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ORIGINAL ARTICLE

SLC19A1 May Serve as a Potential Biomarker for Diagnosis and Prognosis in Osteosarcoma

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SUMMARY

Background: **Osteosarcoma is the most frequent primary malignant tumor of bone. SLC19A1 has been explored as a novel biomarker in some cancers. In this research, the diagnostic and prognostic value of SLC19A1 expression in osteosarcoma was evaluated by bioinformatics analysis. Data were sourced from the Gene Expression Omnibus (GEO) database.**

Methods: **Gene expression data and clinical materials of patients with osteosarcoma were collected from GSE42352 and GSE21257 datasets. The mRNA expression of SLC19A1 was compared between osteosarcoma cells and mesenchyme stem cells with the Wilcoxon rank-sum test. Moreover, receiver operating characteristic (ROC) curve analysis was performed to determine the diagnostic merit of SLC19A1 for osteosarcoma. The relationship between SLC19A1 and clinicopathological characteristics was analyzed using logistic regression. Besides, the correlation between SLC19A1 and survival rate was assessed using Kaplan-Meier and Cox regression. The biological functions of SLC19A1 were annotated and evaluated through gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA).**

Results: **SLC19A1 was significantly highly expressed in osteosarcoma cells ($p < 0.001$). The ROC curve showed an area under the curve of 0.899, which indicated a high diagnostic value. High SLC19A1 expression showed a negative correlation with Huvos grade [odds ratio (OR) = 0.09 for III vs. I, $p = 0.014$]. Kaplan-Meier survival analysis showed that the overall survival (OS) of the patients with high SLC19A1 expression was significantly poorer than the low SLC19A1 expression group ($p = 0.016$). The univariate analysis revealed that high SLC19A1 expression was associated with poor OS [$p = 0.013$, hazard ratio (HR) = 6.74, 95% CI = 1.49 - 30.46]. The multivariate analysis revealed that SLC19A1 expression ($p = 0.014$, HR = 8.03, 95% CI = 1.52 - 42.51) was independently correlated with OS. GSEA showed that genes in high expression group of SLC19A1 were enriched in KEGG pathways, including "Glyoxylate and dicarboxylate metabolism", "Oxidative phosphorylation", "Aminoacyl tRNA biosynthesis", "Base excision repair", "Pyrimidine metabolism" and "Proteasome". GSVA further suggested their importance in the progression of osteosarcoma.**

Conclusions: **SLC19A1 may be a potential biomarker for diagnosis and prognosis in osteosarcoma.**
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KEY WORDS

diagnosis, SLC19A1, osteosarcoma, prognosis, biomarker

INTRODUCTION

Osteosarcoma derives from mesenchymal tissues and is the most common primary malignant bone tumor in children and adolescents [1,2]. Current main treatments for osteosarcoma include surgery, chemotherapy, and radiotherapy [3]. With the current treatment for osteosarcoma, the average 5-year survival rate is only 60 - 70%, and there appears to be a plateau over the past three decades in survival data [4]. Although previous studies have concentrated on the mechanism of osteosarcoma, the pathogenesis of osteosarcoma is still unclear. Therefore, uncovering the pathogenesis and identifying reliable biomarkers are critically necessary. SLC19A1, also known as the reduced folate carrier 1 (RFC1), has been previously characterized as a critical transporter of reduced folates and a subset of cyclic dinucleotides [5]. Recent studies found that DNA accumulation in cytosol acted as an essential immunostimulatory signal connected with cancer [6]. SLC19A1 is a critical transporter of cyclic di-nucleotides (CDNs) into cells. Recognition of SLC19A1 has implications for the immunotherapeutic therapy of cancer [7,8]. Previous research findings have revealed that SLC19A1 plays a critical role in various cancer types [9-11]. So far, the relationship between SLC19A1 and osteosarcoma has not been reported.

