constructed, which may facilitate the prognosis and treatment of osteosarcoma in clinical practice.

Introduction

Osteosarcoma (OS) is a malignancy derived from primitive osteogenic mesenchymal cells. It is the most common type of malignant bone tumor among children and adolescents [1]. OS occurs mostly in the metaphysis of long bones, especially around the knees, and is characterized by high rates of metastasis and progression [2]. Along with the improvement of neoadjuvant therapy and surgical resection, more than two-thirds of patients with localized lesions are likely to achieve long-term survival [3; 4]; however, approximately 30% of patients with non-metastasis at diagnosis suffer from concomitant lung

metastasis after comprehensive treatment. The 5-year survival rate of patients with distant metastasis at diagnosis is still unfavorable, and effective therapeutic interventions for patients with metastatic OS or chemoresistance are lacking [5]. Therefore, it is important to identify new prognostic biomarkers for early diagnosis and to investigate novel targets for OS treatment.

Abnormal glycometabolism has been reported as one of the hallmarks of how malignant tumor cells arise [6]. It is characterized by increased glucose consumption for rapid proliferation and invasion [7]. Aerobic glycolysis, also termed the Warburg effect, is widely and preferentially used in the glycometabolism pathway in tumor cells [8]. Multiple studies suggested that aerobic glycolysis is a critical step in the initiation, proliferation, and migration of OS [9-11]. Considering these findings, targeting the glycolytic pathway may be an attractive therapeutic option for the treatment of OS; however, the function and underlying mechanisms of glycometabolism in OS development remain largely unknown.

In this study, we performed a comprehensive bioinformatics analysis to screen glycometabolism-related genes in OS progression, and investigated the potential molecular mechanisms of abnormal glycometabolism-mediated OS development to explore novel therapeutic strategies for the treatment of OS.

Materials And Methods

Data acquisition

The expression profile and corresponding clinical data of OS samples were downloaded from the TARGET database (TTD, <http://bidd.nus.edu.sg/group/ttd/ttd.asp>) [12]. The dataset includes 85 OS samples with survival information that were divided into training (n=43) and testing (n=42) cohorts with a proportion of 1:1 randomly. Moreover, the GSE39055 dataset, that included 36 OS samples with survival information was downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) as a validation cohort. According the GO Annotation, glycometabolism genes were identified in the molecular signatures database (MSigDB) (<https://www.gsea-msigdb.org/gsea/msigdb/index.jsp>) [13]. Then eight glycometabolism gene sets were extracted after removing overlapping genes. The entire glycometabolism gene sets contained 291 genes, and 282 genes remained after removing genes whose expression was zero in 50% of the samples.

Construction and validation of a glycometabolism-related genes signature

Univariate Cox analysis was performed to assess the prognostic value of glycometabolism-related genes which with a $P < 0.05$ were considered potential prognostic genes. Next, the Least Absolute Shrinkage and Selection Operator (LASSO) regression algorithm was used to construct an optimal glycometabolism-related gene signature. The risk score for each patient was calculated as follows:

$$[\text{risk score} = \sum_{i=1}^n \beta_i \times \text{Exp}_{\text{gene}}(i)]$$

, where n is the number of genes in this prognosis model, beta (β) is the

regression coefficient, and *Exp_{gene}* is the expression level of each gene. The patients from the training cohort were divided into high- and low-risk groups based on the median value of the risk score. Moreover, Kaplan-Meier curves and receiver operating characteristic (ROC) curves in testing and validation cohort were used to assess the prognostic capacity of the gene signature.

Evaluation of the relationship between glycometabolism-related gene signature and clinical characteristic

Clinical data were extracted from the whole TARGET cohort (including samples in the training and testing cohorts), including gender and age. The correlation between the glycometabolism-related gene signature and clinical characteristics calculated by *t*-test. A result of $P < 0.05$ indicates that the risk score is significantly different between the two groups. Meanwhile, we also analyzed the correlation between the risk score and clinical characteristics in the cohorts, indicating that the risk score was significantly different between the two groups.

Independent prognostic analysis and construction of a nomogram

The univariate and multivariate Cox regression analyses were used to assess whether the risk score could be used as an independent prognostic factor in the whole TARGET cohort (including samples in the training and testing cohorts). The results of multivariate regression analysis were used to establish a nomogram to predict the 1-, 3-, and 5-year overall survival. Moreover, the discrimination and accuracy of the nomogram were assessed by the concordance index (C-index), calibration curves, and decision curves.

