OncoTargets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

MALAT1 promotes proliferation, migration, and invasion of MG63 cells by upregulation of *TGIF2* via negatively regulating *miR-129*

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Kai Liu
Yingang Zhang
Liang Liu
Qiling Yuan

Department of Orthopedics, The First Affliated Hospital of Xi'an Jiaotong University, Xi'an, China

Purpose: This article aimed to investigate the mechanism by which *MALAT1* and *miR-129* affected the development of osteosarcoma.

Methods: Tumor tissues and adjacent tissues of 23 osteosarcoma patients were collected. Normal osteoblasts hFOB1.19 and osteosarcoma cells MG63 were cultured. MG63 cells were transfected and grouped: si-negative control (NC) group, si-MALAT1 group, miR-129 NC group, miR-129 mimics group, p-Empty vector group, p-MALAT1 group, p-MALAT1+ miR-129 mimics group, and p-MALAT1+ si-TGIF2 group. Luciferase reporter assay, Cell Counting Kit-8 assay, Transwell assay, quantitative reverse transcription PCR, Western blot, and Pearson correlation analysis were performed.

Results: *MALAT1* expression in tumor tissues and MG63 cells was increased ($P<0.01$). High *MALAT1* expression predicted poor prognosis of osteosarcoma patients. MG63 cells of si-MALAT1 group exhibited much lower cell viability, migration, and invasive cell numbers when compared with si-NC group ($P<0.01$). For MG63 cells of miR-129 mimics group, they had markedly lower cell viability, migration, and invasive cell numbers than miR-129 NC group ($P<0.01$). *miR-129* was targetedly and negatively regulated by *MALAT1*. *TGIF2*, which was targetedly and negatively regulated by *miR-129*, was overexpressed in tumor tissues and MG63 cells ($P<0.01$). *miR-129* overexpresison and *TGIF2* downregulation significantly reversed the enhanced cell viability, migration, and invasion induced by *MALAT1* ($P<0.01$).

Conclusion: *MALAT1* promotes *TGIF2* expression through negative regulation of *miR-129*, which further promotes the proliferation, migration, and invasion of MG63 cells.

Keywords: *MALAT1*, *miR-129*, *TGIF2*, osteosarcoma, invasion, prognosis

Introduction

Osteosarcoma, a primary malignant bone tumor, often occurs in children and adolescents.[1] About 5.6 per million were diagnosed with osteosarcoma according to a 2009 statistic.[2] Up to 80% of patients had metastasized when they were first diagnosed with osteosarcoma. Metastasis caused a serious adverse outcome for osteosarcoma patients and absence of metastasis was a key factor in improving patients' 5-year survival rate.[3] Complete cure could not be achieved by traditional treatment methods. Therefore, the discovery of molecular targets is very important for the research of pathogenesis and therapeutic strategies of osteosarcoma.

lncRNAs are a family of RNA molecules, which are composed of >200 nucleotides.[4] lncRNAs are located in the cytoplasm and nucleus and they do not possess the ability of encoding proteins.[5,6] More and more studies have found that lncRNAs are

Correspondence: Kai Liu
Department of Orthopedics, The First Affliated Hospital of Xi'an Jiaotong University, No 277, Yanta West Road, Xi'an, Shanxi 710061, China
Tel +86 29 8532 3930
Email liukai244@163.com

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/OTT.S182993

involved in the regulation of a variety of biological processes, such as cell proliferation, differentiation, migration, and invasion.[7,8] Osteosarcoma occurrence and development have also been found to be regulated by a variety of genes, including lncRNAs. For examples, Ma et al[9] suggested that lncRNA *TUG1* in osteosarcoma tissues was markedly higher than corresponding adjacent tissues. High level of *TUG1* is closely related to tumor size and poor prognosis, which might be used as a candidate molecular indicator for monitoring osteosarcoma. Lv et al[10] revealed that lncRNA *XIST* might stimulate osteosarcoma cells proliferation and invasion through directly repressing *miR-320b*. They proposed *XIST* to be a biomarker and target for osteosarcoma diagnosis and treatment. Osteosarcoma is a disease usually treated by surgery and a long-course chemotherapy treatment. Previous research had found that, in colorectal cancer, *MALAT1* was associated with poor response to oxaliplatin-based chemotherapy.[11] Its suppression could also improve diffuse large B-cell lymphoma chemotherapy sensitivity by enhancing autophagy-related proteins.[12] However, the relevant mechanism of *MALAT1* affecting osteosarcoma progression is still not clear. miRNA is a kind of small non-coding RNA, the aberrant expression of which has been proved to be involved in multiple tumors initiation and progression.[13] *miR-129* acted as a tumor suppressor in several cancers, including hepatocellular carcinoma, breast cancer, and colon cancer, etc.[14–17] Few reports have documented the influence and mechanism of *MALAT1* and *miR-129* on osteosarcoma progression.

In this paper, we researched the expression of *MALAT1* and *miR-129* in osteosarcoma and investigated their effects on proliferation, migration, and invasion of osteosarcoma cells. More importantly, the relationship between *MALAT1* and *miR-129* was also deeply explored in order to provide guidance for molecular therapy of osteosarcoma.

Methods

Clinical samples

This study enrolled 23 osteosarcoma patients, and their tumor tissues and paracancerous normal tissues were obtained. All patients were diagnosed with osteosarcoma for the first time between October 2016 and August 2017, and none of them had received medical treatment or had radiochemical history of osteosarcoma. The clinicopathological characteristics of 23 patients are listed in Table 1. The study was conducted with the approval of all patients and the Ethics Committee of our hospital.

Table 1 The relationship between patients' clinicopathological characteristics and *MALAT1* expression level

Clinicopathological characteristics	*MALAT1* expression (mean ± SD)	*t*-value	*P*-value
Gender		−0.76	0.46
Male (n=13)	2.62±0.26		
Female (n=10)	2.71±0.31		
Age		2.74	0.01*
<10 years (n=18)	2.94±0.41		
>10 years (n=5)	2.39±0.34		
Lymph node metastasis		2.53	0.02*
Positive (n=6)	2.86±0.35		
Negative (n=17)	2.51±0.27		
Tumor size		−2.45	0.02*
<2 cm^3 (n=15)	2.31±0.46		
>2 cm^3 (n=8)	2.77±0.36		
Infiltration		2.30	0.03*
Positive (n=7)	2.65±0.40		
Negative (n=16)	2.29±0.32		
Differentiation		2.526	0.021*
Low-medium (n=12)	2.72±0.45		
High (n=9)	2.25±0.38		

Note: *$P<0.05$.

Cell culture

Normal osteoblasts (hFOB1.19) and osteosarcoma cells (MG63) were purchased from Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. All cells were cultured in DMEM supplemented with 10% FBS and were maintained in a 5% CO_2 incubator. It should be noted that the culture environment of hFOB1.19 cells was 34°C whereas MG63 cells were cultured at 37°C.

Cell transfection

MG63 cells were maintained in 6-well plates at a density of 1×10^5 cells/mL. When the confluence reached 40%–60%, the cells were transfected by *MALAT1* siRNA (si-negative control [NC] group), *MALAT1* siRNA NC (si-MALAT1 group), *miR-129* mimics (miR-129 NC group), and *miR-129* NC (miR-129 mimics group). All transfection plasmids were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). In addition, *MALAT1* overexpression vector and its empty vector were also constructed (Shanghai Jima Gene Co., Ltd., Shanghai, China) to transfect MG63 cells, which were named as p-MALAT1 group and p-Empty vector group, respectively. Meanwhile, MG63 cells were subjected to co-transfection by *MALAT1* overexpression vector and *miR-129* mimics (p-MALAT1+ miR-129 mimics group)

or by *MALAT1* overexpression vector and *TGIF2* siRNA (p-MALAT1+ si-TGIF2 group). All transfections were carried out by using Lipfectamine 2000 transfection kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the instructions. Cells of each group were collected for 48 hours after transfection.

