Research Paper

TIAM2 promotes proliferation and invasion of osteosarcoma cells by activating the JAK2/STAT3 signaling pathway

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HIGHLIGHTS

• TIAM2 is significantly upregulated in both osteosarcoma tissues and cell lines.
• TIAM2 promotes proliferation and invasion of osteosarcoma both in vitro and vivo.
• TIAM2 knockdown inhibited the tumorigenicity of osteosarcoma cells by downregulating of phosphorylation of JAK2/STAT3.
• The role of TIAM2 in promoting osteosarcoma progression is achieved by activating JAK2/STAT3 signaling pathway.

ABSTRACT

T-cell lymphoma invasion and metastasis 2 (TIAM2) plays a critical role in the malignancy development of many human cancers. However, the specific regulatory mechanism of TIAM2 in osteosarcoma has not yet been explored. In this study, we investigated how TIAM2 affects the proliferation and invasion of osteosarcoma cells and the underlying molecular mechanism. We performed data mining of publicly available datasets to examine whether the expression of TIAM2 is associated with osteosarcoma. We knocked down the expression of and overexpressed TIAM2 in osteosarcoma cells. The proliferative capacity of cells in each group was determined by the Cell Counting Kit-8 assay. Wound healing and Transwell invasion assays were performed to evaluate the migration and invasion abilities of the TIAM2 knockdown and overexpressed osteosarcoma cells. Determination of the function of TIAM2 in vivo was performed in nude mice. Data mining confirmed that TIAM2 expression is associated with poor prognosis in osteosarcoma. TIAM2 expression levels were significantly higher in osteosarcoma cells, and TIAM2 expression knockdown reduced proliferation and invasion abilities. Animal experiments demonstrated that TIAM2 promotes tumor growth. Additional experiments suggested that TIAM2 was significantly related to the activation of the JAK2/STAT3 pathway, and subsequent mechanistic experiments further confirmed this. Our findings suggest that TIAM2 promotes the proliferation and invasion capacities of osteosarcoma cells by activating the JAK2/STAT3 signaling pathway. TIAM2 may serve as a potential prognostic target for patients with OS.

1. Introduction

Osteosarcoma (OS) is the most common type of primary malignant tumor of the bone tissue, mainly affecting children and adolescents, and is highly metastatic [1]. Distant metastatic progression and subsequent relapse remain the leading causes of death in patients with OS [2]. The current treatment for OS consists of surgery combined with chemotherapy; however, for patients with metastatic disease or relapse after treatment the prognosis remains poor, with a five-year survival rate of <30% [3]. Therefore, further research on the pathogenesis of osteosarcoma will help to identify novel therapeutic targets and improve the survival rate of patients with OS.

Invasion and metastasis of malignant tumors are the cause of death in most cancer patients, and numerous genes and molecular pathways are included in these processes [4]. Rac family small GTPase 1 (Rac1) regulates a variety of cellular biological behaviors and plays a crucial role in regulating tumor aggressiveness [5]. Guanine nucleotide exchange factor (GEF) is also involved in many important biological processes, such...
as playing a significant role in GTPase-induced signals [6]. Previous studies have shown that the TIAM family TIAM1 and TIAM2 are GEFs that can activate Rac1 [7]. TIAM1 can induce cell spreading and affect neurite outgrowth in a rac-dependent manner in neuroblastoma cells [8]. TIAM2 is a Rac1 specific GEF, which was identified originally as a homologue of TIAM1 [9]. TIAM2 expression upregulates N-cadherin and vimentin, thus enhancing epithelial-to-mesenchymal transition (EMT) and leading to the enhancement of proliferation and invasion in hepatoma cells [10]; knockdown of TIAM2 expression suppresses the invasion and motility of lung cancer cells [11]. These studies suggest that the TIAM family is highly associated with the proliferation and invasion of several tumors. However, the expression of TIAM2 in osteosarcoma has not been explored and its effect on the proliferation and invasion of osteosarcoma remains unclear.

In the present study, we examined the expression of TIAM2 both in vivo and vitro to explore the role of TIAM2 in the proliferation, migration, and invasiveness of OS cells and tissues and the molecular mechanism of its action.

2. Materials and methods

2.1. Publicly available datasets and analysis

The publicly available datasets GSE21257 and GSE39055 were downloaded from the Gene Expression Omnibus (GEO) database, with GSE21257 containing 19 primary OS samples and 34 metastatic OS samples and GSE39055 containing 37 osteosarcoma samples with clinical follow-up. The prognostic value of TIAM2 in OS was further confirmed using The Cancer Genome Atlas (TCGA) dataset. Samples without follow-up were excluded to produce a final dataset of 81 osteosarcoma samples (TCGA cohort) for subsequent analysis.