Functional enrichment analysis

The “limma” package was used to analyze the all gene expression profiles between the high- and low-risk groups in the training and testing cohort. Gene set enrichment analysis (GSEA) was performed to analysis the biological functions of the gene. Gene Ontology (GO; <http://www.geneontology.org>) and Kyoto Encyclopedia of Genes and Genomes (KEGG <http://www.kegg.jp/> or <http://www.genome.jp/kegg/>) pathway enrichment analyses were performed using the ClusterProfiler package [14,15]. We also compared the single-sample GSEA (ssGSEA) scores between the high- and low-risk groups based on gene expression profiles involved in the top 10 GO and KEGG terms [16].

Immune cell analysis

To analyze the differences in the proportion of 28 immune cell types between the high- and low-risk groups, ssGSEA was performed in the training and testing cohorts. Then we used “psych” package of R performed a correlation analysis between the proportion of the 28 immune cells and the gene signature.

Construction of a competitive endogenous RNA (ceRNA) network

Micro RNAs (miRNAs) that can regulate genes in the signature were predicted based on the miRanda software. Then long non-coding RNAs (lncRNAs) were predicted through the miRanda software by

miRNAs. To improve the accuracy of the competitive endogenous RNA (ceRNA) network, we further screened the results using the following criteria: combined score > 200 was set as the screening criteria of lncRNA-miRNA interactions, and combined score > 200 and MFE score < -200 were set as the screening criteria of miRNA-gene interactions. Finally, a ceRNA network was visualized by Cytoscape.

Statistical analysis

All the above analyses were completed by the R software. A time-dependent ROC analysis was performed by the “pROC” package [17]. In addition, a nomogram was constructed by the “rms” packages [18]. P -value < 0.05 was considered statistically significant.

Results

Identification of the four glycometabolism-related gene signatures

Univariate Cox regression analysis identified 19 glycometabolism-related genes in the training set, which were correlated with the survival of patients with OS (Figure 1A). LASSO regression analysis identified four glycometabolism-related genes, including *PRKACB*, *SEPHS2*, *GPX7*, and *PFKFB3* (Figure 1B and 1C). Subsequently, combining the gene expression and corresponding regression coefficient, a risk-scoring model was established using the following formula: [risk score = expression level of *PRKACB* × (-0.10894429) + expression level of *SEPHS2* × (-0.01563243) + expression level of *GPX7* × 0.32559346 + expression level of *PFKFB3* × 0.55009921]. According to the median risk score, patients were divided into high- and low-risk groups (Figure 1D). The K-M survival analysis showed that patients in the high-risk group had significantly shorter overall survival than those in the low-risk group. Furthermore, patients with low-risk scores had a survival advantage over those with a high-risk score (Figure 1E), and the area under the ROC curve (AUC) was 0.904, 0.939, 0.900, 0.900, and 0.919 for 1-, 2-, 3-, 4-, and 5-year survival, respectively (Figure 1F).

Validation of the glycometabolism-related gene signature

The Kaplan-Meier curve indicated that patients of the high-risk group had a significantly shorter overall survival than patients of the low-risk group, and the overall survival was significantly decreased with increasing risk score (Figure 2A and 2B), in the testing cohort. The AUC for 1-, 2-, 3-, 4-, and 5-years survival was 0.687, 0.730, 0.729, 0.729, and 0.718, respectively (Figure 2C). The Kaplan-Meier curve showed fewer deaths among patients of the low-risk group than patients of the high-risk group, in the GSE39055 cohort (Figure 2D). The number of patients died rose as the risk score increased (Figure 2E). The AUC for 1-, 2-, 3-, 4-, and 5-years reached 0.907, 0.825, 0.743, 0.743, and 0.743, respectively (Figure 2F). These results indicated that the gene prognostic signature had a robust predictive performance.

Correlation analysis of risk score and clinical features

To assess the relationship between the risk score and clinical features, we profiled the risk score, gender, and age by t -test. The correlation between the risk score and clinical features in the training cohort is

shown in Figure 3A. Similarly, the correlation between the risk score and clinical features was also analyzed in the testing and validation cohort (Figure 3B and 3C). The results showed that age and gender were not correlated with the risk score in the training cohort (Table S1), and neither age nor gender was correlated with the risk score in the testing and validation cohort (Table S2 and 3).