In the current study, we compared the mRNA expression of SLC19A1 between osteosarcoma cells and mesenchymal stem cells. Moreover, we analyzed the prognostic value of SLC19A1 and searched for the relationship between SLC19A1 and clinical features as well as overall survival (OS). Furthermore, the biological pathways related to SLC19A1 were explored by gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA).

We found SLC19A1 was highly expressed in osteosarcoma and was associated with poor survival. Some metabolism and genetic information processing pathways were significantly associated with the SLC19A1 expression phenotype using GSEA and GSVA. Our results indicate that SLC19A1 is considered as a diagnostic and prognostic biomarker in osteosarcoma. This study will contribute to the understanding of the molecular mechanisms underlying tumorigenesis and progression in osteosarcoma.

MATERIALS AND METHODS

Data Sources and Data Preprocessing

The analysis was performed on the raw gene expressions of the osteosarcoma datasets and clinical information obtained from the Gene Expression Omnibus data repository (http://www.ncbi.nlm.nih.gov/geo) [12]. GSE42352 is a microarray dataset containing 12 mesenchymal stem cells and 19 osteosarcoma cells.

GSE21257, a much larger microarray dataset of osteosarcoma, included a total of 53 osteosarcoma samples with relatively complete clinical data. Finally, 46 samples in GSE21257 remained for further analysis after the exclusion of 7 samples without adequate clinical information. The characteristics of patients, including gender, age, subtype, grade, and tumor location, were recorded. We focused on expression differences of SLC19A1 between osteosarcoma cells and mesenchymal stem cells in GSE42352.

Gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA)

In GSE21257, 46 samples were divided into high and low expression groups based on the median expression of SLC19A1. GSEA was carried out to analyze significant survival differences between SLC19A1 high and low groups on GSEA software [13]. By running GSEA, normalized enrichment scores (NES) and nominal p-value (NOM p-value) were generated for the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways enrichment analysis in each phenotype. Gene sets were considered significantly enriched with NOM p-value < 0.05 and a false discovery rate value (FDR) < 0.25. Additionally, the "GSVA" R package was utilized to detect the pathways that are most associated with each phenotype [14]. A p-value < 0.01 was considered statistically significant. We downloaded the reference gene set "c2.cp.kegg.v6.2.symbols.gmt" from the Molecular Signature Database (MSigDB, http://software.broadinstitute.org/gsea/msigdb/index.jsp).

Statistical analysis

Statistical analysis was conducted using R (version. 3.6.0, https://www.r-project.org/). Wilcoxon test was performed to validate expression levels SLC19A1 between osteosarcoma cells and mesenchymal stem cells. A p-value < 0.05 was considered statistically significant. To validate the possibility of SLC19A1 as a diagnostic biomarker, we outlined the receiver operating characteristic (ROC) curves. The area under the ROC curve (AUC) was computed with the "pROC" R package. The association between clinical characteristics and SLC19A1 expression was analyzed with logistic regression. Cox regression was used for the correlation between clinical characteristics and survival. Multivariate

Table 1. Clinical characteristics of the osteosarcoma patients.

Age (year), n (%)	**< 12**	**11 (23.91)**
	≥ 12	**35 (79.09)**
Gender, n (%)	**Female**	**16 (34.78)**
	Male	**30 (65.22)**
Tumor location, n (%)	**Femur**	**25 (54.35)**
	Fibula	**2 (4.35)**
	Humerus	**5 (10.87)**
	Tibia	**14 (30.43)**
Histological subtype, n (%)	**Osteoblastic**	**29 (63.04)**
	Chondroblastic	**5 (10.87)**
	Fibroblastic	**4 (8.70)**
	Giant cell rich	**1 (2.17)**
	Sclerosing	**2 (4.35)**
	Telangiectatic	**2 (4.35)**
	Anaplastic	**2 (4.35)**
	Pleomorphic	**1 (2.17)**
Huvos grade, n (%)	**I**	**12 (26.09)**
	II	**16 (34.78)**
	III	**13 (28.26)**
	IV	**5 (10.87)**

Table 2. SLC19A1 expression associated with clinical characteristics (logistic regression).