Luciferase reporter gene assay

The binding sites of *MALAT1* and *miR-129* were 3′UTR region according to Starbase online prediction software. The wild-type (WT) and mutant-type (MT) of *miR-129* sequences containing *MALAT1* 3′UTR binding sites were designed by Shanghai Jima Gene Co., Ltd. All sequences were constructed into pmirGLO vector (Promega Corporation, Fitchburg, WI, USA). MG63 cells were pre-transfected with *MALAT1* siRNA or its NC, and subsequently transfected with WT *miR-129* vector or MT *miR-129* vector. Furthermore, the binding sites of *TGIF* and *miR-129* were also predicted by Target Scan, and these two genes were combined in 3′UTR region. *TGIF2* WT and MT containing *miR-129* 3′UTR sequences were obtained from Shanghai Jima Gene Co., Ltd. They were incorporated into pmirGLO vector to transfect MG63 cells after these cells were transfected with *miR-129* mimics or *miR-129* NC. All cells were incubated in the incubator for 48 hours at 37°C, 5% CO_2. Luciferase activity was measured by using Dual-luciferase Reporter Assay Kit (Promega).

Cell Counting Kit-8 (CCK-8) assay

MG63 cells were seeded in 96-well plates (1×10^5 cells/mL), with 100 µL cell suspension in each well. After being incubated for 24, 48, 72, and 96 hours at 37°C, 5% CO_2, the cells were incubated again for 2 hours after adding 10 µL CCK-8 solution into each well. OD 450 value of each well was measured by ELISA. In this study, normal MG63 cells were set as Control group. Cell viability of other groups was considered as the percentage of Control group.

Transwell assay

Invasion ability was measured with 24-well Transwell chamber. The upper chamber was pre-coated with a layer of Matrigel. DMEM containing 10% FBS was added into the lower chamber, while a total of 2×10^4 MG63 cells were inoculated in the upper chamber. Cells were cultured at 37°C, 5% CO_2 for 24 hours. Then cells on the upper layer of the membrane were gently wiped off with a cotton swab, and those on the lower layer of the membrane were fixed by formaldehyde for 5 minutes. After being stained with crystal violet (0.1%) for 10 minutes, the cells on the lower layer of the membrane were observed under a microscope and five fields were randomly selected to count invading cell numbers.

Migration ability was also detected by using the same procedure mentioned before, but the upper chamber was without Matrigel.

Quantitative reverse transcription (qRT)-PCR

Trizol reagent (Thermo Fisher Scientific) was used to extract total RNA in tissues and cells according to the instructions. Reverse Transcription Kit (Promega) was used to synthesize cDNA templates. qRT-PCR was conducted under the following conditions by applying the Fast Start Universal SYBR Green Master (Roche, Palo Alto, CA, USA): initial denaturation at 94°C for 2 minutes, followed by 40 cycles of 94°C for 20 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. Relative expression of candidate genes was calculated by $2^{-\Delta\Delta CT}$ method. Primers involved in this article were as follows: *MALAT1*-F, 5′-GGGTGTTTACGTAGACCAGAACC-3′, *MALAT1*-R, 5′-CTTCCAAAAGCCTTCTGCCTTAG-3′. *miR-129*-F, 5′-GTTGGGGAGATTTAGTTTGTT-3′, *miR-129*-R, 5′-CCTACTCCAATTCCCCCTATAATAC-3′, *U6*-F, 5′-CTCGCTTCGGCAGCACATATACT-3′, *U6*-R, 5′-ACGCTTCACGAATTTGCGTGTC-3′, *TGIF2*-F, 5′-GTACTTGCACCGCTACAACG-3′, *TGIF2*-R, 5′-GGCATTGATGAACCAGTTAC-3′, *GAPDH*-F, 5′-GTCGATGGCTAGTCGTAGCATCGAT-3′, *GAPDH*-R, 5′-TGCTAGCTGGCATGCCCGATCGATC-3′. *MALAT1* and *miR-129* expression was normalized to *U6*, while *TGIF2* expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

Western blot analysis

Tissues (ground in liquid nitrogen) and cells were lysed by RIPA buffer to collect total protein. Bicinchoninic Acid Protein Assay Kit (Pierce, Rockford, IL, USA) was applied to determine the concentration of these protein samples. Protein samples were transferred onto polyvinylidene difluoride membranes after being separated by SDS-PAGE. These membranes were exposed to 5% skim milk for 2 hours before being incubated with primary antibody (mouse anti-rabbit TGIF2 antibody, 1:1,000, Santa Cruz, Dallas, TX, USA) and secondary antibody (goat anti-mouse IgG secondary antibody, 1:5,000; Zhongshang Jinqiao Biotechnology Co., Ltd., Beijing, China). Kodak film developer (Fujifilm,

Tokyo, Japan) was selected to detect immunoblots. GAPDH was set as internal control.

Statistical analyses

SPSS 19.0 was used to process data in this study and all data were presented in the form of mean ± SD. The differences between two groups were compared by *t*-test and one-way ANOVA was used to compare differences among multiple groups. Correlation analysis of two genes expression was performed using Pearson correlation analysis. $P<0.05$ was considered statistically significant. In this paper, all experiments were repeated three times.

Ethics approval and consent to participate

This study was conducted after obtaining the ethical committee approval of The First Affliated Hospital of Xi'an Jiaotong University and written informed consent from the patients and complies with the Declaration of Helsinki.

Results

Upregulated *MALAT1* in osteosarcoma predicted poor prognosis

qRT-PCR was conducted to determine *MALAT1* expression in tissues and cells. The results showed significantly increased *MALAT1* relative expression in osteosarcoma tumor tissues than that in adjacent tissues ($P<0.01$) (Figure 1A). At the same time, we also analyzed the relationship between patients' clinicopathological characteristics and *MALAT1* level. As shown in Table 1, patients' gender was not a factor that affected *MALAT1* expression. However, low age, positive lymph node metastasis and infiltration, large tumor size, and low-medium differentiation were significantly associated with high *MALAT1* expression level ($P<0.05$), indicating that high *MALAT1* expression predicted poor prognosis of osteosarcoma patients. In addition, *MALAT1* relative expression in MG63 cells was also remarkably higher than that found in hFOB1.19 cells ($P<0.01$) (Figure 1B).

MALAT1 promoted MG63 cells proliferation, migration, and invasion

MG63 cells were transfected by *MALAT1* siRNA and its NC. Compared with si-NC group, *MALAT1* relative expression in MG63 cells of si-MALAT1 group was significantly decreased ($P<0.01$) (Figure 2A). CCK-8 assay revealed markedly lower cell viability of MG63 cells in si-MALAT1 group than that found in si-NC group at 48–96 hours ($P<0.01$) (Figure 2B). In addition, the number of migrating and invading cells of si-MALAT1 group was remarkably decreased when compared with si-NC group ($P<0.01$) (Figure 2C and D). Silencing of *MALAT1* markedly inhibited MG63 cells proliferation, migration, and invasion ability.

miR-129 inhibited proliferation, migration, and invasion of MG63 cells

miR-129 relative expression in tumor tissues was much lower than that in adjacent tissues ($P<0.01$) (Figure 3A). At the same time, significantly declined *miR-129* relative expression was found in MG63 cells when compared with that found in hFOB1.19 cells ($P<0.01$) (Figure 3B). Transfection with *miR-129* mimics successfully increased the expression level of *miR-129* in MG63 cells ($P<0.01$) (Figure 3C). Furthermore, MG63 cells of miR-129 mimics group exhibited significantly lower MG63 cells viability, migration, and invasion ability than that of miR-129 NC group ($P<0.01$) (Figure 3D–F).