Independent prognostic value of glycometabolism-related gene signature

To assess the independent prognostic value of the glycometabolism-related gene signature, univariate and multivariate Cox regression analyses were used on the risk score and clinical characteristics. Univariate analysis identified that the risk score was an independent factor affecting overall survival (Figure 4A). Then, multivariate analysis showed that the risk score was an independent prognostic factor (Figure 4B). To better predict the prognosis of OS patients in different years after diagnosis, a new nomogram was constructed based on the independent prognostic factors (Figure 4C). When the total score of the patient rose, the prognosis became poor. The C-index of the new risk model was 0.76, and the calibration curve was close to the ideal curve, indicating that the nomogram had a good predictive ability (Figure 4D). Furthermore, the decision curve analysis results indicated that the model can be applied (Figure 4E).

Functional enrichment analysis

We conducted GSEA to determine the biological functions of DEGs between the high- and low-risk groups. The results of GO enrichment analysis showed that the DEGs between the high- and low-risk groups were mainly enriched in pathways of the actin filament organization, actin polymerization or depolymerization, activation of innate immune response, adaptive immune response, antigen processing and presentation, and antigen receptor-mediated signaling pathway. The top 10 GO biological processes are shown in Figure 5A. Results from KEGG analysis showed that the DEGs were mainly involved in allograft rejection, antigen processing and presentation, autoimmune thyroid disease, cell adhesion molecule cams, chemokine signaling pathway, complement and coagulation cascades, cytokine-cytokine receptor interaction, graft-versus-host disease, hematopoietic cell lineage, and Leishmania infection pathway (Figure 5B). The pathway score of each sample was calculated by ssGSEA, and the score of the high- and low-risk groups was displayed in the heat map (Figure 5C and 5D).

Immune cell analysis

We next evaluated immune infiltration to describe their immune landscape, and an abundance of 28 immune-correlated cell populations were computed in the high- and low-risk groups using the ssGSEA (Figure 6A). Computation showed differential expression in immune cells between the high- and low-risk groups (Figure 6B). *SEPHS2* was positively correlated with 14 immune cells, *PRKACB* was positively correlated with 17 immune cells, and *GPX7* was negatively correlated with 11 immune cells (Figure 6C).

Construction of a ceRNA network

MiRNAs and lncRNAs correlated with the prognostic genes were screened out using the miRanda software. Then a glycometabolism-related ceRNA network was established by integrating lncRNA-miRNA interactions with miRNA-gene interactions. This network contained four prognostic genes, 148 miRNAs, and 91 lncRNAs (Figure 7).

Discussion

In this study, data acquired from the MSigDB, TARGET and GEO database were used for bioinformatics analysis. Four glycometabolism-related prognostic genes (*PRKACB*, *SEPHS2*, *GPX7*, *PFKFB3*) were identified using the univariate Cox regression analysis followed by LASSO regression analysis. A risk model was established based on these genes. Further analysis showed that the risk model had a good predictive performance and application value. Moreover, immune cell analysis indicated that three prognostic genes including *SEPHS2*, *GPX7*, and *PFKFB3*, were correlated with different proportions of immune cells. To reveal the upstream regulatory mechanisms of these four genes, a ceRNA network was created to show the targeting miRNAs and corresponding lncRNAs related to the prognostic genes. These results may shed new light on the underlying mechanisms of OS from the perspective of glycometabolism.

Glycometabolism in tumor cells is emerging as enhanced glucose uptake and aerobic glycolysis [8]. Aerobic glycolysis allows the conversion of glucose into pyruvate eventually contributing to the production of lactate. This energy metabolic reprogramming promotes energy generation and thus facilitates OS cells proliferation, invasion, and chemoresistance [19]. Several factors including glycolytic enzymes (e.g., GLUT1), oncogenes (e.g., KRT17), transcription factors (e.g., HIF1 α), tumor suppressors (e.g., p53), and related signaling pathways have been reported to regulate the glycometabolism in OS cells and play pivotal roles in overall survival of patients [20–22]. These factors may help formulate therapeutic strategies.