2.2. Cell culture and inhibitor treatment

The human osteoblast (hFOB1.19) and osteosarcoma cell lines (U2OS and 143B) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640; Gibco, USA) containing 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin/streptomycin (Solarbio, Beijing, China). The cells were propagated at 37 °C in 5% CO2. The culture medium was replaced every 3 d.

JAK inhibitor AG490 (10 μM, MedChemExpress, USA) was diluted in DMSO, and cells were incubated with AG490 for 37°C at the specified concentrations. As a control, cells were treated with the equivalent volume of DMSO in parallel. We set up four groups to explore the effect of AG490 on OS cells: Vector, vector + AG490, TIAM2-OE, TIAM2-OE + AG490.

2.3. Plasmids

The full-length cDNA sequence of human TIAM2 (hTIAM2) were obtained by the National Center for Biotechnology Information (NCBI). The pcDNA3.1-hTIAM2-Flag recombinant plasmid were constructed by cloning the entire coding region of hTIAM2 into the NotI and Xhol sites of the pcDNA3.1-Flag plasmid (GeneCopoeia Inc., USA). Details of the clonal design, transformation, and vector plasmid replication were performed by transforming the pcDNA3.1-hTIAM2-Flag plasmid into E. coli DH5α. In order to over-express TIA2, the pcDNA3.1-hTIAM2-Flag plasmid was sent to Shanghai Hanheng Biotechnology Co., Ltd. for adenovirus packaging.

2.4. Cell transfection

The TIAM2-siRNA (5'-GAACGUAGGCUAGGGUUAAA-3') and siNC were purchased from GenePharma, Shanghai, China. The siRNA and vector plasmids were transfected into U2OS and 143B cells (6-well plates, 2 × 10^5 cells/well) using Lipofectamine™ 2000 (Thermo Fisher Scientific Inc, USA) according to the manufacturer’s instructions. The transfected OS cells were selected using puromycin. Briefly, 143B cells were seeded in 6-well plates (2 × 10^5 cells/well), and TIAM2 shRNA lentivirus (10 μL; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to each well (virus titer: 6 × 10^8TU/mL, shTIAM2 group), with nonspecific shRNA lentivirus added as a negative control (shNC group). Stably transfected 143B cells were selected using puromycin (1.5 μg/mL) and verified by western blotting and qRT-PCR.

2.5. RNA extraction and RNA sequencing

Total RNA from hFOB1.19, U2OS, and 143B cells was isolated using TRIzol® Reagent (Sigma-Aldrich, SF, CA, USA) following the manufacturer’s protocol. The RNA was subsequently sent to the Beijing Genomics Institute (BGI, China) for RNA-seq. In a nutshell, RNA integrity (RNA content >80 ng/μL and RIN >7.0) was determined using the Agilent 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, CA, USA), after which library preparation was initiated. A 2 × 50 bp pair-end RNA sequencing was subsequently performed on the BGISEQ-500 platform (BGI, Beijing, China).

2.6. Differential gene expression analysis

Differentially expressed genes (DEGs) between siTIAM2 and the control group were identified using R software and edgeR package. DEGs were identified with a cutoff of |log2(fold change)| > 1 and adjusted P-value <0.05.

2.7. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses

The DEGs were introduced into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) for GO and KEGG analyses, and the screening condition was P < 0.05.

2.8. Quantitative real time-polymerase chain reaction (qRT-PCR)

Qualified RNAs were reverse transcribed to complementary DNAs (cDNAs) and subjected to qRT-PCR using SYBR® Premix Ex Taq™ (Takara, Tokyo, Japan). The PCR system (25 μL) contained 12.5 μL SYBR, 1 μL of 10 μM forward and reverse primers, 2 μL cDNA, and 9 μL sterile distilled water. The qRT-PCR was performed using the following thermal cycling conditions according to the manufacturer’s instructions: Predenaturation at 95 °C for 30 s, followed by 40 cycles of 5 s at 95 °C, 20 s at 60 °C; and an extension for 5 min at 72 °C. The sequences of the two primers used are listed in Table 1. The qRT-PCR was carried out on Smart Cycler II (Cepheid, USA). The relative gene expression was calculated using the 2-ΔΔCq method.