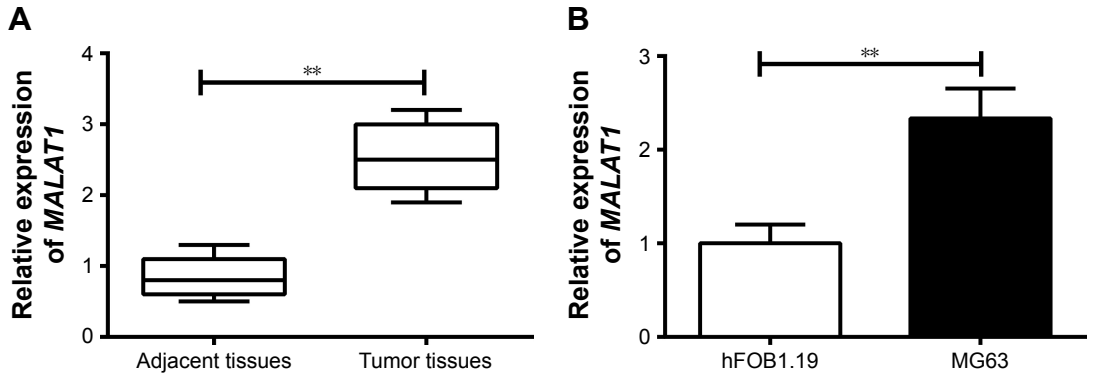

Figure 1 *MALAT1* was upregulated in osteosarcoma tumor tissues and MG63 cells.
Notes: (**A**) *MALAT1* relative expression was significantly increased in osteosarcoma tumor tissues compared with adjacent tissues. (**B**) *MALAT1* relative expression was significantly increased in normal osteoblastic cells (hFOB1.19) compared with in osteosarcoma cells (MG63). $^{**}P<0.01$.

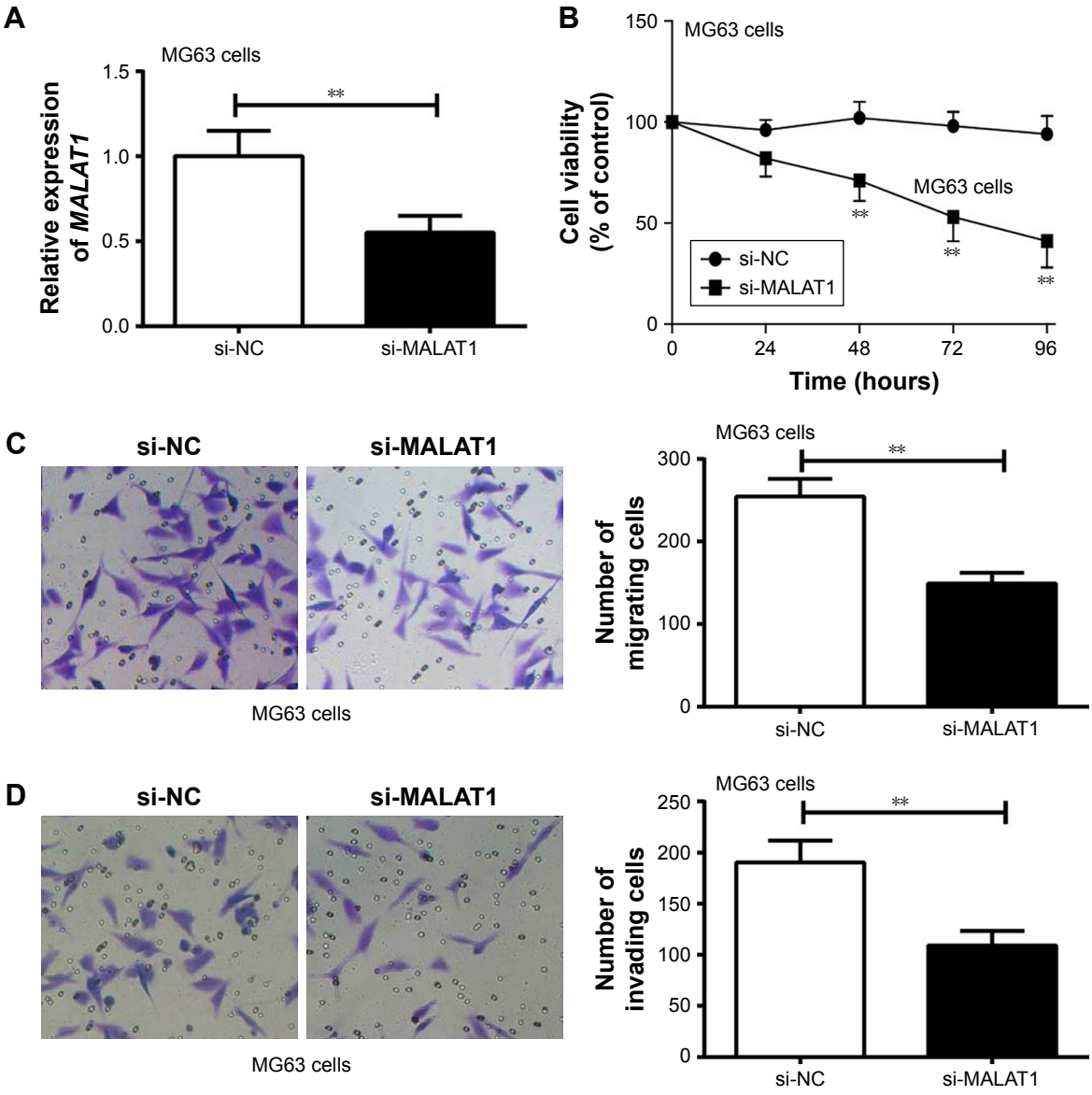

Figure 2 *MALAT1* promoted MG63 cells proliferation, migration, and invasion.
Notes: (**A**) *MALAT1* relative expression in si-MALAT1 group was significantly decreased compared with si-NC group. (**B**) Silencing of *MALAT1* significantly decreased MG63 cells viability. (**C**) Silencing of *MALAT1* remarkably decreased MG63 cells migration ability (200× magnification). (**D**) Silencing of *MALAT1* remarkably decreased MG63 cells invasion ability (200× magnification). **$P<0.01$.
Abbreviation: NC, negative control.

miR-129 was targetedly and negatively regulated by *MALAT1*

Correlation analysis between *MALAT1* and *miR-129* expression in tumor tissues was investigated. The result indicated an obviously negative correlation between *MALAT1* and *miR-129* expression levels ($P<0.01$) (Figure 4A). After being transfected by *MALAT1* siRNA or its NC, *miR-129* relative expression in MG63 cells of si-MALAT1 group was significantly higher than that of si-NC group ($P<0.01$) (Figure 4B). Starbase online prediction results showed that *MALAT1* was directly bound to *miR-129* at 3′UTR region. The binding site is shown in Figure 4C. Luciferase reporter gene assay also demonstrated significantly increased relative luciferase activity in WT+ si-MALAT1 group when compared with WT+ si-NC group ($P<0.01$). However, no statistical significance was found in relative luciferase activity between MT+ si-MALAT1 group and MT+ si-NC group (Figure 4D). All these results indicated that *miR-129* was directly and negatively regulated by *MALAT1*.

