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Monoacylglycerol Lipase (MAGL) Inhibition Impedes the Osteosarcoma Progression by Regulating Epithelial Mesenchymal Transition

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Osteosarcoma is a primary malignancy of mesenchymal origin, and its metastasis and multidrug resistance remain major problems affecting the therapeutic effect. This study aimed to evaluate the efficacy and underlying mechanism of monoacylglycerol lipase (MAGL) on osteosarcoma progression. MAGL expression was downregulated by shMAGL or JZL184 (an MAGL inhibitor) and upregulated through plasmid. RT-PCR and Western blot were utilized to determine the expression of target molecules. CCK-8 assay, transwell assay and ROS assay were performed to investigate the inhibitory effect of MAGL on the growth and metastasis of osteosarcoma cells. The role of JZL184 on tumor growth was examined in cisplatin-resistant MG-63 (MG-63/R) xenograft model. MAGL was highly expressed in osteosarcoma cells and tissues. MAGL knockdown significantly impeded the proliferation, clone formation, invasion and migration of MG-63 cells, whereas opposite result was observed in 143B cells with MAGL overexpression. Likewise, an MAGL inhibitor JZL184 displayed reduced viability, clone formation, invasion and migration of osteosarcoma cells. Western blot of the epithelial mesenchymal transition (EMT)-related proteins indicated that MAGL knockdown or JZL184 could upregulated E-cadherin expression and downregulated vimentin expression. *In vitro* and *in vivo* experiments indicated that JZL184 re-sensitized MG-63/R cells to cisplatin. In summary, MAGL regulated osteosarcoma by modulating EMT, and JZL184 might be a promising agent for osteosarcoma patients who are resistant to cisplatin.

Keywords: cisplatin; epithelial mesenchymal transition; JZL184; monoacylglycerol lipase; osteosarcoma
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Introduction

Osteosarcoma is a primary malignancy of mesenchymal origin that occurs most frequently in children and adolescents, and is prone to occur in the epiphyseal region with abundant blood flow (Corre et al. 2020). According to relevant statistics, the incidence of osteosarcoma is gradually increasing worldwide (Zhao et al. 2021). Currently, the treatment of osteosarcoma usually fails due to chemoresistance and the development of metastasis, accompanied by poor prognosis, high recurrence rate and metastasis risk (Luo et al. 2020). Patients with metastatic osteosarcoma are usually resistant to conventional chemotherapy and present a major challenge in the current treatment of osteosarcoma (Natarajan et al. 2021). The recurrence, metastasis and multi-drug resistance of osteosarcoma have become the main problems restricting the therapeutic effect of osteosarcoma, which is due to the lack of understanding of the pathogenesis of osteosarcoma and the lack of treatment methods that can eradicate the tumor. Therefore, identifying a new targeted treatment for osteosarcoma is urgently required.

Abnormal lipid metabolism is one of the causes of a variety of chronic diseases and tumorigenesis. Accumulating evidence has revealed that abnormal lipid metabolism plays a dual part in tumorigenesis, specifically in the progression of tumors (Li et al. 2019). As a hydrolase involved in lipid metabolism, monoacylglycerol lipase (MAGL) can not only decompose triacylglycerols into fatty acids and glycerol in lipid metabolism to provide energy for the body, but also hydrolyze 2-arachidonic acid glycerides and regulate the signal transduction of cannabinoid system

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in vivo (Gil-Ordonez et al. 2018). MAGL is known to be implicated in numerous pathophysiological processes, such as chronic pain, neurodegenerative diseases and inflammatory response (Poli et al. 2019). MAGL has been identified as a vital player in the initiation and progression of various malignancies. Carbonetti et al. (2019) have shown that MAGL can promote nuclear receptor activation and prostate cancer metastasis by regulating the expression of fatty acid binding protein 5 (FABP5). MAGL has been reported to be highly expressed in a variety of cancers, promoting the occurrence of tumors (Nomura et al. 2011). Inhibition of MAGL activity might reduce the metastasis and invasion ability of tumor cells and hinder the progress of tumors, which provides new ideas for tumor therapy (Pagano et al. 2017). However, the biological function and potential mechanisms of MAGL expression in osteosarcoma have not been further determined.