2.9. Wound healing assays

The transfected osteosarcoma cells were seeded in six-well plates and cultured to 90% cell density. Next, a scratch was created using a 200 μL pipette tip. The scratches were observed and photographed every 24 h using inverted microscopy (Olympus, Tokyo, Japan), and the migration distance was measured.

| Primer sequences used for qRT-PCR. |
|------------------------------------|
| GAPDH: F: 5'-CTGAGTACGTCGTGGAGTCC-3' |
| R: 5'-GTCCTTGGTGCCAGTGAT-3' |
| TIAM2: F: 5'-TACACCTGAGGGAAGCATA-3' |
| R: 5'-ACACGTTCCATAATCGCCTCA-3' |
2.10. Transwell invasion assays

Dual-chamber invasion plates (BD Biosciences, San Jose, CA, USA) were used for Transwell invasion assays. The membrane pore size measured 8 \( \mu \)m and was coated with Matrigel. The transfected osteosarcoma cells were suspended in serum-free medium and added to the upper chamber (1 \( \times \) 10^5 cells/well). The subchamber was filled with RPMI 1640 medium supplemented with 10% FBS. After 48 h, cells in the lower chamber were stained with 1% crystal violet. Migrated cells were counted using inverted microscopy.

2.11. Cell Counting Kit-8 (CCK-8) assays

Different groups of 143B and U2OS cells were seeded into 96-well plates (4 \( \times \) 10^3 cells/well). One hundred microliters of CCK-8 reagent (Dojindo Laboratories Co., Ltd., Kumamoto, Japan) were added per well on d 1, 2, 3, 4, and 5. After cell culture in the dark for 1 h at 37°C, the optical density (OD) was measured at 450 nm to plot the cell viability curves.

Fig. 1. T-cell lymphoma invasion and metastasis 2 (TIAM2) is highly expressed in osteosarcoma (OS) tissue and is correlated with the prognosis of OS. A: TIAM2 expression levels of diverse human tumor types. B, C: Immunohistochemical (IHC) results showing that the expression of TIAM2 was significantly upregulated in OS tissue (200×); Assessment of staining intensity by positive cells ratio. D: The protein levels of TIAM2 in hFOB1.19, U2OS, and 143B cells detected using western blotting. Quantification of the band density ratio of TIAM2 to GAPDH. E: The mRNA expression of TIAM2 determined using qRT-PCR in hFOB1.19, U2OS, and 143B cells. G-I: Survival curve analysis of TIAM2 high- and low-expression groups in three databases. * \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \).
2.12. Western blotting

Total protein was extracted from the hFOB1.19, U2OS, and 143B cells. Proteins were separated using 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked in TBST buffer containing 5% nonfat milk for at least 1 h and incubated with primary antibodies (anti-TIAM2, ab199426; anti-GAPDH, ab9485; Abcam, UK) overnight at 4 °C. The membranes were washed four times with TBST and incubated with secondary antibodies (Biosharp, No. BL023A, Wuhan, China) for 1 h at room temperature. After three washes in TBST, the protein bands were visualized using enhanced chemiluminescence (ECL) reagents (Servicebio, Wuhan, China) and quantified by densitometry using image J (National Institutes of Health, USA).

Fig. 2. Expression of TIAM2 promotes proliferation, migration, and invasion of OS cells. A: Western blot showed TIAM2 protein levels extracted from TIAM2-OE and the control group in 143B and U2OS cells. B: Quantification of the band density ratio of TIAM2 to GAPDH. C, D: Detection of cell proliferation using the CCK-8 assay. E-F, H-I: The wound distance of TIAM2-OE and the control group (0 h, 24 h, and 48 h) determined using wound healing assays. G: The number of migrated cells in the TIAM2-OE and control groups determined using the transwell assay. *P < 0.05, **P < 0.01.
2.13. Xenograft tumor model

Five-week-old male athymic nude mice were purchased and housed in the Centre for Animal Experiments, Wuhan University Renmin Hospital (Wuhan, China). All animal experiments were approved by the Animal Care and Use Committee of Wuhan University Renmin Hospital (approval number 20150419). Mice were subcutaneously injected with $4 \times 10^6$ stably transfected 143B cells or control cells suspended in 150 μL serum-free PBS. Mice were sacrificed four weeks after transfaction and all tumors were harvested. Tumor volumes were monitored by a graduated cylinder filled with water.