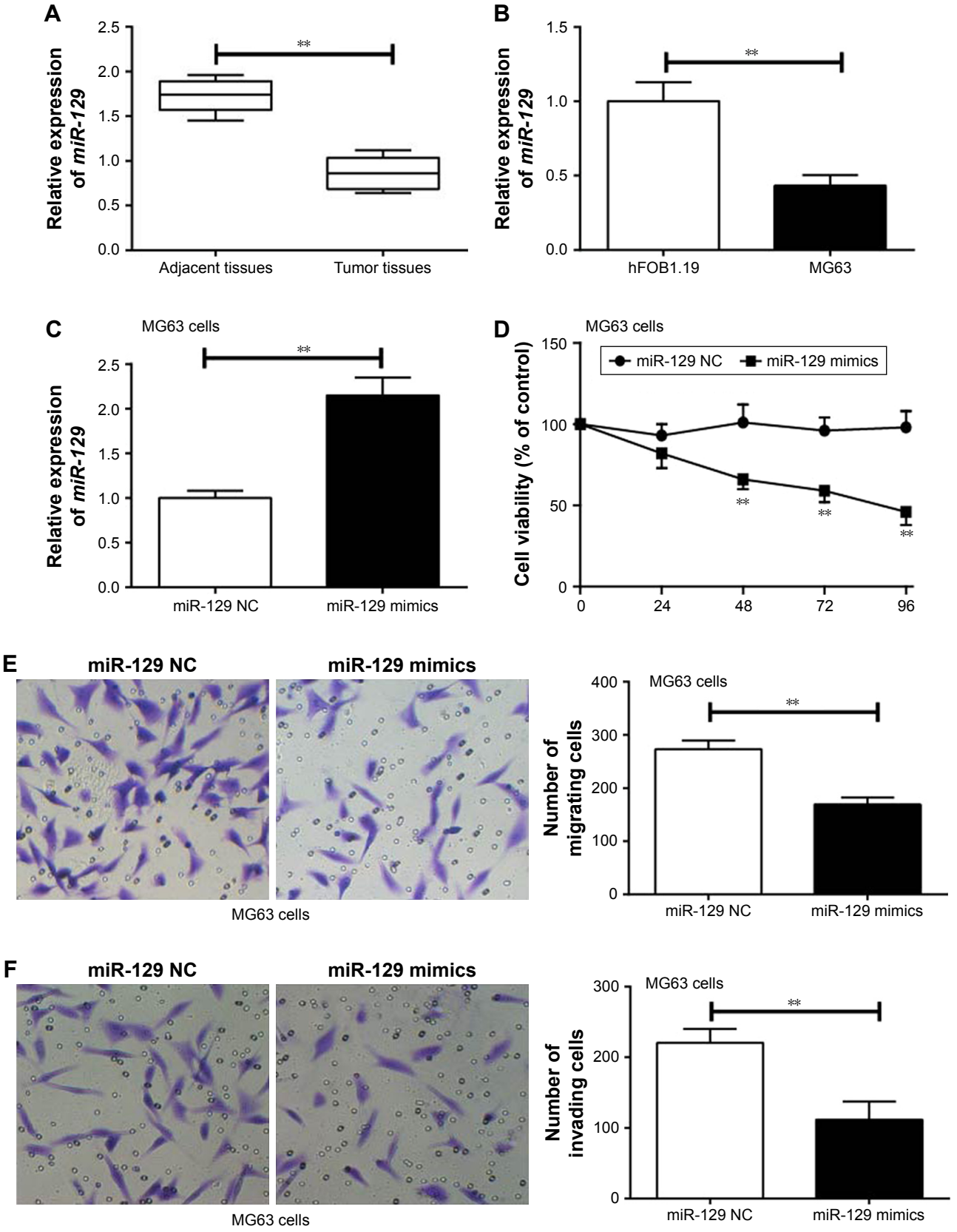

Figure 3 *miR-129* inhibited proliferation, migration, and invasion of MG63 cells.

Notes: (**A**) *miR-129* relative expression in tumor tissues was much lower than that found in adjacent tissues. (**B**) Significantly declined *miR-129* relative expression was found in MG63 cells when compared with that found in hFOB1.19 cells. (**C**) The relative expression of *miR-129* was markedly higher in MG63 cells of the miR-129 mimics group than that of the miR-129 NC group. (**D**) Overexpression of *miR-129* significantly impaired MG63 cells viability. (**E**) Overexpression of *miR-129* significantly impaired MG63 cells migration ability (200× magnification). (**F**) Overexpression of *miR-129* significantly impaired MG63 cells invasion ability (200× magnification). $^{**}P<0.01$.

Abbreviation: NC, negative control.

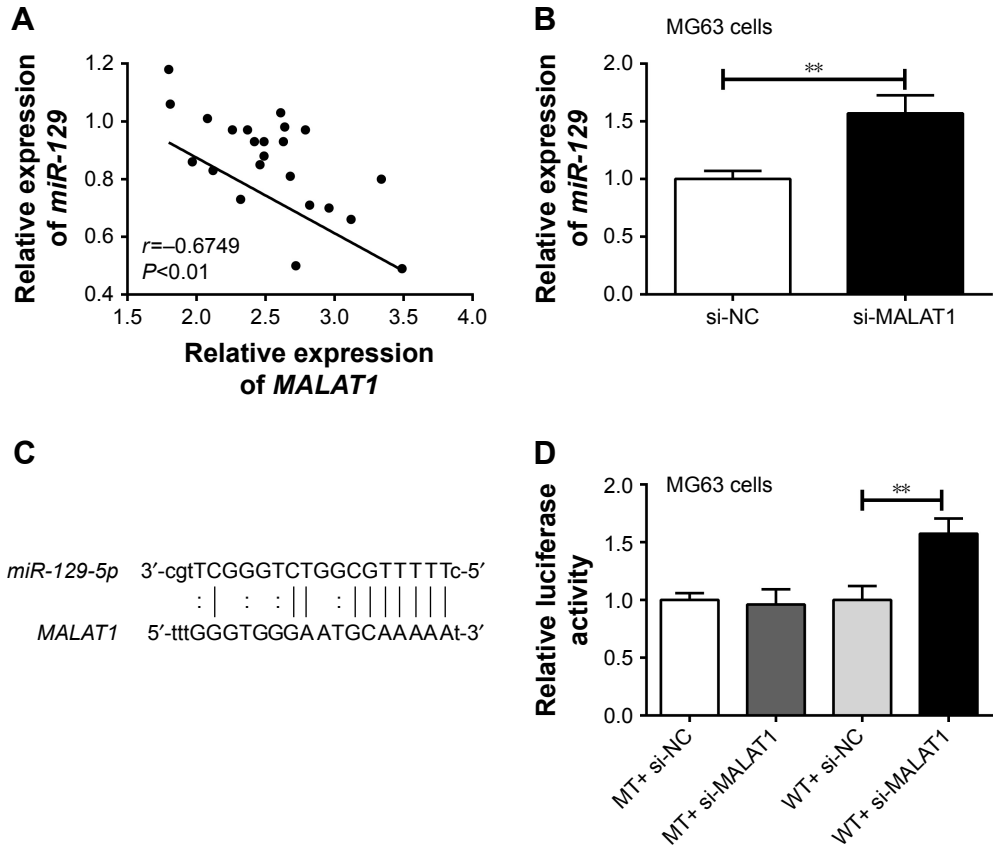

Figure 4 *miR-129* was targetedly and negatively regulated by *MALAT1*.
Notes: (**A**) Negative correlation between *MALAT1* and *miR-129* expression levels was found by Pearson correlation analysis. (**B**) *miR-129* relative expression in MG63 cells of si-MALAT1 group was significantly higher than that of si-NC group. (**C**) *MALAT1* directly binds to *miR-129* at 3′UTR region. (**D**) Luciferase reporter gene assay further confirmed that *miR-129* was directly and negatively regulated by *MALAT1*. $^{**}P<0.01$.
Abbreviations: MT, mutant-type; NC, negative control; WT, wild-type.