Clinical characteristics	Odds ratio in SLC19A1 expression	p-value
Age (≥ 12 vs. < 12)	**1.27 (0.32 - 5.16)**	**0.730**
Gender (male vs. female)	**0.68 (0.20 - 2.30)**	**0.537**
Tumor location (humerus vs. femur)	**1.18 (0.17 - 10.16)**	**0.869**
Histological subtype (Osteoblastic vs. Fibroblastic)	**0.81 (0.09 - 7.54)**	**0.846**
Huvos grade (III vs. I)	**0.09 (0.01 - 0.52)**	**0.014** *

* **- Underlined values indicate statistical significance with p < 0.05.**

Cox analysis was performed to identify independent risk factors of survival.

RESULTS

Clinical characteristics

The clinical characteristics of 46 patients were obtained from GSE21257, including patients' gender, age, subtype, Huvos grade, survival status, and tumor location of osteosarcoma (Table 1).

SLC19A1 was expressed at high levels in osteosarcoma cells

The mRNA expression of SLC19A1 was then compared between 19 osteosarcoma cells and 12 mesenchymal stem cells. The results showed that the expression of SLC19A1 was significantly increased in osteosarcoma cells ($p < 0.001$) (Figure 1), indicating SLC19A1 may have diagnostic values for osteosarcoma patients. ROC curve estimation was performed to evaluate the ability of SLC19A1 in discriminating tumor cells from normal cells (Figure 2; AUC: 0.899).

Table 3. Univariate analysis and multivariate analysis of the correlation of SLC19A1 expression with survival.

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.61	0.47 - 5.58	0.451			
Gender	1.41	0.52 - 3.79	0.500			
Histological subtype	1.15	0.84 - 1.59	0.385			
Tumor location	1.07	0.76 - 1.50	0.701			
Huvos grade	0.52	0.30 - 0.92	0.025 *	0.55	0.31 - 0.98	0.041 *
SLC19A1	6.74	1.49 - 30.46	0.013 *	8.03	1.52 - 42.51	0.014 *

* - Underlined values indicate statistical significance with $p < 0.05$.

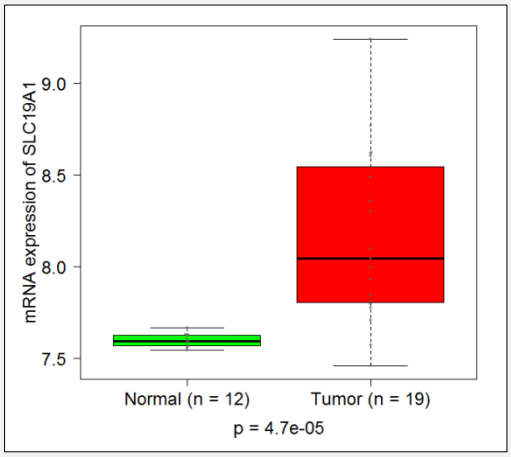

Figure 1. Differential expression analysis of SLC19A1 between osteosarcoma and normal cells.

Expression levels of SLC19A1 were significantly upregulated in osteosarcoma cells in comparison to mesenchymal stem cells in the GSE42352 ($p < 0.001$).

Association with SLC19A1 expression and clinical characteristics

We explored the relationship between SLC19A1 expression and clinical characteristics (Table 2). High SLC19A1 expression showed a negative correlation with the Huvos grade [odds ratio (OR) = 0.09 for III vs. I, p = 0.014]. Univariate analysis showed that high SLC19A1 expression was correlated with poor prognostic features. The results showed that high expression of SLC19A1 predicts poor prognosis in osteosarcoma.