Based on the biological characteristics of osteosarcoma, this study explored the influence of MAGL on the occurrence and development of osteosarcoma, thereby providing a potential target for osteosarcoma.

Materials and Methods

Patient samples and cell culture

Osteosarcoma tissues or normal adjacent tissues were collected from 3 patients in the Zhejiang Taizhou Municipal Hospital. Signed written informed consents were obtained from all the patients, and the procedure was approved by the Ethics Committee of Zhejiang Taizhou Municipal Hospital.

Human osteosarcoma cell lines [MG-63, cisplatin-resistant MG-63 (MG-63/R), Saos2, 143B and U2OS] and normal osteoblast cell line (hFOB 1.19) were purchased from Shanghai Cell Biology Institute of Chinese Academy of Sciences (Shanghai, China). Cells were grown in DMEM media supplemented with 10% fetal bovine serum (Gibco, Life Technologies, New York, NY, USA) under 5% CO_2 at 37˚C. Cells (2×10^5 cells/well) were seeded into 6-well plates and transiently transfected with short-hairpin RNA targeting MAGL (MAGL-shRNA: 5'-CAACTTTCAAGGTCCTTGC-3', GenePharma, Suzhou, China) and control shRNA (sh-NC), or MAGL pMSCVpuro vector (GenePharma) or control vector using Lipofectamine 2000 reagent (Thermo Fisher Scientific, Inc., Shanghai, China).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

After 48 h of transfection, total RNA was extracted from cells or tissue samples using TRIzol® reagent (Thermo Fisher Scientific, Inc.) and reversely transcribed to cDNA using M-MLV reverse transcriptase (Promega, Beijing, China). The quantitative PCR was performed using SYBR Green PCR Master Mix (Thermo Fisher Scientific, Inc.). Relative expression was calculated using the $2^{-\Delta\Delta Cq}$ method. The primers were as follows: MAGL: forward 5'-CCGCAGAGCATTCCCTACCA-3' and reverse 5'-GCTGCAACACATCCCTGACG-3'; β-actin: forward, 5'-CAGGGCGTGATGGTGGGCA-3' and reverse 5'-CAAACATCATCTGGGTCATCTTCTC-3'. β-actin was used as a reference gene.

Western blot

Proteins from cells or tissue samples were extracted using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). The proteins obtained were subjected to sodium dodecyl sulfate–polyacrylamides gel electrophoresis, transferred to PVDF membranes (Millipore, Burlington, MA, USA) and blocked with 5% skim milk. The membrane was incubated with the following primary antibodies: anti-MAGL (1:1,000, Santa Cruz Biotech, Santa Cruz, CA, USA), anti-E-cadherin (1:1,000, Abcam, Cambridge, UK), anti-vimentin (1:1,000, Abcam), anti-GAPDH (1:5,000, Abcam) and anti-β-actin (1:5,000, Abcam) overnight at 4°C. The specific bands were detected using enhanced chemiluminescence detection reagents with a Bio-Rad imaging system (Bio-Rad, Hercules, CA, USA).

CCK-8 assay

MG-63, MG-63/R and 143B cells were cultured into 96-well plate (1,000 cells/well in 100 μL volume) for 24 h at 37˚C with 5% CO_2. Then, CCK-8 reagent 10 μL (Dojindo Molecular Technologies, Inc., Rockville, MD, USA) was added to 90 μL of DMEM to generate a working solution, of which 100 μL was added per well and incubated for 1 h. The absorbance was measured at a wavelength of 450 nm using a microplate reader.

Colony formation assay

Appropriate number of MG-63, MG-63/R and 143B cells were plated in 6-well plates and incubated for 24 h under standard conditions, respectively. After the corresponding different treatments, they were incubated for 10 to 14 days to form colonies. Colony forming was scored under a light microscope following fixed with 4% formaldehyde and stained with 1% crystal violet solution (Sigma-Aldrich, St. Louis, MO, USA).