2.14. Immunohistochemistry (IHC) assay

Tumor tissues were fixed with 4 % paraformaldehyde and embedded in paraffin. Sections (4 μm) were deparaffinized and rehydrated before antigen retrieval and blocking of endogenous peroxidase using hydrogen peroxide. The sections were incubated with primary antibodies (TIAM2, purchased from Abcam; Ki-67, A00254-1; STAT3, PB0540; p-STAT3, BM4269; Boster Biological Technology, China) overnight at 4 °C and incubated at 37 °C with horseradish peroxidase-conjugated secondary antibody (Servicebio, China). Reactions were visualized using DAB (3,3-diaminobenzidine) and the sections were counterstained with hematoxylin and eosin (HE). Images were photographed by light microscope.

2.15. Statistical analyses

Experimental results in our study are presented as the mean ± standard deviation (SD). Student’s $t$-test was used to analyze differences between two groups and one-way ANOVA was used to compare more than two groups. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 13.0, and figures were created using GraphPad Prism 8.0 (San Diego, CA, USA).
significance was set at $P < 0.05$.

3. Results

3.1. TIAM2 is upregulated and associated with poor survival in patients with OS

We analyzed data from multiple tumor tissues using the TIMER database and found significant differences in TIAM2 expression between tumor and normal tissues (Fig. 1A). To determine the expression of TIAM2 in OS, tumor and paracancerous tissues were analyzed using IHC. The results show that TIAM2 expression was markedly upregulated in osteosarcoma tissues (Fig. 1B, C). We detected the expression of TIAM2 in three cell lines (hFOB1.19, 143B, and U2OS) using western blot and qRT-PCR. The expression of TIAM2 was obviously increased in the two commonly used osteosarcoma cell lines (143B and U2OS) compared with that in normal human osteoblasts (hFOB1.19; Fig. 1D, E). In parallel, we analyzed datasets from TCGA and GEO to determine the clinical
significance of TIAM2 expression. In the TCGA osteosarcoma dataset and two independent GEO datasets (GSE39055 and GSE21257), the survival rate of the high TIAM2 expression group was obviously lower than that of the low TIAM2 expression group. Taken together, TIAM2 expression is related to the poor prognosis of osteosarcoma (Fig. 1F–I).

3.2. Expression of TIAM2 promotes proliferation, migration, and invasion of OS cells

We designed a series of experiments to investigate whether the expression of TIAM2 promotes the proliferation, metastasis, and invasion ability of osteosarcoma cells. The results showed that the protein level of TIAM2 was significantly higher in the TIAM2-overexpression (OE) group than in the control group (Fig. 2A, B). We used a CCK-8 assay to determine the influence of TIAM2 overexpression on osteosarcoma cell proliferation. In both the 143B and U2OS cell lines, TIAM2 overexpression significantly promoted the proliferation of OS cells (Fig. 2C, D). We performed wound healing assays to observe the influence of TIAM2 overexpression on cell migration. Overexpression of TIAM2 significantly increased the migratory capacity of OS cells (Fig. 2E-F, H-I). Using Transwell assays, we observed that the number of migrated cells in the TIAM2-OE group was significantly higher than that in the control group (Fig. 2G). In summary, these results suggest that TIAM2 promotes proliferation, migration, and invasion of OS cells.

3.3. Silencing of TIAM2 suppresses proliferation, migration, and invasion of OS cells

Since TIAM2 acts as a tumor promoter in OS progression, we explored whether silencing the TIAM2 gene might have the opposite effect. TIAM2-siRNAs and siRNA-negative (siNC) were transfected into the two cell lines. In both the 143B and U2OS cells, western blotting showed that the protein level of TIAM2 in siTIAM2 cells was significantly lower than that in the siNC cells (Fig. 3A, B). Compared with the proliferation ability of cells in the control group, that of the siTIAM2 cells was significantly inhibited (Fig. 3C, D), indicating that silencing TIAM2 could suppress the proliferation of OS cells. Furthermore, silencing TIAM2 suppressed the migration and invasion abilities of OS cells (Fig. 3E–I). In conclusion, these findings propose that silencing of TIAM2 undermines the aggressiveness of OS cells.

3.4. Differential gene expression, GO enrichment, and KEGG pathway analyses

To reveal the underlying molecular mechanism, we performed RNA-seq after silencing TIAM2. When comparing the two groups, there were 989 up-regulated genes and 678 down-regulated genes (Fig. 4A, B). GO analysis of biological processes showed that the DEGs were enriched in regulation of RNA metabolic process, RNA biosynthetic processes, nucleic acid-templated transcription. Cellular component analysis indicated that DEGs were abundant in the intracellular part, intracellular
Molecular function analysis indicated that DEGs were mainly located in protein binding (Fig. 4 C). Furthermore, KEGG analysis showed that the DEGs were enriched in pathways in cancer, PI3K-Akt and MAPK signaling pathways (Fig. 4 D). Moreover, Gene Set Enrichment Analysis (GSEA) revealed the negative enrichment of JAK/STAT pathway after TIAM2 knockdown (Fig. 4 E).