Prognostic role of SLC19A1 expression in osteosarcoma

In this study, the median follow-up time of 46 osteosarcoma patients was 71.2 months. Overall survival of patients with high SLC19A1 expression was significantly poorer than the low SLC19A1 expression group (p = 0.016) (Figure 3A - 3C). Univariate analysis of prognostic factors for OS was performed with the Cox regression model (Table 3). High SLC19A1 expression was correlated with worse OS (p = 0.013, HR = 6.74, 95% CI = 1.49 - 30.46). High Huvos grade correlated with better OS (p = 0.025, HR = 0.52, 95% CI = 0.30 - 0.92). Results of multivariate analysis showed that SLC19A1 expression (p = 0.014, HR = 8.03, 95% CI = 1.52 - 42.51) and Huvos grade (p = 0.041, HR = 0.55, 95% CI = 0.31 - 0.98) were independently associated with OS (Table 3). The above results indicated that SLC19A1 was a prognostic biomarker and high levels of SLC19A1 predicted poor prognosis.

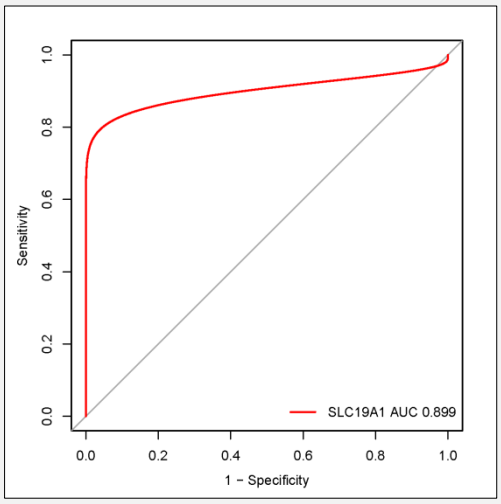

Figure 2. ROC analysis of SLC19A1 expression in osteosarcoma cells and mesenchymal stem cells.

The ROC curve has an AUC of 0.899. ROC, receiver operating characteristic; AUC, area under the ROC curve.

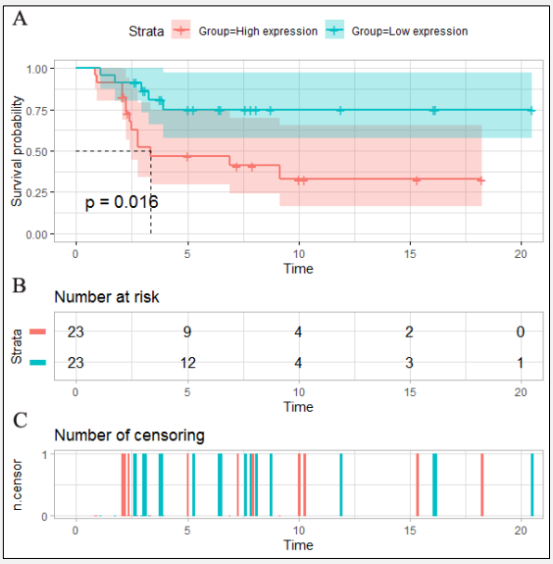

Figure 3. High expression of SLC19A1 is associated with poor OS in patients with osteosarcoma.

(A) The Kaplan-Meier curves, (B) number at risk, and (C) number of censoring of OS in osteosarcoma. OS, overall survival.

SLC19A1-related signaling pathways based on GSEA and GSVA

We performed GSEA to search signaling pathways activated in osteosarcoma enriched in the high SLC19A1 expression data sets. As shown in Figure 4, we focused on the six significantly enriched KEGG pathways, including "Glyoxylate and dicarboxylate metabolism", "Oxidative phosphorylation", "Aminoacyl tRNA bio-