Migration and invasion assays

MG-63, MG-63/R and 143B cells were seeded into the upper chamber containing an uncoated or Matrigel-coated insert (BD Biosciences, San Diego, CA, USA) to measure cell migration and invasion, respectively. The lower chambers were filled with DMEM containing 10% FBS. Following cell culture for 15 h, the cells at the upper side of the membrane were wiped off, and the cells in the lower side of the membrane were fixed in methanol and stained with 0.1% crystal violet. Five randomly chosen fields of view were imaged from each well using light microscopy.

Measurement of ROS

MG-63 and MG-63/R cells were cultured with 2.5 μM of 2',7'-dichlorofluorescein diacetate (DCFH-DA, Sigma-Aldrich Pty. Ltd., St. Louis, MO, USA) for 30 min at 37˚C. After cells were stimulated at 485 nm, the fluorescence intensities were measured at 530 nm via a flow cytometer (BD Biosciences).

Tumor xenograft model

Totally, 9 BALB/c nude mice (4-5 weeks old) were purchased from Hangzhou Ziyuan Experimental Animal Technology Co. (Hangzhou, China). MG-63/R cells were injected subcutaneously into the mice. When tumor volume reached 65 mm^3, mice were divided into MG-63/R group, cisplatin group and cisplatin + JZL184 group (n = 3 per group). Tumor volumes were measured every 3 days via following formula: V (mm^3) = 0.5 × length × $width^2$. At the end of the experiment, all mice were euthanatized by cervical dislocation. Animal experimental procedures were approved by the Wenzhou Medical University Animal Policy and Welfare Committee.

Statistical analysis

All statistical analyses in this study were performed using Prism8.0.2 (GraphPad). For two group comparison, Student's t-test was employed. Multiple group comparison was performed by one-way ANOVA test. Data are shown as mean ± standard deviation (SD). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

MAGL is highly expressed in osteosarcoma

To investigate the role of MAGL in human osteosarcoma, RT-qPCR and Western blot were performed, and a respective 3.359- and 1.796-fold increase in the mRNA and protein expression of MAGL was evident in osteosarcoma tissues compared with normal adjacent tissues (Fig. 1A, B). Subsequently, we determined the expression of MAGL in human osteosarcoma cell lines and normal osteoblast cell line. The results showed that MAGL gained a higher expression in MG-63, Saos2, 143B and U2OS cells than hFOB 1.19 cells, among which MAGL expression was the highest in MG-63 cells and lowest in 143B cells (all p <