This study identified four glycometabolism-related prognostic genes including *PRKACB*, *SEPHS2*, *GPX7*, and *PFKFB3*. The risk model based on the four prognostic genes showed a good predictive performance in OS patients. Previous studies have suggested that these glycometabolism-related genes are involved in tumorigenesis. Lower *PRKACB* expression was found in colorectal carcinoma and breast tumor tissues, and was significantly associated with unfavorable overall survival in patients with such tumors [23, 24]. It was shown that *SEPHS2* was elevated in breast tumor samples, and *SEPHS2* overexpression was correlated with malignancy. This suggests that *SEPHS2* may serve as a prognostic marker and therapeutic target for patients with breast cancer [25, 26]. *GPX7* is a member of the glutathione peroxidase (GPx) family with weak GPx activity [27]. *GPX7* has been confirmed to inhibit tumorigenesis and function as a tumor suppressor [27–30]. This study investigates the prognostic value of these three genes in OS. Accumulated studies have demonstrated that *PFKFB3* is a key regulator of glycolysis in tumorigenesis, angiogenesis chemoresistance, and tumor microenvironment [31]. Furthermore, several studies indicated that upregulating *PFKFB3* accelerates cell growth and metastasis, making it a potential biomarker for OS [32–34]. Few studies have focused on the molecular mechanisms of these genes in the

pathogenesis of OS. However, their roles in regulating the glycometabolism of OS are warranted in further studies.

To identify more details about the genes mentioned, patients collected from TARGET were divided into high- and low-risk groups based on the median value of the risk score. GSEA was performed to investigate the biological functions of DEGs between groups. These DEGs were significantly enriched in multiple biological processes and signaling pathways. Both GO and KEGG analyses indicated that DEGs were significantly associated with the immune-related processes and pathways. The tumor immune microenvironment has been implicated in the occurrence and progression of OS [35]. Local tumor-infiltrating immune cells function as key regulators in OS cells growth and invasion [36]. In the present study, 26 immune cells were found differentially expressed between groups. Further analysis showed that three genes, including *PRKACB*, *SEPHS2*, and *GPX7*, were significantly associated with different immune cells. The results indicated that the three genes may be involved in the tumorigenesis of OS by regulating the glycometabolism and immune microenvironment.

In order to analysis of the functions of these genes deeply, we constructed the lncRNA-miRNA network. CeRNA network is a prevalent form of post-transcriptional regulation of gene expression in mammals [37]. The mRNA, lncRNA, pseudogene, and circular RNA can affect the stability or translation activity of target RNAs by competitively binding to miRNA. An increasing number of studies have confirmed that ceRNA regulatory network is widely involved in the occurrence and development of OS [38; 39]. To further understand the upstream mechanisms of prognostic genes, a ceRNA was performed to identify the target miRNAs and the corresponding lncRNAs. The results showed that a total of 148 miRNAs and 91 lncRNAs were linked with these four prognostic genes. The ceRNA networks may facilitate a better understanding of the pathophysiologic process of OS.

Conclusions

In summary, our research identified a novel signature based on four glycometabolism-related genes and constructed a risk model that can predict the survival of patients with OS. This study provided new insights into the role of glycometabolism-related genes in the molecular mechanisms of OS and may help in the development of novel diagnostic and therapeutic methods.

Declarations

Authors' contributions

This study was conceived by H Sun and FY Wang. FY Wang and K Yang performed the bioinformatic and statistical analysis. K Li and GX Peng participated in figures and charts drawing. H Yang supervised the implement of the current study. Data acquisition was accomplished by Y Xiang from online databases. The funding was provided by H Yang and H Sun. K Yang and FY Wang prepared the original draft. H Sun reviewed and edited the manuscript. The final version of the manuscript was approved by all authors.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors read and approved the final manuscript.

Disclosure Statement

The Authors declare that there is no conflict of interest.

