Progression of human osteosarcoma with altered ANT1/SLC25A4 expression

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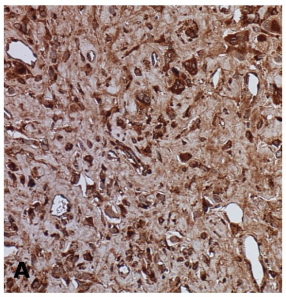
INTRODUCTION: Osteosarcoma (OS) remains the most prevalent primary malignant bone tumor among the pediatric and adolescent population. It is widely acknowledged that early detection and surveillance of neoplastic lesions significantly enhance patient survival outcomes. Recent advancements in genomic analysis have facilitated the identification of differentially expressed genes (DEGs) and the elucidation of critical molecular pathways implicated in OS tumorigenesis, thereby establishing a robust biological framework for early diagnostic and therapeutic interventions. In our recent collaborative investigations, a comprehensive comparative analysis of gene expression profiles derived from osteosarcoma and normal tissue samples within the Gene Expression Omnibus (GEO) database—comprising 25,035 annotated genes—led to the identification of numerous DEGs [1]. Subsequent exploration of these DEGs via the STRING database enabled the construction of a protein-protein interaction (PPI) network, revealing 3 significantly upregulated and 94 downregulated genes. Unpublished bioinformatics analyses further identified 16 hub genes, among which elevated expression of CASQ1, CASQ2, DES, PDLIM3, and SLC25A4 correlated with improved overall survival in OS patients. The gene SLC25A4 encodes the adenine nucleotide translocator 1 (ANT1), a mitochondrial ADP/ATP transporter essential for the regulation of cellular energy metabolism; its dysfunction has been implicated in the dysregulation of apoptosis in malignant cells. The present study reports in vitro findings concerning SLC25A4 expression in established osteosarcoma cell lines and clinical specimens, with the overarching aim of identifying prognostic biomarkers and elucidating their mechanistic roles in osteosarcoma pathogenesis.
METHODS: *Cell Culture:* The human OS cell lines (MG-53 and HOS) were cultured in DMEM medium with 10% FBS and antibiotics at 37°C. To establish *SLC25A4* knockdown or enhancing OS cells, cells were cocultured with siRNA plasmids, overexpressing plasmids, or the corresponding controls using Lipofectomine2000 according to the manufacturer' instructions. Proliferation assay, scratch assay, and trans-well invasion assay were performed on the genetic-modified cells.
Human osteosarcoma histological sections: Histological-processed unstained human tissue sections from 54 OS cases collected for a previous research project were adopted per IRB approval for secondary research. Among the specimens, 32 were from male and 22 female patients.
Histological and immunohistochemical (IHC) Analyses: One section from each case was routinely stained with H&E to examine tumor morphology. IHC staining and analysis of the clinical specimens were performed with antibodies against human ANTI/SLC25A4 (1:100). A computerized image system (Image-Pro+) was employed to quantify the signals and dissemination in the tissues. We adopted H-score (histochemistry score), a histological scoring method to quantitate the immunohistochemistry results. The number of positive cells and their staining intensity in each section was transformed into corresponding intensity values. The formula to calculate the staining intensity is H-SCORE = (percentage of cells of weak intensity×1) + (percentage of cells of moderate intensity×2) + (percentage of cells of strong intensity×3). The maximum value of H-score is 300 and the minimum value 0. H-score values of 150 or more was considered as high expression, where H-score < 150 as low expression.

RESULTS: *Modulation of SLC25A4 expression on OS cells.* Successful transfection of *SLC25A4*-modulating plasmids on the OS cell lines were confirmed by RT-PCR and Western blots. In vitro proliferation assay (MTT) suggested a significantly faster cell growth on the SLC25A4-knockdown cells. Scratch assay (wound healing) and trans-well migration experiment revealed the elevated SLC25A4 expressed cells dramatically retarded cell migration and invasion ($p<0.05$).
IHC staining on human OS specimens. Compared to the normal peri-osteo tissue, majority of the clinical OS specimens (48 of 54) revealed obviously less intensive immunostaining of the ANT1/ *SLC25A4.* However, there was an overexpressed-ANT1 tumor (Fig1A) in spite of other specimens were less intensive ANT1 stained (Fig1B). There was no difference between gender, age (18 years or older), and original tumor site. Although there is a tendency of reverse correlation of the ANT1 expression and OS recurrence/remote metastasis, it could not reach statistical analysis due to the small sample size.



DISCUSSION: Bioinformatics analyses have identified ANT1 (encoded by SLC25A4) as part of a gene cluster implicated in the development and progression of osteosarcoma. Previous research in rhabdomyosarcoma demonstrated that diminished ANT1 expression disrupts mitochondrial function, affecting tumor cell metabolism and apoptotic pathways, thereby contributing to tumorigenesis [2]. Although rhabdomyosarcoma and osteosarcoma share several oncogenic features, the role of SLC25A4 expression in osteosarcoma pathophysiology remains to be fully elucidated. In this preliminary study, restoration of ANT1 expression *in vitro* significantly inhibited osteosarcoma cell proliferation and attenuated cell migration and invasion. Analysis of clinical osteosarcoma specimens further revealed a generally reduced expression of ANT1 relative to normal tissue counterparts. These findings warrant further investigation to establish the relationship between ANT1/SLC25A4 expression levels and disease progression or prognosis, as well as to elucidate the underlying molecular mechanisms.
SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): This study explored the ANT1/ *SLC25A4* expression levels in clinical osteosarcoma tissues and its potential influence in progression/prognosis of osteosarcoma. It may have impact in developing a potential prognostic indicator for human osteosarcoma.

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