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MAT1 facilitates the lung metastasis of osteosarcoma through upregulation of AKT1 expression

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ABSTRACT

Aims: We aimed to elucidate the effects and mechanisms of MAT1 in the progression of osteosarcoma, especially for its lung metastasis.

Main methods: CCK-8 and flow cytometry assays were carried out to detect the proliferation and apoptosis of osteosarcoma cells. Wound healing and transwell assays were used to determine cell migration and invasion abilities. Real time quantitative PCR (RT-PCR) and western blot technologies were applied to detect the expression levels of RNA and protein, respectively.

Key finds: The results showed that both the mRNA and protein expression levels of MAT1 were elevated in osteosarcoma tissues with lung metastasis and metastatic lung tissues, particularly in the metastatic lung tissues, as compared to the osteosarcoma tissues without lung metastasis. High expression level of MAT1 in osteosarcoma patients showed a negative association with the overall survival. In addition, upregulation of MAT1 induced significant increases in cell growth, migration and invasion and an obvious inhibition in cell apoptosis in osteosarcoma MG63 and 143B cells, as well as elevated AKT1 expression level. Moreover, knockdown of AKT1 obviously impaired MAT1-mediated promotions in cell migration and invasion in vitro, as well as repressed tumor growth and reduced the number of metastatic lung tumors in xenografted nude mice.

Significance: This study reveals that high expression of MAT1 closely related to the poor prognosis and malignant clinical process of osteosarcoma patients. MAT1 serves as a promoter in the lung metastasis of osteosarcoma through increasing AKT1 expression. Our study may provide a potent therapeutic target for the lung metastasis of osteosarcoma.

1. Introduction

Osteosarcoma is the most common primary malignant bone tumor affecting children and adolescents. It is mainly derived from the metaphysis of long bone and is inclined to local invasion and distant metastases, with lung metastasis as the most common type [1–3]. Despite that patients' prognosis has been improved due to the application of combination therapy of chemoradiotherapy and surgical resection [4,5], the five-year survival rates of patients with lung metastasis are still dim, with ~30% [6]. Therefore, further exploration of the mechanisms underlying the lung metastasis in osteosarcoma is of importance.

It's well documented that cyclin-dependent kinases (CDKs) play important roles in modulating cell proliferation via controlling cell cycle [7]. The phosphorylation of CDKs is executed by CDK-activating kinase (CAK) [8,9], which is a trimeric complex consisting of CDK7 [10], Cyclin H [11], and the accessory protein, MAT1 (MNAT1) [12,13]. MAT1 gathers CAK and determines the substrate specificity of CAK, and then regulates cell cycle G1 exit [14]. Through MAT1, CAK interacts with and phosphorylates retinoblastoma tumor suppressor protein (Rb), a proliferation repressor and a differentiation promoter [15,16]. However, MAT1 deletion impedes the phosphorylation of Rb and induces G1 arrest in osteosarcoma cells [14], suggesting that MAT1 may play a role in the progression of osteosarcoma. Noticeably, inhibition of the expression and phosphorylation of Rb plays an important role in the metastasis of various kinds of cancers. For example, Arima et al. [17] demonstrated that downregulation of Rb with siRNA interference in breast cancer MCF-7 cells destroyed cell-cell adhesion and promoted the epithelial-to-mesenchymal transition (EMT), which was closely implicated in the metastasis of primary tumors. Berman et al. [18] found that mice with Rb and p53 double mutant were viable and displayed multiple characteristics of human osteosarcomas, including high metastasis. These findings suggest that MAT1 might be involved in the metastasis of osteosarcoma.

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As a result, this study aimed to explore the effects of MAT1 on the metastasis of osteosarcoma, especially for lung metastasis, as well as reveal its underlying mechanisms.

2. Materials and methods

2.1. Patients

All tissue samples used in this study were obtained from osteosarcoma patients who received an osteosarectomy with/without a pneumonectomy. In detail, 8 osteosarcoma tissues were obtained from patients with no pulmonary metastasis; 8 osteosarcoma tissues were acquired from patients with pulmonary metastasis and another 8 lung tissues were gained from patients with pulmonary metastasis. Experiments involving human samples were approved by the ethical committee of the 2nd Xiangya Hospital, Central South University (Changsha, Hunan, China).

2.2. Immunohistochemistry

Paraffin-embedded tissues were sliced into 4 μm thickness sections and proceeded to the following procedures bases on a previous report [19]: dewaxing and hydrating; incubation with 3% H_2O_2; antigen repairing with Tris-EDTA; incubation with 5% goat serum to block the slides; incubation with anti-MAT1 antibody (1:100 dilution; No. ab174687, Abcam, MA, USA) overnight at 4 °C, and incubation with the corresponding secondary antibody. 3, 3′-diaminobenzidine tetrachloride (Sigma, MO, USA) was served as a substrate. Harri's hematoxylin solution was used to stain the nucleus.

The staining of MAT1 protein was evaluated by two investigators according to both the proportion of positive staining tumor cells and the staining intensity. The proportion of positive stained tumor cells was scored as: 0 (no positive tumor cells), 1 (< 10%), 2 (10–50%), 3 (50–75%) and 4 (> 75%). The staining intensity was scored as: 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). The final score was obtained by multiplying the staining intensity score and positive staining score. The score of MAT1 staining ≥6 was considered as MAT1 high expression and < 6 was thought as MAT1 low expression.

2.3. Cell culture and treatment

Human osteosarcoma cell lines MG63 and 143B were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). MG63 cells were maintained in Eagle's Minimum Essential Medium (Gibco, CA, USA), while 143B cells were cultured in Earle's BSS with 0.015 mg/ml 5-bromo-2′-deoxyuridine, all filled with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA).