TGIF2 was upregulated in osteosarcoma tumor tissues and MG63 cells, and its expression might be affected by *MALAT1* and *miR-129*

As shown in Figure 5A and B, the relative expression of *TGIF2* mRNA and protein in osteosarcoma tumor tissues was dramatically higher than those in adjacent tissues ($P<0.01$). Meanwhile, when compared with hFOB1.19 cells, MG63 cells had much higher *TGIF2* mRNA and protein relative expression ($P<0.01$) (Figure 5C and D). Pearson correlation analysis revealed that there was a positive correlation between *TGIF2* mRNA and *MALAT1* expression ($P<0.01$), while a negative correlation was observed between *TGIF2* mRNA and *miR-129* expression ($P<0.05$) (Figure 5E and F).

TGIF2 was directly and negatively regulated by *miR-129*

Compared with miR-129 NC group, MG63 cells of miR-129 mimics group had significantly lower *TGIF2* mRNA and protein relative expression ($P<0.01$) (Figure 6A and B). We speculated that *TGIF2* might be regulated by *miR-129*. Therefore, Target Scan online forecasting was used to verify this speculation. As seen in Figure 6C, *miR-129* was directly bound to *TGIF2* at 3′UTR region. Luciferase reporter assay suggested that there was no statistical significance in luciferase activity between MT+ NC group and MT+ mimics group. However, the luciferase activity was found to be significantly decreased in the WT+ mimics group when compared with the WT+ NC group ($P<0.01$) (Figure 6D), indicating that *TGIF2* was targetedly and negatively regulated by *miR-129*.

MALAT1 promoted proliferation, migration, and invasion of MG63 cells by upregulating *TGIF2* via negatively regulating *miR-129*

This research still studied the interaction mechanism of *MALAT1*, *miR-129*, and *TGIF2* affecting osteosarcoma progression. When compared with p-Empty vector group, p-MALAT1+ miR-129 mimics group, and

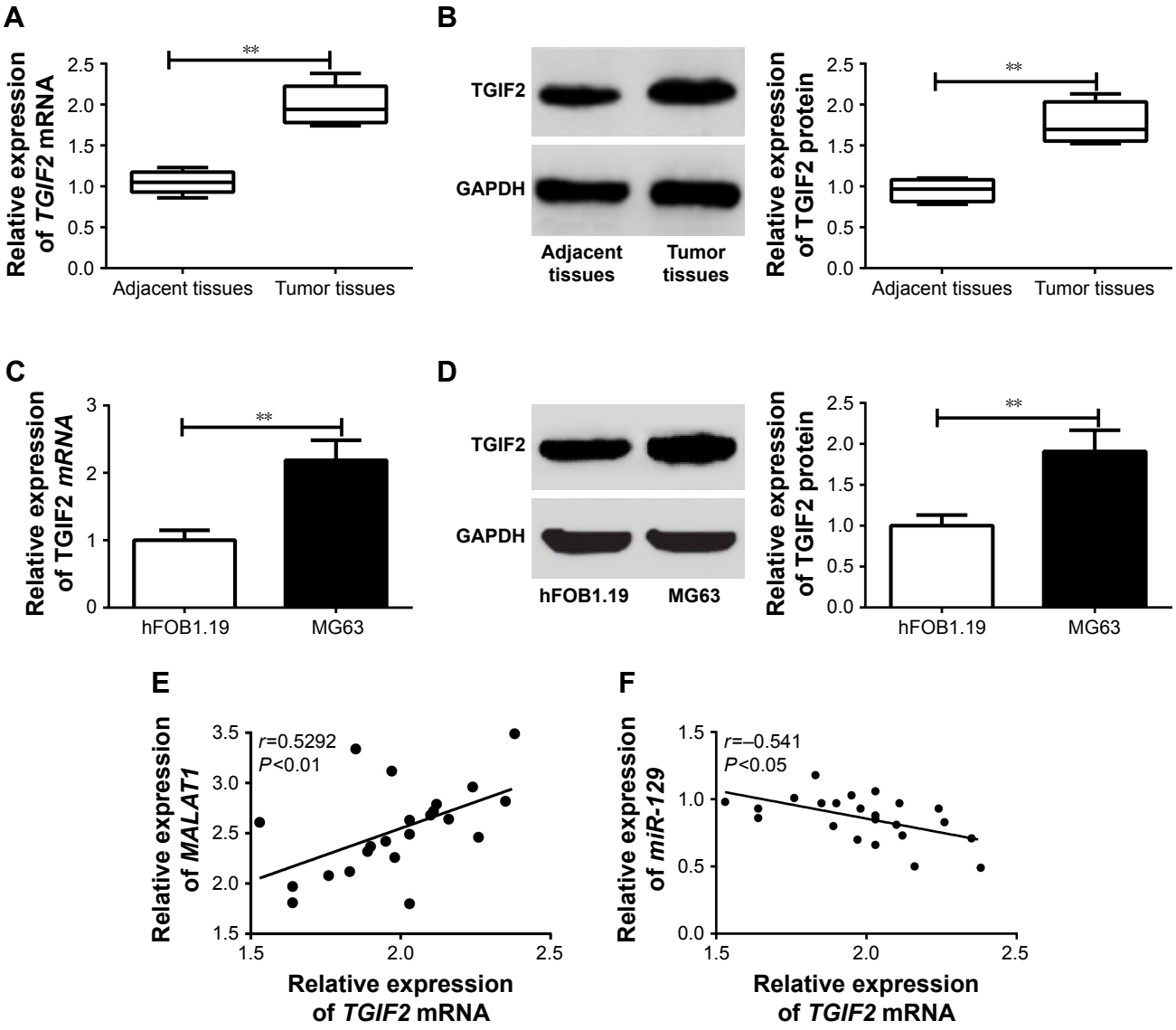

Figure 5 *TGIF2* was upregulated in osteosarcoma tumor tissues and MG63 cells, and its expression might be affected by *MALAT1* and *miR-129*.
Notes: (**A**) *TGIF2* mRNA relative expression in osteosarcoma tumor tissues was higher than that found in adjacent tissues. (**B**) TGIF2 protein relative expression in osteosarcoma tumor tissues was higher than that found in adjacent tissues. (**C**) *TGIF2* mRNA relative expression in MG63 cells was increased when compared with hFOB1.19 cells. (**D**) TGIF2 protein relative expression in MG63 cells was increased when compared with hFOB1.19 cells. (**E**) There was a positive correlation between *TGIF2* mRNA and *MALAT1* expression. (**F**) A negative correlation was observed between *TGIF2* mRNA and *miR-129* expression. $^{**}P<0.01$.
Abbreviation: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

p-MALAT1+ si-TGIF2 group, significantly increased *TGIF2* mRNA and protein relative expression could be seen in p-MALAT1 group ($P<0.01$) (Figure 7A and B). From 48 to 96 hours, the cell viability of p-MALAT1 group was much higher than the other three groups ($P<0.01$) (Figure 7C). Moreover, the number of migration and invasion cells of p-MALAT1 group was the highest, which was markedly higher than that of the other three groups ($P<0.01$) (Figure 7D and E). Thus, *MALAT1* promoted proliferation, migration, and invasion of MG63 cells by upregulating *TGIF2* via negatively regulating *miR-129*.