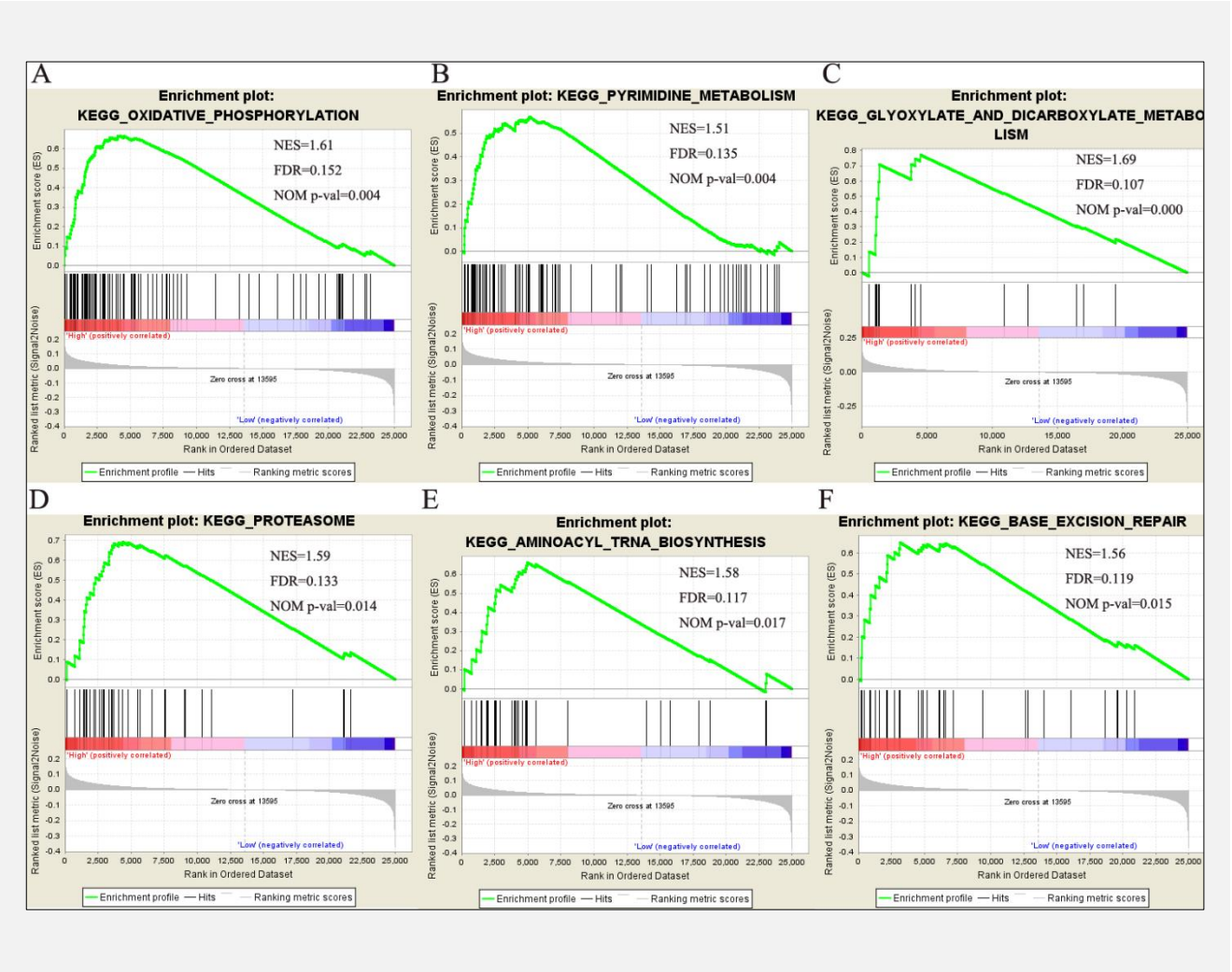

Figure 4. Gene set enrichment analysis (GSEA) curves for SLC19A1 enriched pathways involved in metabolism (A - C) and genetic information processing (D - F).