To upregulate the expression of AKT1 and MAT1 in both MG63 and 143B cells, lentivirus containing human AKT1/MAT1 open reading frame (ORF) were constructed by GenePharma (Shanghai, China). ShRNAs targeting the human AKT1 gene (sh-AKT1; No. TL320260) and MAT1 gene (sh-MAT1; No. TL311433) were purchased from OriGene (Beijing, China) and used to downregulate AKT1 and MAT1, respectively.

2.4. Real time quantitative PCR (RT-PCR)

Total RNA extracted from cells and tissues were obtained by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then, the extracted RNAs were reversely transcribed into the complementary DNA (cDNA) with QuantiTect Reverse Transcription Kit (Qiagen, NY, USA), followed by RT-PCR to detect the expression of MAT1 using SuperRT One Step RT-PCR Kit (CWBIO, Beijing, China). The relative expressions of mRNAs were calculated by the $2^{-\Delta\Delta Ct}$ method, with glyceraldehyde3-phosphate dehydrogenase (GAPDH) level as an internal control. Primer sequences are shown as follows: MAT1-forward: 5′-ACTGCCCTGAGTG TGGTACT-3′, MAT1-reverse: 5′-TGAAATATGTTGACCCAGCTCATCT-3′; GAPDH-forward: 5′-TTTGCGTCGCCAGGTGAAGA-3′, GAPDH-reverse: 5′-AGTTAAAAGCAGCCCTGGTGA-3′.

Table 1
Relationship between MAT1 expression and the clinicopathologic features of osteosarcoma patients.

Clinicopathological features	n	High expression	Low expression	*P* value
Gender				0.536
Male	26	12	14	
Female	21	12	9	
Age (years)				0.171
≤21	42	20	22	
> 21	5	4	1	
Tumor size (cm^3)				0.025
≤20	27	10	17	
> 20	20	14	6	
Metastasis				0.029
Yes	26	17	9	
No	21	7	14	
Lung metastasis				0.002
Yes	19	15	4	
No	28	9	19	
Clinical stage				0.013
I/II	20	6	14	
III	27	18	9	

2.5. Western blot analysis

For protein isolation, tissue samples and cells were first incubated with RIPA lysis buffer for 30 min at 4 °C. The mixture was then centrifuged at 12,000 ×*g* for 20 min and the supernatant was carefully collected. After protein concentration being quantified with BCA Protein Assay (Bio-Rad Laboratories, CA, USA), 20–30 μg proteins were separated by electrophoresis via 10% polyacrylamide gels, and were then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Next, the membranes were incubated with 5% non-fat milk and probed overnight at 4 °C with the following primary antibodies, including MAT1 (1:500 dilution; No. ab174687, Abcam, MA, USA), ROCK1 (1:3000 dilution; No ab45171, Abcam, MA, USA), α-SMA (1:2000 dilution; No. 14968, Cell Signaling Technology, CA, USA), N-cadherin (1:500 dilution; No. ab18203, Abcam, MA, USA), E-cadherin (1:500 dilution; No. ab15148, Abcam, CA, USA), Vimentin (1:1000 dilution; No. 3932, Cell Signaling Technology, CA, USA), AKT1 (1:1000 dilution; No. 2967, Cell Signaling Technology, CA, USA) and GAPDH (1:50000 dilution; Santa Cruz Biotechnology, TX, USA). After incubation with the second antibody (1:50000 dilution; Santa Cruz Biotechnology, TX, USA), the protein signals in membranes were examined by using chemiluminescent ECL reagent (Millipore, Billerica, MA, USA).

2.6. Cell counting kit-8 (CCK-8) assay

MG63 and 143B cells were seeded in 96-well plates at a density of 3×10^3 cells/well overnight, and the cells were transfected with different vectors: sh-MAT1, sh-NC, OE-MAT1 or OE-NC. Next, a CCK-8 kit (Beyotime, Beijing, China) was applied to detect the proliferation potential of these cells, according to the manuscript's description. The absorbance at 450 nm was measured with a plate reader (model 680; Bio-Rad, Hertfordshire, UK) every 24 h after cell transfections until 72 h.