Discussion

Rapid growth and metastasis of osteosarcoma was the leading cause of death.[18] The discovery of effective prognostic factors and therapeutic targets are important tasks in improving the prognosis of patients with osteosarcoma. The regulation effect of lncRNAs in various tumors has attracted the attention of researchers. lncRNA *MALAT1* is located on chromosome 11q13, which has been pointed out to be involved in the regulation of multiple cancers.[19] Tee et al[20] suggested that, under hypoxic conditions, overexpression of *MALAT1* stimulated tumor-driven angiogenesis by promoting

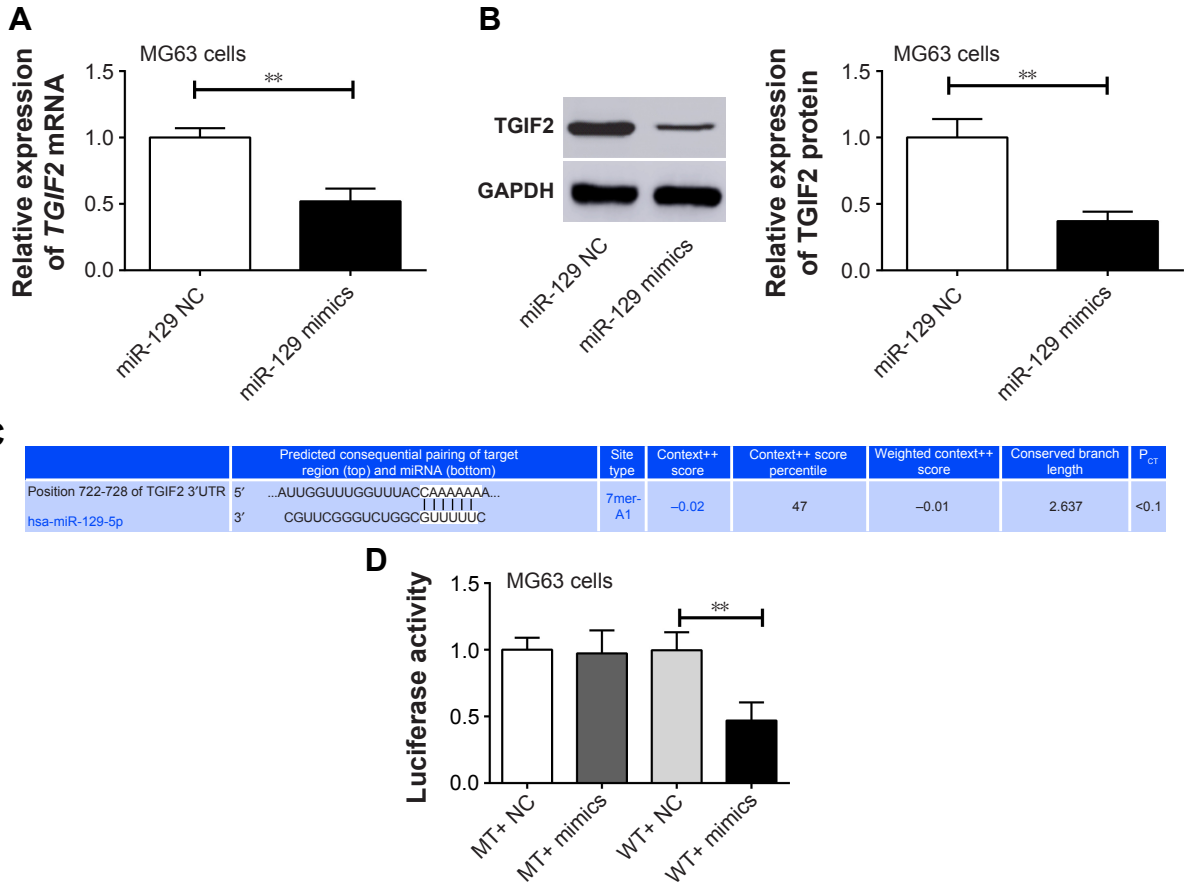

Figure 6 *TGIF2* was negatively regulated by miR-129.

Notes: (**A**) MG63 cells of miR-129 mimics group had significantly lower *TGIF2* mRNA relative expression than miR-129 NC group. (**B**) MG63 cells of miR-129 mimics group had significantly lower TGIF2 protein relative expression than miR-129 NC group. (**C**) *miR-129* directly binds to *TGIF2* at 3′UTR region. (**D**) *TGIF2* was targetedly and negatively regulated by *miR-129* according to luciferase reporter assay. $^{**}P<0.01$.

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MT, mutant-type; NC, negative control; PCT, probability of conserved targeting; WT, wild-type.

fibroblast growth factor 2 expression and vasculature formation. In gastric cancer, high *MALAT1* expression predicted deep tumor invasion.[21] Jadaliha et al[22] considered that *MALAT1* was a metastasis driver, which could also serve as a prognostic marker for breast cancer patients. High level of *MALAT1* was still closely related to shorter survival rate in nephrectomy patients.[23,24] Existing literatures have also found abnormal expression of *MALAT1* in osteosarcoma. Through Kaplan–Meier survival analysis, Huo et al[3] reported that high serum *MALAT1* expression of osteosarcoma patients exhibited declined survival rate, and *MALAT1* promoted migration of osteosarcoma cells and invasion by negatively regulating *EZH2*. Li et al[25] illustrated that upregulation of *MALAT1* was related to unfavorable prognosis in osteosarcoma patients. *MALAT1* could also enhance proliferation of osteosarcoma cells by inhibiting *miR-205* and stimulating *SMAD4*. In this study, we observed that *MALAT1* was aberrantly overexpressed in osteosarcoma tissues and cells, which enhanced osteosarcoma cells proliferation, migration, and invasion by upregulating *TGIF2* via suppressing *miR-129*.

miR-129 acted as a tumor suppressor in a variety of cancers, such as glioma, gastric cancer, and renal cell carcinoma.[26–28] In this study, *miR-129* was downregulated in osteosarcoma tissues and cells, and the proliferation, migration, and invasion ability of osteosarcoma cells was remarkably decreased after miR-129 was overexpressed. A previous study has also shown that osteosarcoma cells with high *miR-129* expression had decreased migration and invasion ability.[29] Several articles also reported the relationship between *MALAT1* and *miR-129* in tumors. For example, Zuo et al[30] demonstrated that *MALAT1* promoted the progression of triple-negative breast cancer cells through mediating the expression of *miR-129*. In colon cancer, *miR-129* was found to be directly regulated by *MALAT1*. *MALAT1* could cause extremely elevated expression of *HMGB* by sponging *miR-129*, thereby promoting the progression of colon cancer.[16]