3.5. TIAM2 gene expression affects the phosphorylation of key components in the JAK2/STAT3 pathway

Since the JAK/STAT signaling pathway was significantly enriched after silencing TIAM2, and osteosarcoma progression is associated with dysregulation of this pathway [12,13], we further explored the influence of TIAM2 gene silencing and overexpression on the JAK2/STAT3 pathway. Silencing of TIAM2 inhibited the phosphorylation of key components in this pathway, namely, the production of p-JAK2 and p-STAT3 (Fig. 5 A–C), which may explain the reduction of OS cell proliferation and invasion capacities. In contrast, TIAM2 overexpression promoted JAK2 and STAT3 phosphorylation in this pathway (Fig. 5D–F). The results were similar in both the 143B and U2OS cell lines, suggesting that TIAM2 is associated with the JAK2/STAT3 pathway in OS.

3.6. TIAM2 regulates tumor biological behavior via the JAK2/STAT3 signaling pathway

Based on the above results, we performed animal experiments to evaluate the function of TIAM2 in vivo. We constructed stable shTIAM2 cells, and Western blot analysis confirmed that TIAM2 was significantly downregulated (Fig. 6A, B). The two groups of cells stably expressing shTIAM2 and shNC were subcutaneously inoculated into nude mice, and tumor growth was observed for four consecutive weeks. The results showed that tumor size, volume, and weight were obviously reduced in the shTIAM2 group compared to those in the shNC group (Fig. 6C–E). Next, we performed HE and IHC staining of the tumor tissues from shTIAM2 and shNC mice. It was clearly found that the tumor tissue in

![Fig. 6. Silencing of TIAM2 can slow tumor growth. A: Western blot showed TIAM2 protein levels extracted from shTIAM2 and the control group in 143B cells. B: Quantification of the band density ratio of TIAM2 to GAPDH. C: Tumors obtained from nude mice after dissection. D: The real-time tumor volume. E: Comparison of tumor weight between the two groups. F-G: Hematoxylin-eosin (HE) and IHC staining of tumor tissues (400×); Assessment of staining intensity by positive cells ratio (F). *P < 0.05, **P < 0.01.](image-url)
Fig. 7. JAK2 inhibitor can attenuate the tumor-promoting effects of TIAM2. A, B: Detection of cell proliferation using the CCK-8 assay. C-F: The wound distance of the four groups determined using wound healing assays. G: The number of migrated cells in the four groups determined using the transwell assay. *P < 0.05, **P < 0.01.
the shTIAM2 group was smaller, tumor proliferation ability was poor, and the p-STAT3 content was lower compared with the relevant values in the tissues of the shNC group (Fig. 6F-G). These results suggest that TIAM2 plays a role in promoting tumor growth in vivo, further supporting a role for TIAM2 expression in accelerating OS cell proliferation and invasion; this effect is achieved via the JAK2/STAT3 pathway.

3.7. JAK2 inhibitor attenuates the tumor-promoting effects of TIAM2

To further confirm the mechanism of TIAM2 promotion of the proliferation, migration, and invasion of OS cells, we added a JAK2 inhibitor (AG490) to OS cells. In both the 143B and U2OS cell lines, overexpression of TIAM2 promoted OS cell proliferation, but inhibition of JAK2 significantly attenuated this effect (Fig. 7A, B). Similarly, the invasion and migration abilities of OS cells were dramatically reduced in the JAK2 inhibitor group compared with those in the TIAM2-OE group (Fig. 7C-G). These results led to the conclusion that JAK2 inhibitors attenuate the tumor-promoting effects of TIAM2.

4. Discussion

OS, the most frequent bone tumor among malignant mesenchymal cells, is a growing threat to young people. Although many treatments are available, such as surgery combined with postoperative adjuvant chemotherapy, the five-year survival rate of patients with OS is only 65–70 %, and even lower for patients with distant metastasis [14]. Tumor metastasis and invasion are the leading causes of extremely high cancer mortality and are complex biological processes involving many molecular pathways [15]. Therefore, metastasis-based targeting strategies are crucial for anticancer therapies. However, the research progress in recent years has not been satisfactory. Consequently, it is imperative to study the molecular mechanisms underlying OS progression [16].