2.7. Flow cytometry assay

The apoptosis of MG63 and 143B cells was determined by using

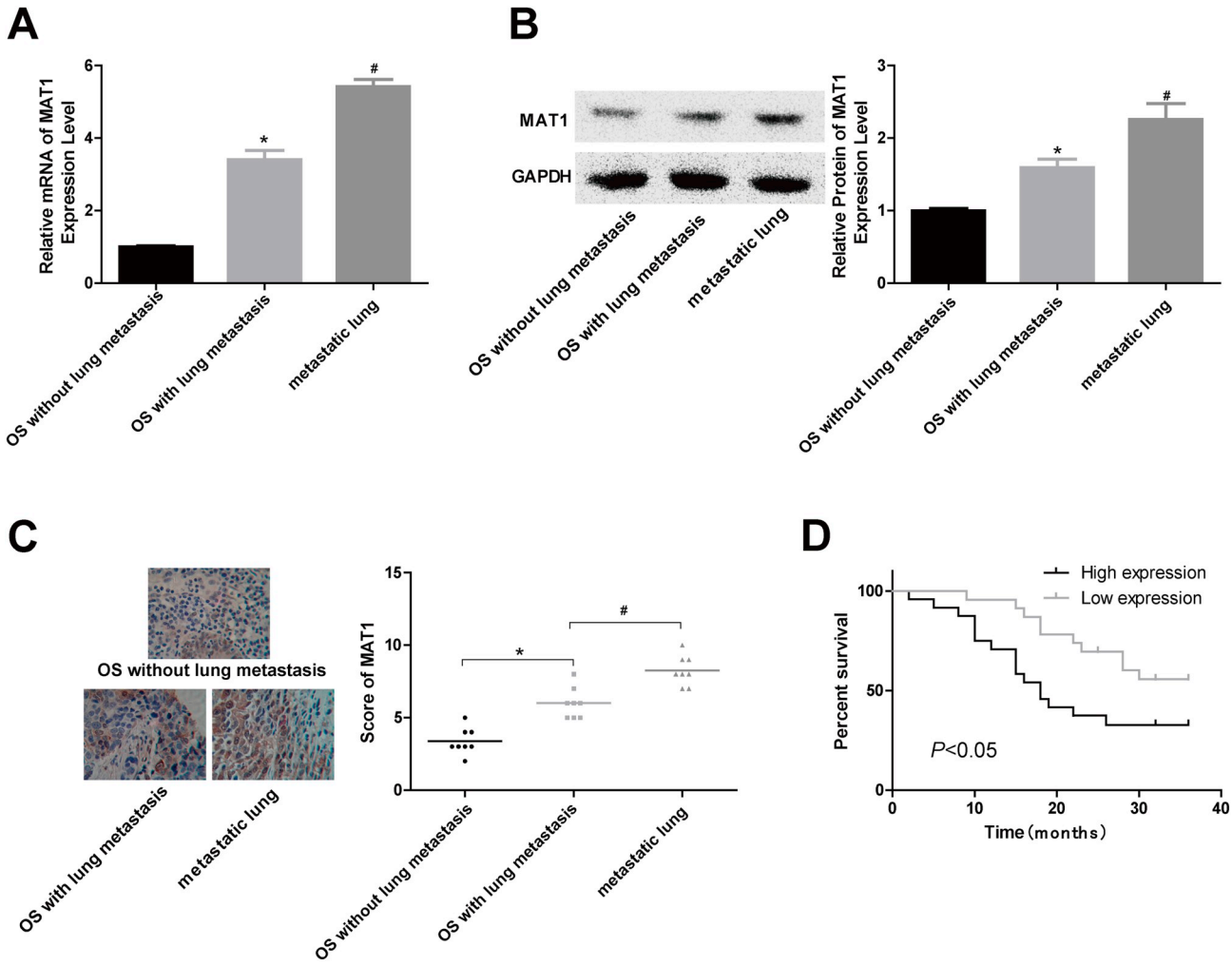

Fig. 1. MAT1 was highly expressed in the metastasis lung tissues of osteosarcoma. (A–B) Protein and mRNA levels of MAT1 in 8 cases of osteosarcoma tissues with or without lung metastasis and 8 cases of metastasis lung tissues were determined by western blot and RT-PCR, respectively. (C) Immunohistochemistry was used to analyze the expression of MAT1 protein in 8 cases of osteosarcoma tissues with or without lung metastasis and 8 cases of metastasis lung tissues of osteosarcoma. For Fig. 1A–C, $^{*}P < 0.05$, OS (osteosarcoma) with lung metastasis group versus OS without lung metastasis group; $^{\#}P < 0.05$, metastatic lung group versus OS with lung metastasis group. (D) Kaplan-Meier analysis of the overall survival in osteosarcoma patients with MAT1 high expression (n = 24) or low expression (n = 23).

flow cytometry assay with Annexin V (FITC)/Propidium Iodide (PI) apoptosis detection kit (KeyGEN Biotech, Jiangsu, China), according to a previous study [20].

2.8. Wound healing assay

The migration abilities of MG63 and 143B cells with different treatments were assessed by wound healing assay. In brief, 1×10^5 cells were seeded in each well of a 24-well plate after 24 h of cell transfection with sh-NC, sh-MAT1, OE-NC, OE-MAT1, sh-AKT1 or OE-MAT1 + sh-AKT1. Subsequently, a 1-mm-wide symmetrical wound was formed using a 20 μl of pipette. After the floated cells were removed via washing with PBS, the adhesive cells were incubated at 37 °C for 24 h with serum-free medium. Six random fields were recorded to evaluate cell migration in a microscope (100× magnification; Abcam, Cambridge, MA, USA) at 0 and 24 h after drawing the wound.

2.9. Transwell assay

The invasion potentials of MG63 or 143B cells were determined using 24-well Transwell chambers (Costar, Massachusetts, USA) coated with Matrigel. After 24 h of different cell transfections, 1×10^5 cells suspended with 200 μl serum-free medium were seeded into the upper chamber of an 8 μm insert. At the same time, 600 μl cell culture medium containing 15% FBS were added into the lower chamber. After incubation at 37 °C for 48 h, cells on the surface of the upper chamber were wiped off with cotton tips, and the invasive cells sticking to the lower membrane surface were fixed with cold methyl alcohol for 15 min and then stained with crystal violet for 8 min in the room temperature. The number of invasive cells in six randomly selected fields was counted under a microscope.

2.10. In vivo tumorigenicity assay

Animal study was performed in accordance with institutional guidelines of the Research Ethics Committee of the 2nd Xiangya Hospital of Central South University, China. Four-week male BALB/c athymic nude mice purchased from Beijing Vital River Laboratory Animal Technology (Beijing, China) were used to build the in vivo tumor xenograft model. Before animal experiments, 143B cells were infected with NC, OE-MAT1, OE-MAT1 + sh-AKT1, sh-MAT1 or sh-MAT1 + OE-AKT1, and were selected by G418 (100 μg/ml), G418 (100 μg/ml), G418 (100 μg/ml) + puromycin (7 μg/ml), puromycin (7 μg/ml) or G418 (100 μg/ml) + puromycin (7 μg/ml) for 14 days to build the stably transfected cell lines. Then, 5×10^6 stable 143B cells undergone the above treatments were injected subcutaneously in flanks