(A) Oxidative phosphorylation, (B) Pyrimidine metabolism, (C) Glyoxylate and dicarboxylate metabolism, (D) Proteasome, (E) Aminoacyl tRNA biosynthesis, (F) Base excision repair. NES - normalized enrichment scores, NOM p-value - nominal p-value, FDR - false discovery rate.

synthesis", "Base excision repair", "Pyrimidine metabolism", and "Proteasome". GSVA confirmed the above pathways were significantly upregulated in the high expression group, further suggesting their importance in the progression of osteosarcoma (Figure 5).

DISCUSSION

Currently, in the comprehensive treatment of osteosarcoma, the primary approach is prosthesis replacement surgery, supplemented with chemotherapy. However, the patients' prognosis is unsatisfactory [15]. High local aggressiveness, considerable histological heterogeneity, widespread genetic instability, rapid recurrence and metastasis, and lack of specific biomarkers impose significant challenges for the treatment of osteosarcoma [16]. Despite intense ongoing research, the molecular mechanism underlying tumorigenesis and progression remains unclear [17]. Identifying a diagnostic and prognostic biomarker may contribute to the early diagnosis and effective therapies for osteosarcoma. Thus, the present study focused on the potential role of SLC19A1 in osteosarcoma.

In this study, bioinformatics analysis of expression microarray data sets demonstrated that SLC19A1 was highly expressed in osteosarcoma and was associated with poor survival. Additionally, increased SLC19A1 in osteosarcoma tissues was positively correlated with clinical Huvos grade. Moreover, ROC analysis confirmed the diagnostic value of SLC19A1 in osteosarcoma. Furthermore, the results of univariate and multivari-

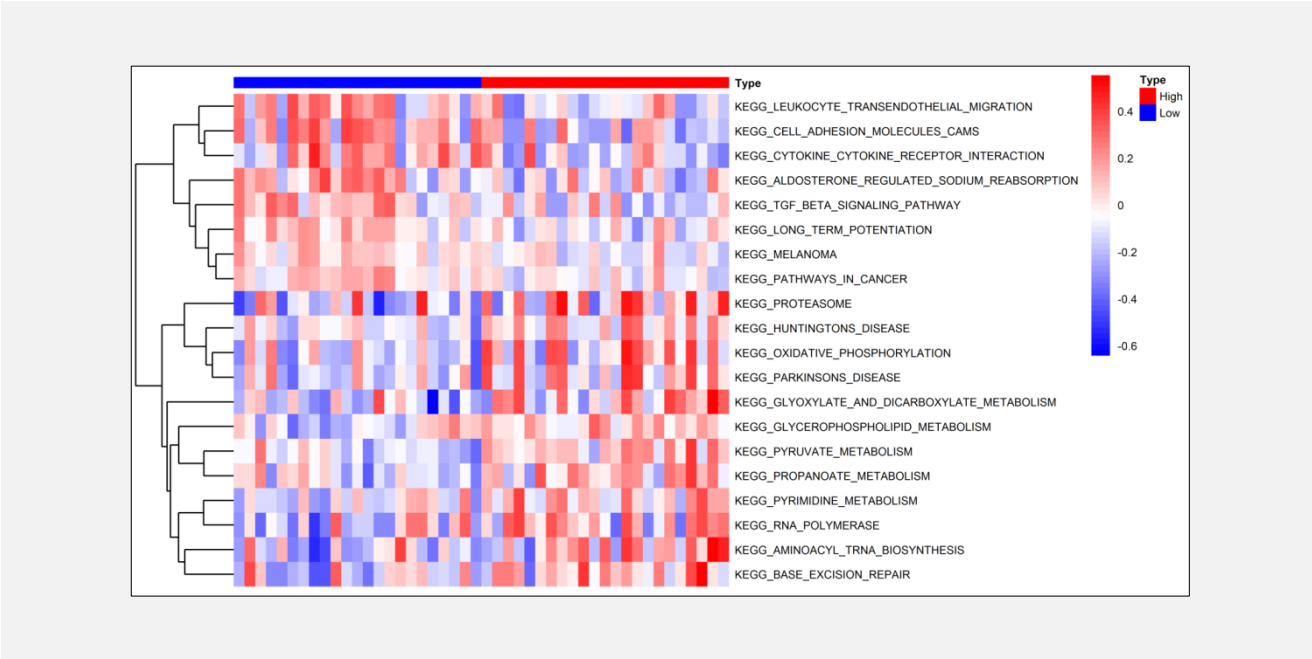

Figure 5. Gene set variation analysis (GSVA) of SLC19A1.