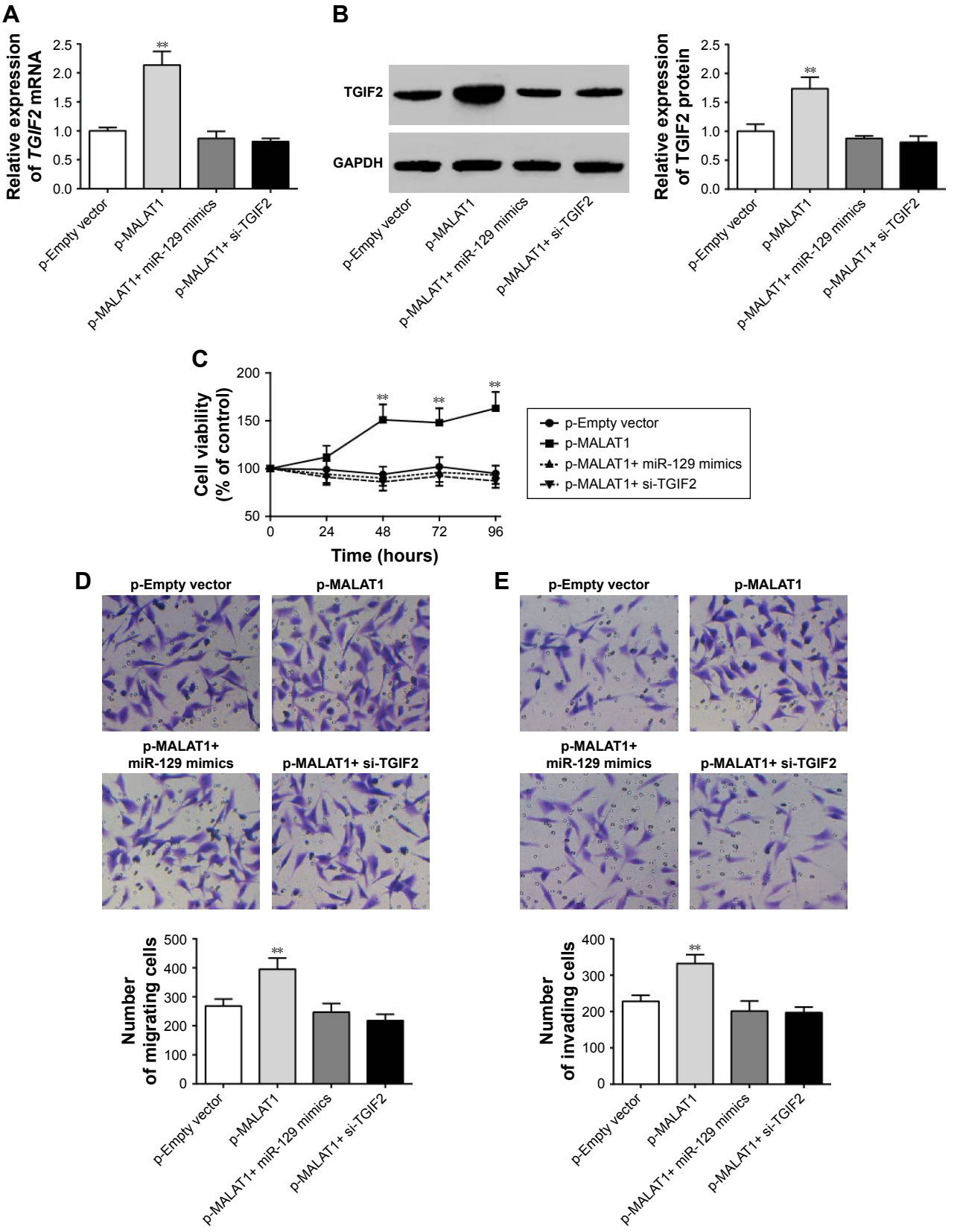

Figure 7 *MALAT1* promoted proliferation, migration, and invasion of MG63 cells by upregulating *TGIF2* via negatively regulating *miR-129*.

Notes: (**A**) Significantly increased *TGIF2* mRNA relative expression was found in p-MALAT1 group compared with the other three groups. (**B**) Significantly increased TGIF2 protein relative expression could be seen in p-MALAT1 group compared with the other three groups. (**C**) From 48 to 96 hours, the cell viability of p-MALAT1 group was much higher than the other three groups. (**D**) The number of migration cells of p-MALAT1 group was markedly higher than the other three groups (200× magnification). (**E**) The number of invasion cells of p-MALAT1 group was dramatically higher than the other three groups (200× magnification). $^{**}P<0.01$ when compared with the other three groups.

Abbreviation: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Other studies have also reported that *MALAT1/miR-129* axis could be used as candidate biomarker in glioma.[31] Currently, only one published literature was found to have studied the relationship between *miR-129* and *MALAT1* in osteosarcoma. Liu et al[32] found that *MALAT1* promoted osteosarcoma cells growth through suppression of *miR-129* expression. Our study further revealed that in osteosarcoma, *miR-129* was negatively and targetedly regulated by *MALAT1*.

TGIF2 acts as an oncogene, the upregulation of which was first discovered in ovarian cancer.[33] It has also been confirmed to be upregulated in many malignant tumors, including multiple myeloma cancer, non-small-cell lung cancer, prostate cancer, and so on.[33–35] Accumulated studies have found that *TGIF2* was regulated by multiple miRNAs in tumors. It could be negatively regulated by *miR-34* in gastric and breast cancer, and multiple myeloma.[34,36,37] In non-small-cell lung cancer, *TGIF2* was targeted and inhibited by *miR-541-3p*. It reversed the inhibition of growth and metastasis of non-small lung cancer cells induced by *miR-541-3p* overexpression.[33] In glioma, *TGIF2* expression was also found to be inhibited by *miR-129*,[38] which was similar to the findings of this study.

There was a limitation in this research. We performed correlation analysis of expression relationships between *MALAT1* and *miR-129* or *TGIF2* by Pearson correlation analysis. However, some potential interfering causes might also affect the correlation between these genes expression. Due to some limitations, we are currently unable to conduct related research. This will be further explored in our future research.