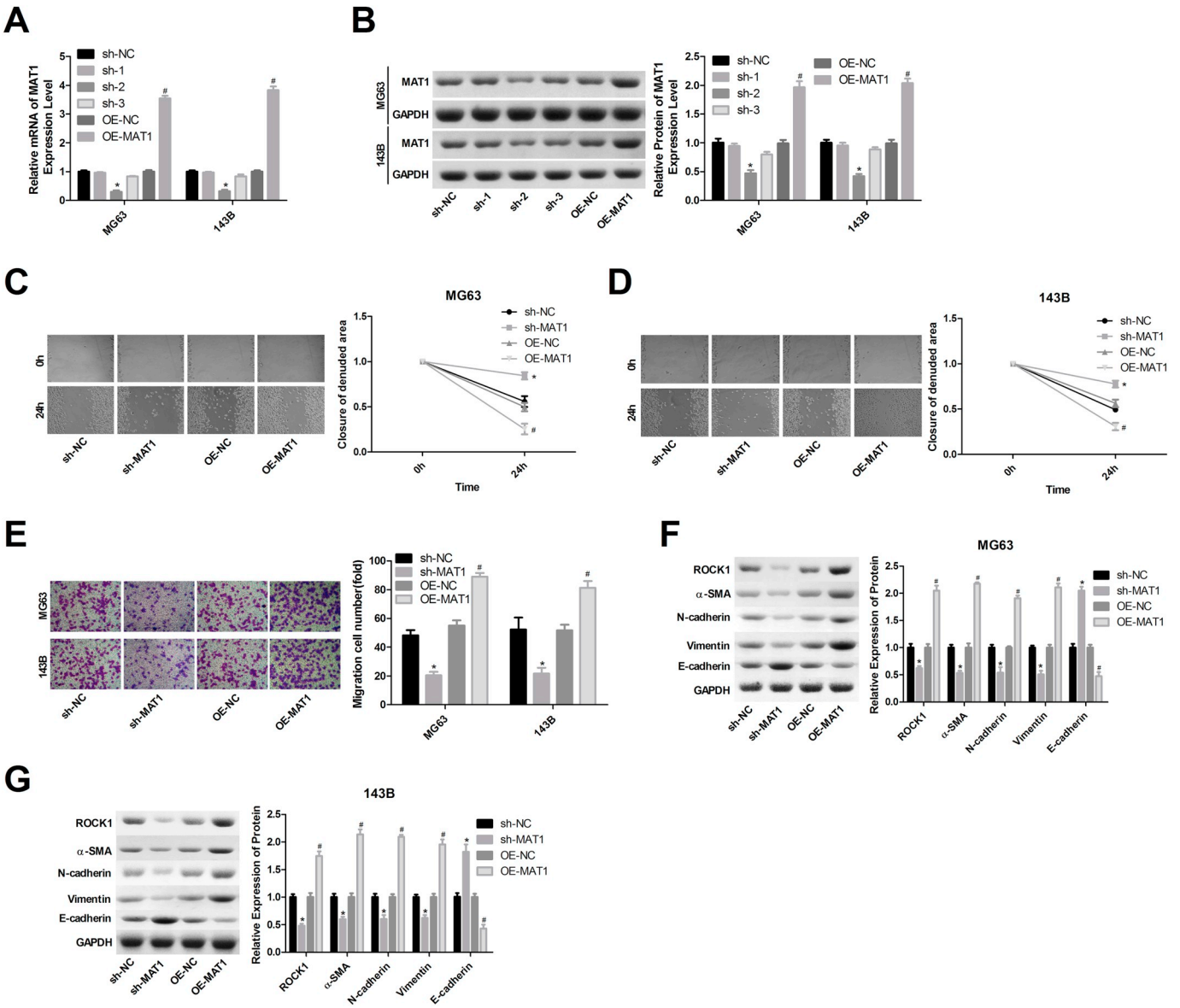

Fig. 2. Overexpression of MAT1 promoted the migration and invasion of MG63 and 143B cells. (A–B) RT-PCR and western blot were performed to measure the mRNA and protein expression levels of MAT1 after MG63 and 143B cells were transfected sh-1, sh-2, sh-3, sh-NC, OE-MAT1 or OE-NC for 48 h or 24 h. All the experiments were performed three times. ($^{*}P < 0.05$, sh-1/sh-2/sh-3 group versus sh-NC group; $^{\#}P < 0.05$, OE-MAT1 group versus OE-NC group) MG63 and 143B cells were transfected OE-NC, OE-MAT1, sh-NC or sh-MAT1, then (C–D) Wound healing assay was performed to detect the migration ability of MG63 and 143B cells after 0 h and 24 h of cell transfection. (E) Transwell chamber coated with Matrigel was used to assess cell invasion after 48 h of cell transfection. (F-G) Western blot analysis of the expressions of ROCK1, α-SAM, N-cadherin, E-cadherin and Vimentin after 48 h of cell transfection. All the experiments were performed three times. ($^{*}P < 0.05$, sh-MAT1 group versus sh-NC group; $^{\#}P < 0.05$, OE-MAT1 group versus OE-NC group).

of mice, with 6 mice in each group. After 28 days of the implantation, mice were killed and the lung tissues were took out to assess the number of tumors which originated from the osteosarcoma cells.

2.11. Statistical analysis

Data were obtained from at least three independent experiments and expressed as mean ± standard deviation (SD). SPSS 22.0 software (Chicago, IL, USA) was used to do data analysis. Enumeration data were analyzed using the chi square test (χ^2 test). Data conformed to Gaussian distribution was analyzed by the *t*-test or one-way analysis of variance (ANOVA). A P value < 0.05 was considered statistically significant.

3. Results

3.1. High expression of MAT1 closely associates with osteosarcoma patients' advanced clinical stage and lung metastasis

To explore the effects of MAT1 in the lung metastasis of osteosarcoma, we first divided osteosarcoma patients into two groups, MAT1 high expression group (n = 24) and MAT1 low expression group (n = 23), according to the expression levels of MAT1 obtained from immunohistochemistry staining of MAT1. The results showed that the high expression level of MAT1 closely associated with advanced clinical stage ($P = 0.013$) and high incidence of lung metastasis ($P = 0.002$) (Table 1), suggesting that MAT1 might play an important role in the lung metastasis of osteosarcoma.