References

1. Czarnecka AM, Synoradzki K, Firlej W, et al. Molecular biology of osteosarcoma. *Cancers (Basel)*. 2020;12:2130.
2. Misaghi A, Goldin A, Awad M, et al. Osteosarcoma: a comprehensive review. *SICOT J*. 2018;4:12.
3. Whelan JS, Davis LE. Osteosarcoma, chondrosarcoma, and chordoma. *J Clin Oncol*. 2018;36:188–93.
4. Isakoff MS, Bielack SS, Meltzer P, et al. Osteosarcoma: current treatment and a collaborative pathway to success. *J Clin Oncol*. 2015;33(27):3029–35.
5. He H, Ni J, Huang J. Molecular mechanisms of chemoresistance in osteosarcoma (Review). *Oncol Lett*. 2014;7:1352–62.
6. Ganapathy-Kanniappan S, Geschwind JF. Tumor glycolysis as a target for cancer therapy: progress and prospects. *Mol Cancer*. 2013;12:152.
7. Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu Rev Cell Dev Biol*. 2011;27:441–64.
8. Spencer NY, Stanton RC. The Warburg effect, lactate, and nearly a century of trying to cure cancer. *Semin Nephrol*. 2019;39:380–93.
9. Kang Y, Zhu X, Xu Y, et al. Energy stress-induced lncRNA HAND2-AS1 represses HIF1alpha-mediated energy metabolism and inhibits osteosarcoma progression. *Am J Cancer Res*. 2018;8:526–37.
10. Shen S, Yao T, Xu Y, et al. CircECE1 activates energy metabolism in osteosarcoma by stabilizing c-Myc. *Mol Cancer*. 2020;19:151.

11. Wan J, Liu Y, Long F, et al. circPVT1 promotes osteosarcoma glycolysis and metastasis by sponging miR-423-5p to activate Wnt5a/Ror2 signaling. *Cancer Sci.* 2021;112:1707–22.
12. Chen X, Ji ZL, Chen YZ. TTD: Therapeutic Target Database. *Nucleic Acids Res.* 2002;30:412–5.
13. Liberzon A, Subramanian A, Pinchback R, et al. Molecular signatures database (MSigDB) 3.0. *Bioinformatics.* 2011;27:1739–40.
14. Yu G, Wang LG, Han Y, et al. Clusterprofiler: an R package for comparing biological themes among gene clusters. *OMICS.* 2012;16:284–7.
15. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A.* 2005;102:15545–50.
16. Barbie DA, Tamayo P, Boehm JS, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature.* 2009;462:108–12.
17. Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics.* 2011;12:77.
18. Zhou Z, Wang J, Fang L, et al. A nomogram for predicting overall survival in patients with Ewing sarcoma: a SEER-based study. *BMC Musculoskelet Disord.* 2020;21:737.
19. Li G, Li Y, Wang DY. Overexpression of miR-329-3p sensitizes osteosarcoma cells to cisplatin through suppression of glucose metabolism by targeting LDHA. *Cell Biol Int.* 2021;45:766–74.
20. Gomes AS, Ramos H, Soares J, Saraiva L. p53 and glucose metabolism: an orchestra to be directed in cancer therapy. *Pharmacol Res.* 2018;131:75–86.
21. Yan X, Yang C, Hu W, et al. Knockdown of KRT17 decreases osteosarcoma cell proliferation and the Warburg effect via the AKT/mTOR/HIF1alpha pathway. *Oncol Rep.* 2020;44:103–14.
22. Yi W, Tu MJ, Liu Z, et al. Bioengineered miR-328-3p modulates GLUT1-mediated glucose uptake and metabolism to exert synergistic antiproliferative effects with chemotherapeutics. *Acta Pharm Sin B.* 2020;10:159–70.
23. Yao X, Hu W, Zhang J, et al. Application of cAMP-dependent catalytic subunit beta (PRKACB) low expression in predicting worse overall survival: A potential therapeutic target for colorectal carcinoma. *J Cancer.* 2020;11:4841–50.
24. Tang J, Luo Y, Wu G. A glycolysis-related gene expression signature in predicting recurrence of breast cancer. *Aging.* 2020;12:24983–94.
25. Carlisle AE, Lee N, Matthew-Onabanjo AN, et al. Selenium detoxification is required for cancer-cell survival. *Nat Metab.* 2020;2:603–11.
26. Nunziata C, Polo A, Sorice A, et al. Structural analysis of human SEPHS2 protein, a selenocysteine machinery component, over-expressed in triple negative breast cancer. *Scientific reports.* 2019;9:16131.
27. Chen YI, Wei PC, Hsu JL, et al. NPGPx (GPx7): a novel oxidative stress sensor/transmitter with multiple roles in redox homeostasis. *Am J Transl Res.* 2016;8:1626–40.