In this study, we hope to explore a new biomarker that may improve the prognosis of patients with OS. We first performed a pan-cancer analysis, TIAM2 was up-regulated or down-regulated in different tumors, which is similar to our results regarding its significantly abnormal expression in OS tissue and two OS cell lines. Then we constructed two cell models, TIAM2-OE and TIAM2-knockdown, in both the 143B and U2OS cell lines. Using CCK-8, wound healing, and Transwell invasion assays, western blotting, and other experimental methods, we found that the overexpression of TIAM2 promoted proliferation, migration, and invasion of OS cells, whereas TIAM2 silencing suppressed these processes. After studying the biological role of TIAM2 in OS cells in vitro, we verified the effects in vivo using a series of animal experiments. After TIAM2 silencing, the tumors formed in the nude mice were smaller in size, volume, and weight. Our results showed that the expression of TIAM2 was significantly positively correlated with the proliferation, migration and invasion abilities of OS cells. These means that TIAM2 may have a role in promoting OS progression.

The TIAM-family GEFs play key roles in cell proliferation, adhesion, and migration [7]. As a homologue of TIAM1, the aberrant expression of TIAM2 has been reported in many malignancies, and studies have shown that the function of TIAM2 promotes the proliferation and invasion of tumor cells. Chen et al. reported that the expression of TIAM2S (TIAM2 short form transcript) was related to EMT and promoted the proliferation and invasion of hepatoma cells [10]. In a study on lung cancer, Zhao et al. found that TIAM2 may regulate the activation of Rac1 and the regulation of some EMT/invasion-related genes, thereby enhancing the invasion and motility of lung cancer cells [17]. In addition, Li et al. reported that fibroblast TIAM2 may promote lung cancer cell invasion and migration by regulating certain cytokines, such as osteoprotegerin (OPG) [11]. Chan et al. also found that TIAM2S stimulates a proinflammatory immune microenvironment via serotonin-induced immune regulation, which promotes colorectal tumorigenesis [18]. The above results suggest an important role of TIAM2 in tumors, which is partly achieved by mediating Rac1 to regulate cell migration ability. In the study by Woroniuk A et al, TIAM2 was found to mediate Rac1 activity to promote stabilization of the perinuclear actin cap, which is essential for cell migration [19]. Similarly, Rooney C et al found that TIAM2 can regulate microtubule-mediated focal adhesion disassembly, thereby altering cell migration ability [20]. Interestingly, studies have shown that TIAM2-Rac signaling plays a critical role in the trans-endocytosis of ephrin receptor EphB2, which is associated with cell repulsion and may enhance the potential for tumor invasion and metastasis [21,22]. These mechanisms may partially explain the role of TIAM2 in OS, but it still needs further study.

To explore the mechanism by which TIAM2 regulates the biological behavior of OS cells, we performed RNA-seq after silencing TIAM2. We found that the JAK/STAT signaling pathway was significantly enriched using GSEA, suggesting that TIAM2 may act as a tumor promoter via the JAK/STAT signaling pathway. The JAK/STAT signaling pathway is mainly composed of three components: tyrosine kinase-related receptors, JAK, and STAT. It is involved in important biological processes, such as cell development, differentiation, proliferation, apoptosis, and immune regulation, and also plays an important role in many tumors [23]. Xu et al. found that JAK2 is upregulated in lung adenocarcinoma tissues and is related to metastasis [24]. Tang et al. reported that STAT3 promotes the progression of hepatocellular carcinoma [25]. Ge et al. reported that BMSC-derived exosomal LCP1 promotes OS proliferation and metastasis via the JAK2/STAT3 pathway [13]. Sun et al. found that curcumin-mediated inhibition of OS cell proliferation and migration was associated with inactivation of the JAK2/STAT3 pathway [26]. Previous studies have demonstrated that The Rac1 is required for G Protein-Coupled Receptors to activate JAK/STAT signalling [27], and Rac1 can induce STAT3 activation by autocrine IL-6 signaling [28,29]. These findings indicate that OS progression is significantly associated with this pathway. We found that the expression level of TIAM2 was significantly correlated with the phosphorylation of key components of the JAK2/STAT3 pathway. Using rescue assays, we confirmed that JAK2 inhibitors attenuated the tumor-promoting effects of TIAM2. Thus, we conclude that TIAM2 promotes the proliferation and invasion abilities of OS cells via the JAK2/STAT3 signaling pathway.