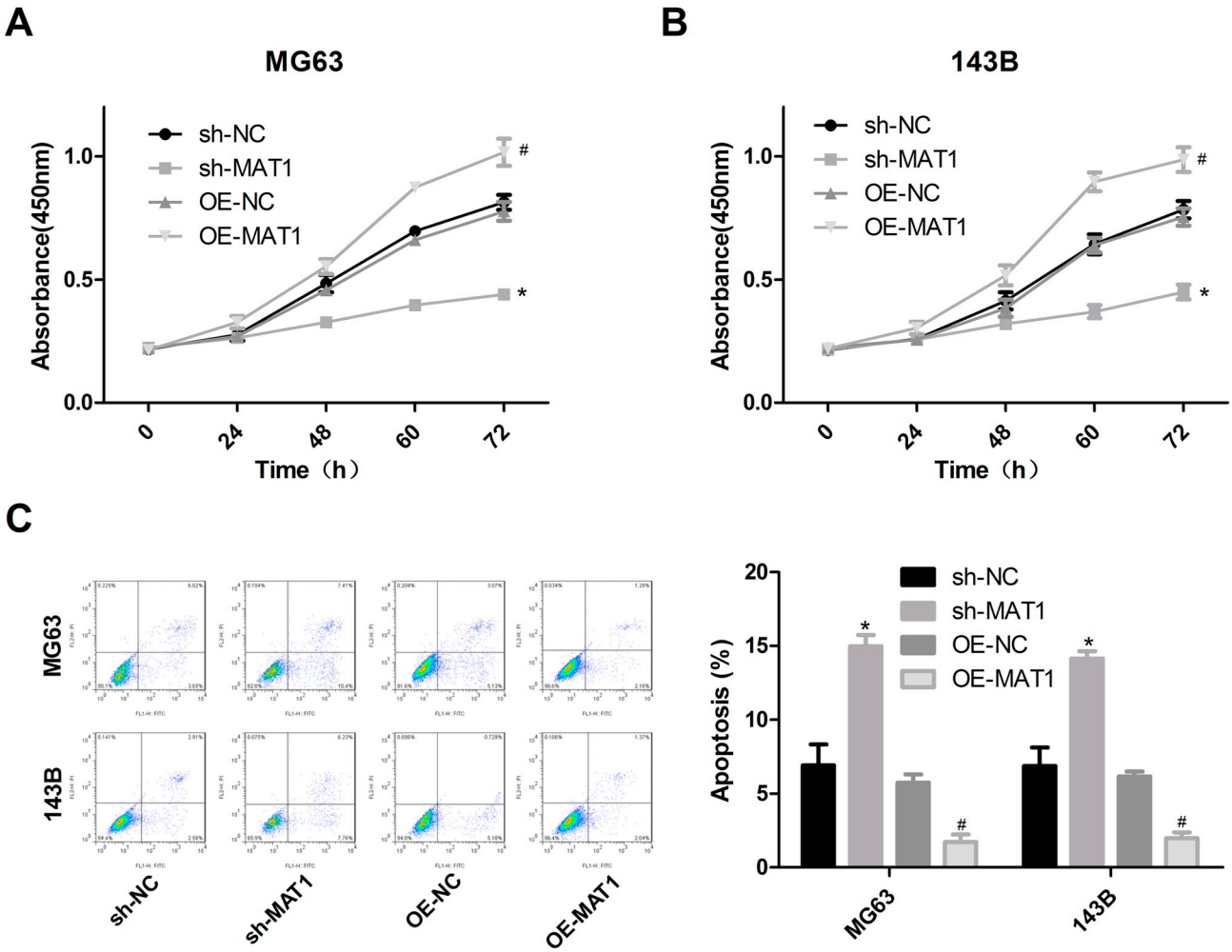

Fig. 3. Analysis of the effects of MAT1 on the growth and apoptosis of MG63 and 143B cells. (A–B) Cell growth ability was determined by CCK-8 assay after MG63 and 143B cells were transfected OE-NC, OE-MAT1, sh-NC or sh-MAT1 for the indicated times. (C) Cell apoptosis was evaluated by flow cytometry with Annexin V/PI staining after 48 h of cell transfection. All the experiments were performed three times. ($^{*}P < 0.05$, sh-MAT1 group versus sh-NC group; $^{\#}P < 0.05$, OE-MAT1 group versus OE-NC group).

3.2. MAT1 is highly expressed in osteosarcoma metastatic lung tissues

To explore the function of MAT1 in the lung metastasis of osteosarcoma, we then assessed the different expression profiles of MAT1 in osteosarcoma tissues without lung metastasis, osteosarcoma tissues with lung metastasis and metastatic lung tissues. Compared to the osteosarcoma tissues without lung metastasis, both the mRNA and protein expressions of MAT1 were elevated in the osteosarcoma tissues with lung metastasis and metastatic lung tissues, especially in the metastatic lung tissues (Fig. 1A–B). Moreover, the average scores of MAT1 staining in the osteosarcoma tissues with lung metastasis and metastatic lung tissues were higher than that in the osteosarcoma tissues with no lung metastasis, especially in the metastatic lung tissues (Fig. 1C). Furthermore, the survival curve used to evaluate the relationship between the overall survival and MAT1 expressions demonstrated that patients with high expression of MAT1 were inclined to have a short overall survival (Fig. 1D). Overall, these findings suggest that MAT1 closely associates the lung metastasis of osteosarcoma, as well as the shorter overall survival.