28. Chen Z, Hu T, Zhu S, et al. Glutathione peroxidase 7 suppresses cancer cell growth and is hypermethylated in gastric cancer. *Oncotarget*. 2017;8:54345–56.
29. Peng D, Belkhiri A, Hu T, et al. Glutathione peroxidase 7 protects against oxidative DNA damage in oesophageal cells. *Gut*. 2012;61:1250–60.
30. Peng D, Hu T, Soutto M, et al. Glutathione peroxidase 7 has potential tumour suppressor functions that are silenced by location-specific methylation in oesophageal adenocarcinoma. *Gut*. 2014;63:540–51.
31. Shi L, Pan H, Liu Z, et al. Roles of PFKFB3 in cancer. *Signal Transduct Target Ther*. 2017;2:17044.
32. Deng X, Deng J, Yi X, et al. Ubiquitin-like protein FAT10 promotes osteosarcoma glycolysis and growth by upregulating PFKFB3 via stabilization of EGFR. *Am J Cancer Res*. 2020;10:2066–82.
33. Deng X, Yi X, Deng J, et al. ROCK2 promotes osteosarcoma growth and metastasis by modifying PFKFB3 ubiquitination and degradation. *Exp Cell Res*. 2019;385:111689.
34. Du JY, Wang LF, Wang Q, et al. miR-26b inhibits proliferation, migration, invasion and apoptosis induction via the downregulation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 driven glycolysis in osteosarcoma cells. *Oncol Rep*. 2015;33:1890–8.
35. Heymann MF, Lezot F, Heymann D. The contribution of immune infiltrates and the local microenvironment in the pathogenesis of osteosarcoma. *Cell Immunol*. 2019;343:103711.
36. Zhang C, Zheng JH, Lin ZH, et al. Profiles of immune cell infiltration and immune-related genes in the tumor microenvironment of osteosarcoma. *Aging*. 2020;12:3486–501.
37. Grull MP, Masse E. Mimicry, deception and competition: The life of competing endogenous RNAs. *Wiley Interdiscip Rev RNA*. 2019;10:e1525.
38. Soghli N, Qujeq D, Yousefi T, et al. The regulatory functions of circular RNAs in osteosarcoma. *Genomics*. 2020;112(4):2845–56.
39. Wang JY, Yang Y, Ma Y, et al. Potential regulatory role of lncRNA-miRNA-mRNA axis in osteosarcoma. *Biomed Pharmacother*. 2020;121:109627.

Figures

Figure 1

Identification of the four glycometabolism related gene prognostic signatures. A, Forest plot of univariate Cox regression analysis to screen survival-associated glycometabolism-related genes. B and C, identification of four glycometabolism-related genes by LASSO regression. D, Kaplan-Meier curves of patients in the training set. E, Risk curves of patients in the training set. F, ROC curves in training set.

Figure 2

Validation of the glycometabolism related gene prognostic signature. A, Kaplan-Meier curve of patients in the testing set. B, Risk curves of patients in the testing set. C, ROC curve in testing set. D, Kaplan-Meier curve of survival in the validation set. E, Risk curves of patients in the validation set. F, ROC curves in validation set.

Figure 3

Correlation analysis of risk score and clinical features. A, the correlation between risk score and clinical features in the training set. B, the correlation between risk score and clinical features in testing set. C, the correlation between risk score and clinical features in validation set.

Figure 4

Independent prognostic value of glycometabolism related gene signature. A, Forest plot of univariate Cox regression analysis to screen factors associated with prognosis. B, Forest plot of multivariate Cox regression analysis to screen factors associated with prognosis. C, Nomogram constructed to predict survival. D, Calibration plot of nomogram. E, Decision curves.

Figure 5

Functional enrichment analysis. A, The top 10 GO terms of DEGs. B, The top 10 KEGG pathways of DEGs. C, Heat map of GO analysis. D, Heat map of KEGG analysis.

Figure 6

Immune cell analysis. A, Heat map of immune-correlated cell populations between high-risk group and low-risk group. B, the expression of immune cells between high-risk group and low-risk group. C, the correlation between four glycometabolism related genes and immune cells.