In summary, our findings suggest that TIAM2 acts as a tumor promoter in OS. TIAM2 can promote the proliferation and invasion of OS cells by activating the JAK2/STAT3 signaling pathway. These results deepen our understanding of OS progression and provide meaningful prognostic targets for OS study.

Author contributions

SC and WG designed the study; SC and XH performed the experiments; SC analyzed the data; XH wrote the manuscript; all authors reviewed the manuscript.

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CRediT authorship contribution statement

Shaohua Cheng: Conceptualization, Methodology, Investigation, Writing – review & editing. Xinghan Huang: Investigation, Writing – original draft, Formal analysis, Validation. Weichun Guo: Conceptualization, Resources, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbo.2022.100461.

References

[1] L. Mirabello, R.J. Troisi, S.A. Savage, Osteosarcoma incidence and survival rates from 1973 to 2004, 115(7) (2009) 1531-1543.
[2] Z. Nie, H. Peng, Osteosarcoma in patients below 25 years of age: An observational study of incidence, metastasis, treatment and outcomes, Oncol. Lett. 16 (5) (2018) 6502-6514.
[3] B. Zhang, Y. Zhang, R. Li, J. Li, X. Lu, Y. Zhang, The efficacy and safety comparison of first-line chemotherapeutic agents (high-dose methotrexate, doxorubicin, cisplatin, and ifosfamide) for osteosarcoma: a network meta-analysis, J. Orthop. Surg. Res. 15 (1) (2020) 51.
[4] Y. Suhail, M.P. Cain, K. Vanaja, P.A. Kurywchak, A. Levchenko, R. Kalluri, Kshitiz, We sincerely thank all members for their meaningful suggestions and comments on this study.
[5] M. Parri, P. Chiarugi, Rac and Rho GTPases in cancer cell motility control, Cell Commun. Signal. 8 (1) (2010) 23.
[6] A. Hall, Rho GTPases and the actin cytoskeleton, Science 279 (5350) (1998) 797–807.
[7] T.R. Shepherd, E.J. Fuentes, Structural and thermodynamic analysis of PDZ-ligand interactions, Methods Enzymol. 488 (2011) 81–133.
[8] C. Rooney, G. White, A. Nazgiewicz, S.A. Woodcock, K.I. Anderson, C. Ballestrem, The guanine nucleotide exchange factor Tiam1 affects neuronal morphology; opposing roles for the small GTPases Rac and Rho, J. Cell Biol. 139 (3) (1997) 797–807.
[9] C.Y. Chiu, S. Leng, K.A. Martin, E. Kim, S. Gorman, D.M. Duhl, Cloning and characterization of T-cell lymphoma invasion and metastasis 2 (TIAM2), a novel guanine nucleotide exchange factor related to TIAM1, Genomics 61 (1) (1999) 56–73.
[10] J.S. Chen, I.J. Su, Y.W. Leu, K.C. Young, H.S. Sun, Expression of T-cell lymphoma invasion and metastasis 2 (TIAM2) promotes proliferation and invasion of liver cancer, Int. J. Cancer 130 (6) (2012) 1502–1513.
[11] S. Li, Y. Ou, S. Liu, J. Yin, W. Zhu, M. Huang, T. Zhu, W. Zhang, H. Zhou, Z. Liu, The fibroblast TIAM1 promotes lung cancer cell invasion and metastasis, J Cancer 10 (8) (2019) 1879–1889.
[12] Y. Hu, R. Luo, J. Zhou, S. Chen, M. Gong, Y. Deng, H. Zhang, Piperlongumine inhibits the progression of osteosarcoma by downregulating the SOCS3/JAK2/STAT3 pathway via miR-30d-5p, Life Sci. 277 (2021), 119501.
[13] X. Ge, W. Liu, W. Zhao, S. Feng, A. Duan, C. Ji, K. Shen, W. Liu, J. Zhou, D. Jiang, Y. Rong, F. Gong, J. Wang, Z. Xu, X. Li, J. Fan, Y. Wei, J. Bai, W. Cai, Exosomal transfer of LCP1 promotes osteosarcoma cell tumorigenesis and metastasis by activating the JAK2/STAT3 signaling pathway, Mol. Ther. Nucleic Acids 21 (2020) 900–915.