3.3. Overexpression of MAT1 promotes cell migration and invasion in osteosarcoma

Next, we investigated MAT1 roles in the migration and invasion of osteosarcoma using gain- and loss-of-function assays. OE-MAT1 transfection significantly increased the mRNA and protein expression levels of MAT1, whereas shRNA-2 transfection induced an obvious decrease in MAT1 expression in both MG63 and 143B osteosarcoma cell lines (Fig. 2A–B). We then carried out wound healing and transwell assays to explore the influence of MAT1 in the migration and invasion of osteosarcoma cells. The results showed that transfection of cells with sh-MAT1 significantly inhibited the migration (Fig. 2C–D) and invasion (Fig. 2E) of MG63 and 143B cells, whereas OE-MAT1 induced significant enhancement in cell migration and invasion abilities (Fig. 2C–E). To further reveal MAT1 roles in the lung metastasis of osteosarcoma, we also detected the expressions of proteins related to cancer cell migration, such as ROCK1, α-SMA, N-cadherin, Vimentin, TWIST and E-cadherin. The results showed that the expression of E-cadherin was decreased while the expressions of ROCK1, α-SMA, N-cadherin, Vimentin and TWIST were increased when MAT1 was overexpressed in both MG63 and 143B cells, and knockdown of MAT1 caused an opposite result (Fig. 2F–G). These results indicate that MAT1 serves as a promoter in osteosarcoma migration.

3.4. Overexpression of MAT1 enhances the growth of osteosarcoma cells

Besides, we also investigated the effects of MAT1 on the growth and apoptosis of osteosarcoma cells via CCK-8 and flow cytometry assays.