Conclusion

MALAT1 was upregulated in osteosarcoma tissues and cells, which enhanced osteosarcoma cells proliferation, migration, and invasion by upregulating *TGIF2* via suppression of *miR-129*. Therefore, *MALAT1* might be a new potential target for diagnosis and treatment of osteosarcoma.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Mukherjee S, Dash S, Lohitesh K, Chowdhury R. The dynamic role of autophagy and MAPK signaling in determining cell fate under cisplatin stress in osteosarcoma cells. *PLoS One*. 2017;12(6):e0179203.
2. Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. *Cancer Treat Res*. 2009;152:3–13.
3. Huo Y, Li Q, Wang X, et al. MALAT1 predicts poor survival in osteosarcoma patients and promotes cell metastasis through associating with EZH2. *Oncotarget*. 2017;8(29):46993–47006.
4. Jain S, Thakkar N, Chhatai J, Pal Bhadra M, Bhadra U. Long non-coding RNA: Functional agent for disease traits. *RNA Biol*. 2017;14(5):522–535.
5. Luo X, Qiu Y, Jiang Y, et al. Long non-coding RNA implicated in the invasion and metastasis of head and neck cancer: possible function and mechanisms. *Mol Cancer*. 2018;17(1):14.
6. Qiu L, Wang T, Xu X, Wu Y, Tang Q, Chen K. Long non-coding RNAs in hepatitis B virus-related hepatocellular carcinoma: regulation, functions, and underlying mechanisms. *Int J Mol Sci*. 2017;18(12):E2505.
7. Yang W, Li Y, He F, Wu H. Microarray profiling of long non-coding RNA (lncRNA) associated with hypertrophic cardiomyopathy. *BMC Cardiovasc Disord*. 2015;15:62.
8. Li H, Liu X, Zhang L, Li X. LncRNA BANCR facilitates vascular smooth muscle cell proliferation and migration through JNK pathway. *Oncotarget*. 2017;8(70):114568–114575.
9. Ma B, Li M, Zhang L, et al. Upregulation of long non-coding RNA TUG1 correlates with poor prognosis and disease status in osteosarcoma. *Tumour Biol*. 2016;37(4):4445–4455.
10. Lv GY, Miao J, Zhang XL. Long noncoding RNA XIST promotes osteosarcoma progression by targeting Ras-related protein RAP2B via miR-320b. *Oncol Res*. 2018;26(6):837–846.
11. Li P, Zhang X, Wang H, et al. MALAT1 is associated with poor response to oxaliplatin-based chemotherapy in colorectal cancer patients and promotes chemoresistance through EZH2. *Mol Cancer Ther*. 2017;16(4):739–751.
12. Li LJ, Chai Y, Guo XJ, Chu SL, Zhang LS. The effects of the long non-coding RNA MALAT-1 regulated autophagy-related signaling pathway on chemotherapy resistance in diffuse large B-cell lymphoma. *Biomed Pharmacother*. 2017;89:939–948.
13. Zhou S, Xiong M, Dai G, et al. MicroRNA-192-5p suppresses the initiation and progression of osteosarcoma by targeting USP1. *Oncol Lett*. 2018;15(5):6947–6956.
14. Zhai J, Qu S, Li X, et al. miR-129 suppresses tumor cell growth and invasion by targeting PAK5 in hepatocellular carcinoma. *Biochem Biophys Res Commun*. 2015;464(1):161–167.
15. Setijono SR, Park M, Kim G, Kim Y, Cho KW, Song SJ. miR-218 and miR-129 regulate breast cancer progression by targeting Lamins. *Biochem Biophys Res Commun*. 2018;496(3):826–833.
16. Wu Q, Meng WY, Jie Y, Zhao H. LncRNA MALAT1 induces colon cancer development by regulating miR-129-5p/HMGB1 axis. *J Cell Physiol*. 2018;233(9):6750–6757.
17. Li J, Wang H, Ke H, Ni S. MiR-129 regulates MMP9 to control metastasis of non-small cell lung cancer. *Tumour Biol*. 2015;36(8):5785–5790.
18. Zhuo B, Li Y, Gu F, et al. Overexpression of CD155 relates to metastasis and invasion in osteosarcoma. *Oncol Lett*. 2018;15(5):7312–7318.
19. Zhai H, Li XM, Maimaiti A, et al. Prognostic value of long noncoding RNA MALAT1 in digestive system malignancies. *Int J Clin Exp Med*. 2015;8(10):18099–18106.
20. Tee AE, Liu B, Song R, et al. The long noncoding RNA MALAT1 promotes tumor-driven angiogenesis by up-regulating pro-angiogenic gene expression. *Oncotarget*. 2016;7(8):8663–8675.
21. Lee NK, Lee JH, Ivan C, et al. MALAT1 promoted invasiveness of gastric adenocarcinoma. *BMC Cancer*. 2017;17(1):46.
22. Jadaliha M, Zong X, Malakar P, et al. Functional and prognostic significance of long non-coding RNA MALAT1 as a metastasis driver in ER negative lymph node negative breast cancer. *Oncotarget*. 2016;7(26):40418–40436.
23. Chen H, Meng T, Liu X, et al. Long non-coding RNA MALAT-1 is downregulated in preeclampsia and regulates proliferation, apoptosis, migration and invasion of JEG-3 trophoblast cells. *Int J Clin Exp Pathol*. 2015;8(10):12718–12727.
24. Sun L, Sun P, Zhou QY, Gao X, Han Q. Long noncoding RNA MALAT1 promotes uveal melanoma cell growth and invasion by silencing of miR-140. *Am J Transl Res*. 2016;8(9):3939–3946.
25. Li Q, Pan X, Wang X, et al. Long noncoding RNA MALAT1 promotes cell proliferation through suppressing miR-205 and promoting SMAD4 expression in osteosarcoma. *Oncotarget*. 2017;8(63):106648–106660.

26. Yang Y, Huang JQ, Zhang X, Shen LF. MiR-129-2 functions as a tumor suppressor in glioma cells by targeting HMGB1 and is down-regulated by DNA methylation. *Mol Cell Biochem*. 2015;404(1–2):229–239.
27. Wang Q, Yu J. MiR-129-5p suppresses gastric cancer cell invasion and proliferation by inhibiting COL1A1. *Biochem Cell Biol*. 2018; 96(1):19–25.
28. Hl X, Feng YY, Jia ZK. MicroRNA-129-5p inhibits the proliferation and migration in renal cell carcinoma via targeting IGF2BP1. *Int J Clin Exp Pathol*. 2016;9:8254–8260.
29. Long XH, Zhou YF, Peng AF, et al. Demethylation-mediated miR-129-5p up-regulation inhibits malignant phenotype of osteogenic osteosarcoma by targeting Homo sapiens valosin-containing protein (VCP). *Tumour Biol*. 2015;36(5):3799–3806.
30. Zuo Y, Li Y, Zhou Z, Ma M, Fu K. Long non-coding RNA MALAT1 promotes proliferation and invasion via targeting miR-129-5p in triple-negative breast cancer. *Biomed Pharmacother*. 2017;95:922–928.
31. Xiong Z, Wang L, Wang Q, Yuan Y. LncRNA MALAT1/miR-129 axis promotes glioma tumorigenesis by targeting SOX2. *J Cell Mol Med*. 2018;22(8):3929–3940.
32. Liu K, Huang J, Ni J, et al. MALAT1 promotes osteosarcoma development by regulation of HMGB1 via miR-142-3p and miR-129-5p. *Cell Cycle*. 2017;16(6):578–587.
33. Lu YJ, Liu RY, Hu K, Wang Y. MiR-541-3p reverses cancer progression by directly targeting TGIF2 in non-small cell lung cancer. *Tumour Biol*. 2016;37(9):12685–12695.
34. Wu S, He X, Li M, et al. MiRNA-34a overexpression inhibits multiple myeloma cancer stem cell growth in mice by suppressing TGIF2. *Am J Transl Res*. 2016;8(12):5433–5443.
35. Zhiping C, Shijun T, Linhui W, Yapei W, Lianxi Q, Qiang D. MiR-181a promotes epithelial to mesenchymal transition of prostate cancer cells by targeting TGIF2. *Eur Rev Med Pharmacol Sci*. 2017;21(21): 4835–4843.
36. Hu Y, Pu Q, Cui B, Lin J. MicroRNA-34a inhibits tumor invasion and metastasis in gastric cancer by targeting Tgif2. *Int J Clin Exp Pathol*. 2015;8(8):8921–8928.
37. Krzeszinski JY, Wei W, Huynh HD, et al. Abstract P6-17-01: MicroRNA-34a suppresses breast cancer bone metastasis by inhibiting osteoclastogenesis and targeting tgif2. *Cancer Res*. 2015;75 (Suppl 9): P6-17-01.
38. Diao Y, Jin B, Huang L, Zhou W. MiR-129-5p inhibits glioma cell progression in vitro and in vivo by targeting TGIF2. *J Cell Mol Med*. 2018;22(4):2357–2367.