GSVA confirmed that the six pathways were also significantly upregulated in the high expression group. KEGG, Kyoto Encyclopedia of Genes and Genomes.

ate Cox analysis suggested that SLC19A1 may be an independent biomarker for osteosarcoma prognosis. Recent studies indicate that the role of the SLC19A1 gene in tumors is controversial. A recent study showed that overexpression of SLC19A1 was associated with more prolonged disease-free survival of the patients in colorectal cancer [9]. Xia et al. demonstrated that SLC19A1 could be used as potential novel biomarkers for prostate cancer [10]. Zheng et al. found that SLC19A1 promoted prostate cancer cell growth and invasion [11].

We further explored the function of SLC19A1 in osteosarcoma by GSEA, and the results revealed that genes in the high expression group of SLC19A1 were significantly enriched in KEGG pathways, including "Glyoxylate and dicarboxylate metabolism", "Oxidative phosphorylation", "Aminoacyl tRNA biosynthesis", "Base excision repair", "Pyrimidine metabolism", and "Proteasome". GSVA further suggested their importance in the progression of osteosarcoma. Among those altered pathway systems, it is especially interesting that the metabolism pathway and genetic information processing pathway were significantly changed in osteosarcoma compared to controls. It has long been thought that the reprogramming of energy metabolism is a hallmark of cancer cells [18]. We found the metabolism pathways included "Glyoxylate and dicarboxylate metabolism", "Oxidative phosphorylation", and "Pyrimidine metabolism" may play critical roles in osteosarcoma. The metabolic profiles of cancer cells usually shift from mitochondrial ATP synthesis via oxidative phosphorylation (OXPHOS) towards a high rate of glycolysis. OXPHOS plays a central role in cellular energy. Many studies have investigated OXPHOS inhibitors as therapeutic targets for cancer treatment with varying results. Rao et al. have suggested that OXPHOS is indeed necessary for lung cancer development [19]. Shi et al. describe a novel OXPHOS inhibitor, Gboxin, that inhibits explicitly primary mouse and human glioblastoma cell growth through rapid and sustained blockade of mitochondrial respiration coupled with oxidative phosphorylation as a new mode of action for antitumor reagent development [20]. These processes of "Aminoacyl tRNA biosynthesis", "Proteasome", and "Base excision repair" are known to play crucial roles in tumorigenesis [21-23]. Our bioinformatics analysis revealed that genes in the high expression group of SLC19A1 were significantly enriched in these three genetic information processing pathways in osteosarcoma. The ubiquitin-proteasome system (UPS) has a central function in managing activities of a wide variety of proteins along with regulation of response to oxidative stress and cell proliferation. The inhibition of the UPS might represent a novel approach for the therapy in malignancies [23]. Future in-depth and systematic investigations will be necessary to understand the underlying mechanism during osteosarcoma progression.

Limitations still exist in the study. First, the sample size in the current study was relatively small. Second, future

studies both *in vitro* and *in vivo* should be conducted to identify the biological roles of SLC19A1. As a result, further experimental validation is needed to confirm these findings.

CONCLUSION

This study demonstrated that the expression of SLC19A1 was significantly increased in osteosarcoma, and SLC19A1 could be identified as an independent risk factor for survival. Our bioinformatic analysis provided good evidence that SLC19A1 might become a valuable biomarker for clinical diagnosis and prognosis of osteosarcoma. Further research is necessary to prove the biological impact of SLC19A1.

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Declaration of Interest:
The authors declare that they have no competing interests.

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