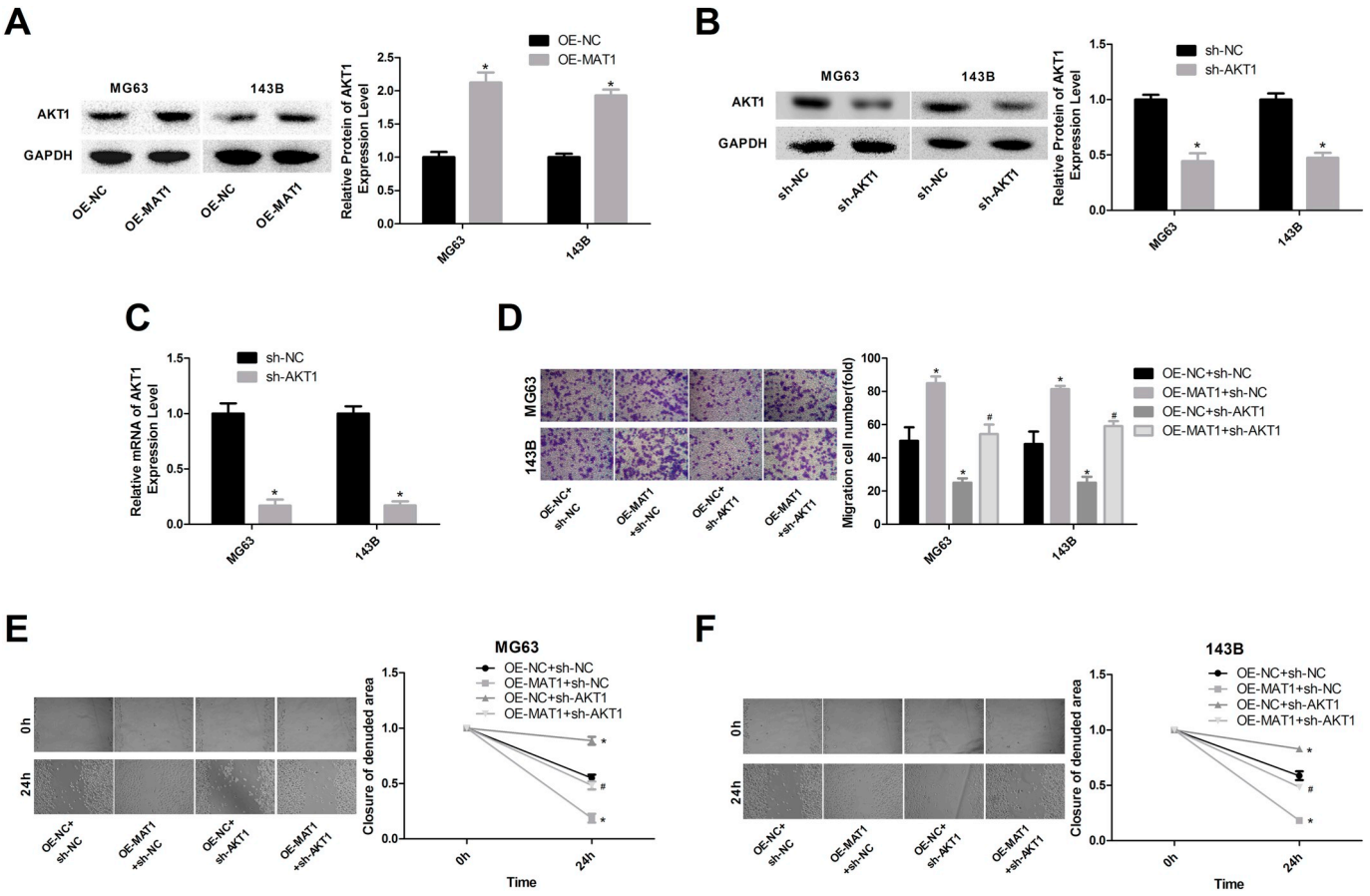

Fig. 4. Upregulation of MAT1 enhanced the migration and invasion of osteosarcoma cells through upregulation of AKT1 in vitro. (A) Western blot technology was carried out to examine the expression of AKT1 protein after MG63 and 143B cells were transfected OE-MAT1 or OE-NC for 48 h. (B–C) Western blot and RT-PCR technologies were carried out to examine the expression of AKT1 protein and mRNA after MG63 and 143B cells were transfected sh-AKT1 or sh-NC for 48 h or 24 h, respectively. (D) Transwell chamber coated with Matrigel was used to assess cell invasion after MG63 and 143B cells were transfected OE-NC + sh-NC, OE-MAT1 + sh-NC, OE-NC + sh-AKT1 or OE-MAT1 + sh-AKT1 for 48 h. (E-F) Wound healing assay was performed to detect the migration of MG63 and 143B cells after 0 h and 24 h of cell transfection. All the experiments were performed three times. For Fig. 3D–F, $^{*}P < 0.05$, OE-MAT1 + sh-NC or OE-NC + sh-AKT1 group versus OE-NC + sh-NC group; $^{\#}P < 0.05$, OE-MAT1 + sh-AKT1 group versus OE-MAT1 + sh-NC group.

The results demonstrated that upregulation of MAT1 significantly enhanced the growth of MG63 (Fig. 3A) and 143B cells (Fig. 3B), and repressed cell apoptosis (Fig. 3C), whereas knockdown of MAT1 reversed these results (Fig. 3A–C). These results illustrate that MAT1 functions as an oncogene in osteosarcoma progression.

3.5. Upregulation of MAT1 facilitates the migration of osteosarcoma through upregulation of AKT1

Next, we explored the molecular mechanisms underlying MAT1 in promoting osteosarcoma metastasis. The expressions of AKT1 in MG63 and 143B cells were markedly elevated when these cells were transfected OE-MAT1 (Fig. 4A), suggesting that AKT1 might take part in the process in which MAT1 deteriorate osteosarcoma. To verify this hypothesis, shRNA targeting human AKT1 gene was applied. As shown in Fig. 4B–C, sh-AKT1 apparently reduced the expression of AKT1 about 60% in protein level and 80% in mRNA level in both MG63 and 143B cells. Moreover, knockdown of AKT1 with shRNA transfection significantly inhibited cell invasion (Fig. 4D) and migration (Fig. 4E–F), as well as rescued the increases in cell invasion and migration induced by MAT1 upregulation in both MG63 and 143B cell lines (Fig. 4D–F). Furthermore, we explored the function of MAT1/AKT1 axis in the metastasis of osteosarcoma in vivo using 143B cells. The results showed that overexpression of MAT1 significantly promoted tumor growth (Fig. 5A–B), increased the expression levels of ROCK1, α-SMA, Vimentin and N-cadherin while decreased E-cadherin expression (Fig. 5C), as well as increased the number of lung metastatic tumor (Fig. 5D). However, these effects were all obviously impaired when AKT1 was downregulated in 143B cells (Fig. 5A–D). These findings demonstrate that MAT1 facilitates the progression and lung metastasis of osteosarcoma via upregulating AKT1.

4. Discussion

Human MAT1 gene (ménage trois 1) is located in chromosome 14q23 and codes a 37 kDa-protein that can activates cyclin-associated kinases through threonine phosphorylation [21]. In the present study, we aimed to explore the effects of MAT1 on the lung metastasis of osteosarcoma. The results reveal that MAT1 servers as an inducer of the lung metastasis in osteosarcoma. Our findings may provide a potent therapeutic target of MAT1 for the lung metastasis of osteosarcoma.

Through immunohistochemical staining, RT-PCR and western blot assays, we observed that MAT1 was overexpressed in the osteosarcoma tissues with lung metastasis, as well as the osteosarcoma metastatic lung tissues, suggesting that MAT1 might be implicated in osteosarcoma metastasis. Research has found that MAT1 plays a crucial role in cell proliferation, differentiation and embryo development [22]. For instance, Liu [23] and Zhang et al. [24] illustrated that knockdown of MAT1 with siRNA or recombinant adenovirus transfection obviously reduced cell proliferation and induced a G0/G1 phase arrest in human