[14] D.J. Harrison, D.S. Geller, J.D. Gill, V.O. Lewis, R. Gorlick, Current and future therapeutic approaches for osteosarcoma, Expert Rev. Anticancer Ther. 18 (1) (2018) 39–50.
[15] D. Spano, C. Heck, P. De Antonellis, G. Christofori, M. Zollo, Molecular networks that regulate cancer metastasis, Semin. Cancer Biol. 22 (3) (2012) 234–249.
[16] M.W. Bishop, K.A. Janeway, R. Gorlick, Future directions in the treatment of osteosarcoma, Curr. Opin. Pediatr. 28 (1) (2016) 26–33.
[17] Z.Y. Zhao, C.G. Han, J.T. Liu, C.I. Wang, Y. Wang, L.Y. Cheng, TIAM2 enhances non-small cell lung cancer cell invasion and motility, Asian Pac. J. Cancer Prev. 14 (11) (2013) 6305–6309.
[18] Y.L. Chan, W.C. Lai, J.S. Chen, J.T. Tseng, P.C. Chuang, J. Jou, C.T. Lee, H.S. Sun, TIAM2 mediates serotonin homeostasis and provokes a pro-inflammatory immune microenvironment permissive for colorectal tumorigenesis, Cancers (Basel) 12 (7) (2020).
[19] A. Woroniuk, A. Porter, G. White, D.T. Newman, Z. Diamantopoulou, T. Waring, C. Rooney, D. Strathdee, D.J. Marston, K.M. Hahn, O.J. Sansom, T. Zech, A. Malliri, STEF/TIAM2-mediated Rac1 activity at the nuclear envelope regulates the perinuclear actin cap, Nat. Commun. 9 (1) (2018) 2124.
[20] C. Rooney, G. White, A. Nazgiewicz, S.A. Woodcock, K.L. Anderson, C. Ballestrem, A. Malliri, The Rac activator STEF (Tiam2) regulates cell migration by microtubule-mediated focal adhesion disassembly, EMBO Rep. 11 (4) (2010) 292–298.
[21] T.N. Gaitanos, J. Koerner, R. Klein, Tiam-Rac signaling mediates endo-oligocysteine of ephrin receptor EphB2 and is important for cell repulsion, J. Cell Biol. 214 (6) (2016) 715–752.
[22] E.B. Pasquale, Eph receptors and ephrins in cancer: bidirectional signalling and beyond, Nat. Rev. Cancer 10 (3) (2010) 165–180.
[23] J.J. O’Shea, S.M. Holland, L.M. Staudt, JAKs and STATs in immunity, immunodeficiency, and cancer, N. Engl. J. Med. 368 (2) (2013) 161–170.
[24] Y. Xu, J. Jin, J. Xu, Y.W. Shao, Y. Fan, JAK2 variations and functions in lung adenocarcinoma, Tumour Biol. 39 (6) (2017).
[25] J.J. Hin Tang, D.K. Hao Thng, J.J. Lim, T.B. Toh, JAK/STAT signaling in immunodeficiency, and cancer, N. Engl. J. Med. 368 (2) (2013) 161–170.
[26] Y. Sun, L. Liu, Y. Wang, A. He, H. Hu, J. Zhang, M. Han, Y. Huang, Curcumin inhibits the proliferation and invasion of MG-63 cells through inactivation of the p-JAK2/p-STAT3 pathway, Oncol. Targets Ther. 12 (2019) 2011–2016.
[27] J. Corry, H.R. Mott, D. Owen, Activation of STAT transcription factors by the Rho-family GTases and ephrin receptor EphB2 and is important for cell repulsion, J. Cell Biol. 214 (6) (2016) 715–752.
[28] Y. Xu, J. Jin, J. Xu, Y.W. Shao, Y. Fan, JAK2 variations and functions in lung adenocarcinoma, Tumour Biol. 39 (6) (2017).
[29] S. Pedletier, F. Dukamel, P. Coulombe, M.R. Popoff, S. Meloche, Rho family GTases are required for activation of Jak/STAT signaling by G protein-coupled receptors, Mol. Cell. Biol. 23 (4) (2003) 1316–1333.
[30] C. Rooney, G. White, A. Nazgiewicz, S.A. Woodcock, K.L. Anderson, C. Ballestrem, A. Malliri, The Rac activator STEF (Tiam2) regulates cell migration by microtubule-mediated focal adhesion disassembly, EMBO Rep. 11 (4) (2010) 292–298.
[31] J. Carry, H.R. Moli, D. Owen, Activation of STAT transcription factors by the Rho-family GTases, Biochem. Soc. Trans. 48 (5) (2020) 2213–2227.
[32] T.R. Fariau, D. Gomez, X.R. Bustelo, D. Bar-Sagi, N.C. Reich, Rac1 mediates STAT3 activation by autocrine IL-6, Proc. Natl. Acad. Sci. U S A 98 (16) (2001) 9014–9019.