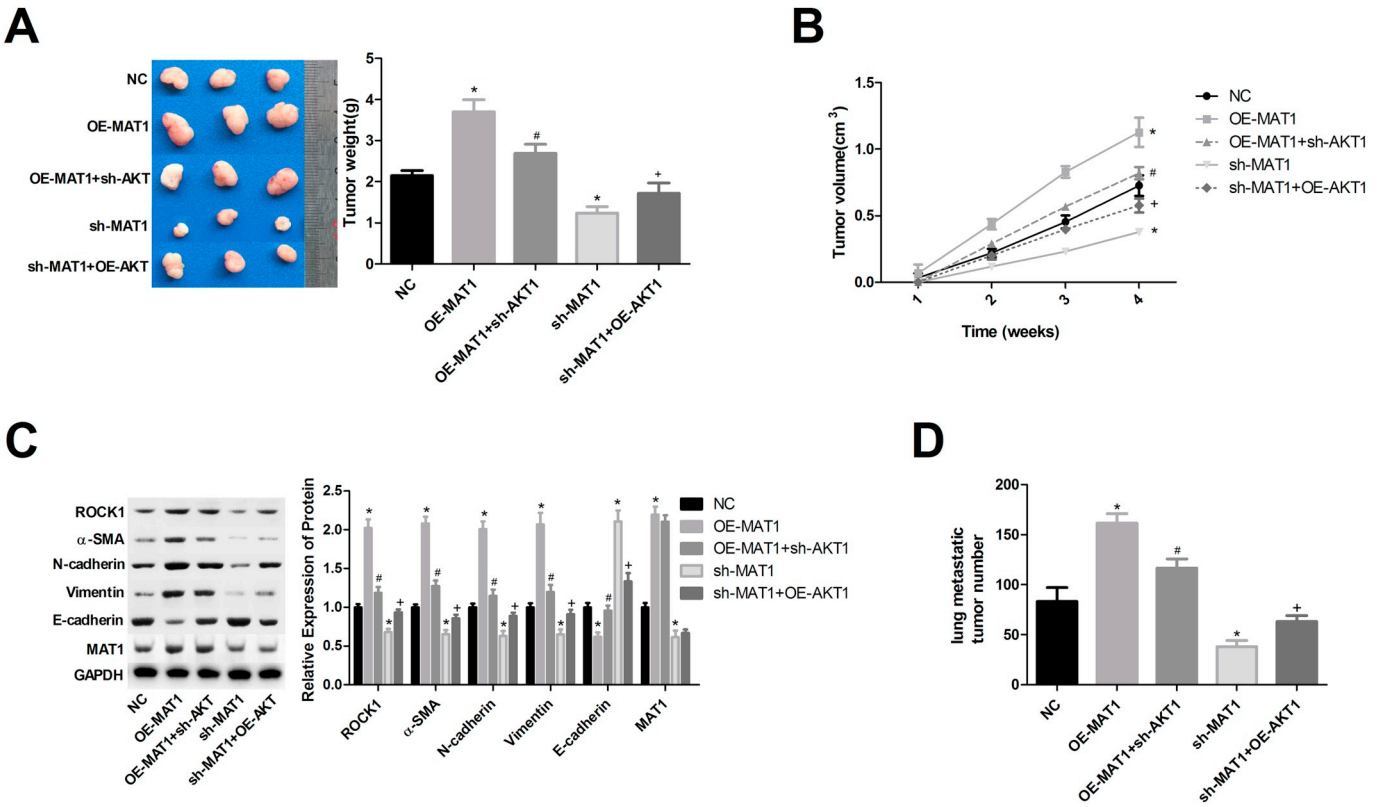

Fig. 5. Upregulation of MAT1 promoted the lung migration of osteosarcoma through up-regulation of AKT1 in vivo. 143B cells were transfected with control vector, lentiv-MTA1, Lentiv-MTA1 + sh-AKT1, sh-MAT1, sh-MTA1 + sh-AKT1, then the cells were selected with puromycin and/or G418 to build stable cell lines. Then the stable cell lines were injected to the nude mice (n = 6 for each group) to perform the tumor-burdened experiments (A-B) Tumor weights and volume were assessed. (C) The protein expressions of ROCK1, α-SMA, N-cadherin, E-cadherin, Vimentin and MAT1 in tumor tissues were detected by western blot. (D) Lung metastatic tumor numbers were shown. ($^{*}P < 0.05$, OE-MAT1/sh-MAT1 group versus NC group; $^{\#}P < 0.05$, OE-MTA1 + sh-AKT1 group vs OE-MTA1 group; $^{+}P < 0.05$, sh-MTA1 + sh-AKT1 group vs sh-MTA1 group).

pancreatic cancer. Patel et al. [25] recently demonstrated that the high expression level of MAT1 was associated with the presence of estrogen receptor in breast cancer. Fejzo et al. [26] revealed that overexpression of ADRM1 enhanced the proliferation and migration of ovarian cancer ES2 cells with an elevated expression of MAT1, indicating that MAT1 might be involved in tumor metastasis. The present study was the first research to explore the function of MAT1 in the metastasis of osteosarcoma and demonstrated that MAT1 expression was positively related to the lung metastasis of osteosarcoma. Moreover, we identified that MAT1 served as an oncogene in the malignant phenotypic transformation of osteosarcoma, as upregulation of MAT1 significantly increased cell growth, migration and invasion and repressed apoptosis in both osteosarcoma MG63 and 143B cells.

Deregulation of phosphatidylinositol 3′-kinase (PI3K)/AKT pathway is frequently detected in osteosarcoma and it is strongly implicated in the occurrence, progression, invasion and metastasis of osteosarcoma [27,28]. For instance, overexpression of the zinc finger transcription factor ZIC2 significantly enhanced the viability and invasion of osteosarcoma cells partly depend on PI3K/AKT signaling pathway activation [29]. Isoliquiritigenin administration repressed the invasion and migration of osteosarcoma U2OS cells through suppressing PI3K/AKT signaling pathway [30]. All of the findings illustrate that PI3K/AKT pathway plays an important role in the metastasis of osteosarcoma. As a key member of PI3K/AKT signaling, the serine/threonine kinase AKT is reported to serve as a promoter in EMT process, cellular motility and invasion in a range of tumors [31–33]. Downregulation of AKT1 was closely implicated in the process in which TIMP3 overexpression repressed osteosarcoma cell migration and invasion [34]. In the present study, we explored whether MAT1 promoted the malignant progression of osteosarcoma through interaction with AKT1. The results showed that overexpression of MAT1 with lentivirus infection obviously increased the protein expression of AKT1 in MG63 and 143B cells. In addition, we observed that AKT1 downregulation significantly neutralized MAT1 roles in promoting cell migration and invasion, suggesting that AKT1 play an indispensable role in MAT1-mediated osteosarcoma progression.

Furthermore, we explored the functions of MAT1/AKT1 axis in the lung metastasis of osteosarcoma using xenografted nude mice. The results demonstrated that MAT1 overexpression significantly enhanced tumor growth and facilitated the lung metastasis of osteosarcoma, with increased expressions of ROCK1, α-SMA, N-cadherin, Vimentin and a decreased expression of E-cadherin, whereas this effect was rescued when AKT1 expression was downregulated, This result further verifies that MAT1 facilitates the progression and metastasis of osteosarcoma in a AKT1-denpendent manner.

5. Conclusion

In conclusion, this study clarifies that MAT1 facilitates the lung metastasis of osteosarcoma through increasing AKT1 expression both in vivo and in vitro. Our study provides theoretical basis for serving MAT1 as a new target for preventing and treating osteosarcoma lung metastasis.

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Declaration of competing interest

The authors declare that there is no any form of interest conflict.

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