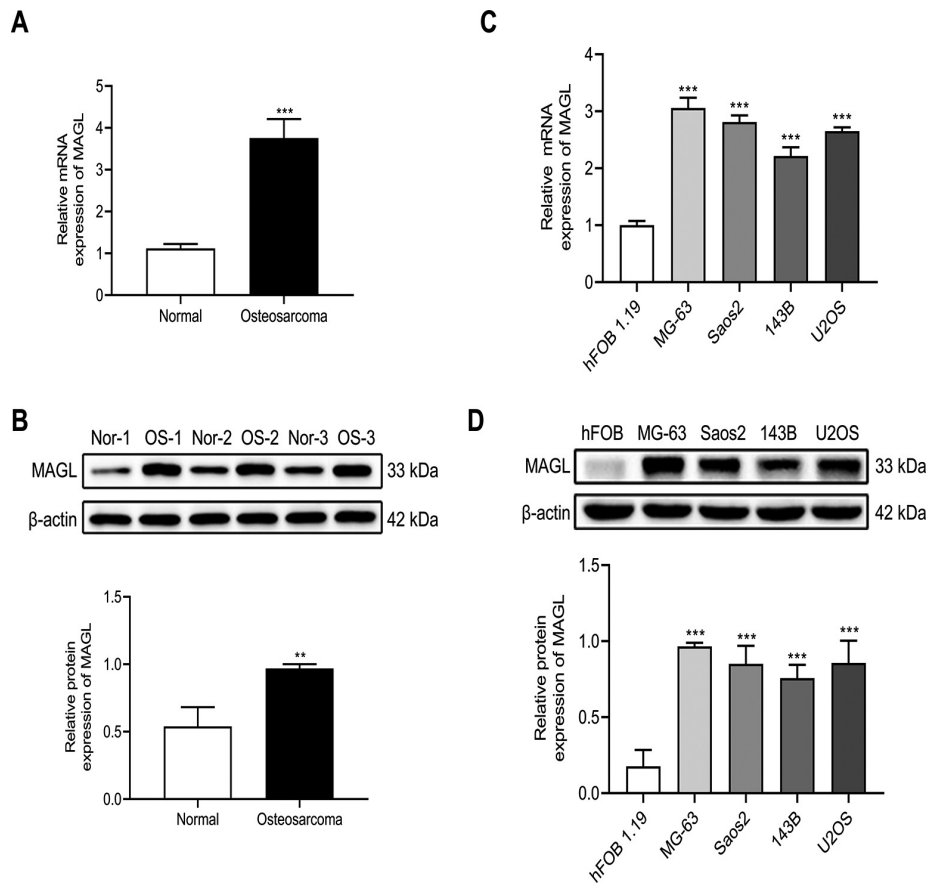

Fig. 1. MAGL is highly expressed in osteosarcoma.
(A) RT-PCR and (B) Western blot were respectively performed to determine the mRNA and protein expression of MAGL in osteosarcoma tissue and adjacent normal tissues from patients. Data are shown as mean ± SD (n = 3). $^{**}p < 0.01$ and $^{***}p < 0.001$, compared with normal group. (C) RT-PCR and (D)Western blot were adopted to detect the protein and mRNA expression of MAGL in normal osteoblast cell line (hFOB 1.19) and human osteosarcoma cell lines (MG-63, Saos2, 143B and U2OS), respectively. Data are shown as mean ± SD (n = 3). $^{***}p < 0.001$, compared with hFOB 1.19 cells.

0.0001) (Fig. 1C, D). We, therefore, selected MG-63 and 143B cells for following experiments.

MAGL knockdown represses the proliferation, migration and invasion of MG-63 cells

Since MAGL expression was upregulated in osteosarcoma tissue and cell lines, we used loss-of-function experiments to determine whether it influences osteosarcoma cell proliferation, migration and invasion. We constructed MG-63 cells with MAGL knockdown using shRNA of MAGL, and effective knockdown of MAGL was verified by qRT-PCR and Western blot (Fig. 2A, B). CCK-8 and colony results indicated that silencing MAGL inhibited MG-63 cell growth and colony formation (Fig. 2C, D). Moreover, MAGL knockdown significantly attenuated the cell migration and invasion, which was corroborated by the increased expression of E-cadherin and decreased expression of vimentin (Fig. 2E, F). These data confirmed that MAGL knockdown attenuated osteosarcoma growth and metastasis *in vitro*.

MAGL overexpression contributes to 143B cell growth and metastasis

To reverse prove the role of MAGL in osteosarcoma, 143B was selected as the low-expression MAGL cell line and was constructed MAGL overexpression through plasmid. Fig. 3A, B show the effective overexpression of MAGL by qRT-PCR and Western blot. MAGL overexpression led to augmented proliferation and clone formation of 143B cells (Fig. 3C, D), as well as stronger metastatic ability, which was reflected in the enhanced migration and invasion abilities in transwell assay (Fig. 3E). In Western blot, vimentin expression was upregulated after MAGL overexpression, while E-cadherin expression was significantly downregulated (Fig. 3F). These data indicated that overexpression of MAGL enhanced the growth ability and metastasis of osteosarcoma cells.

MAGL inhibitor reduces metastatic potential of MG-63 cells

Then we assessed the function of MAGL inhibitor JZL184 on MG-63 cell growth and metastasis. From results of CCK-8 assay, JZL184 decreased the osteosarcoma cell viability in a dose-dependent manner (Fig. 4A). Likewise, stimulation of JZL184 at 5 μM significantly suppressed the clone formation, migration and invasion (Fig. 4B, C). Moreover, consistent with the findings of MAGL knockdown on MG-63 cells, JZL184 administration remarkably upregulated E-cadherin expression and downregulated vimentin expression ($p < 0.001$ and $p < 0.01$, respectively) (Fig. 4D). Overall, these data indicated that MAGL modulated osteosarcoma migration by the epithelial-mesenchymal transition (ETM).

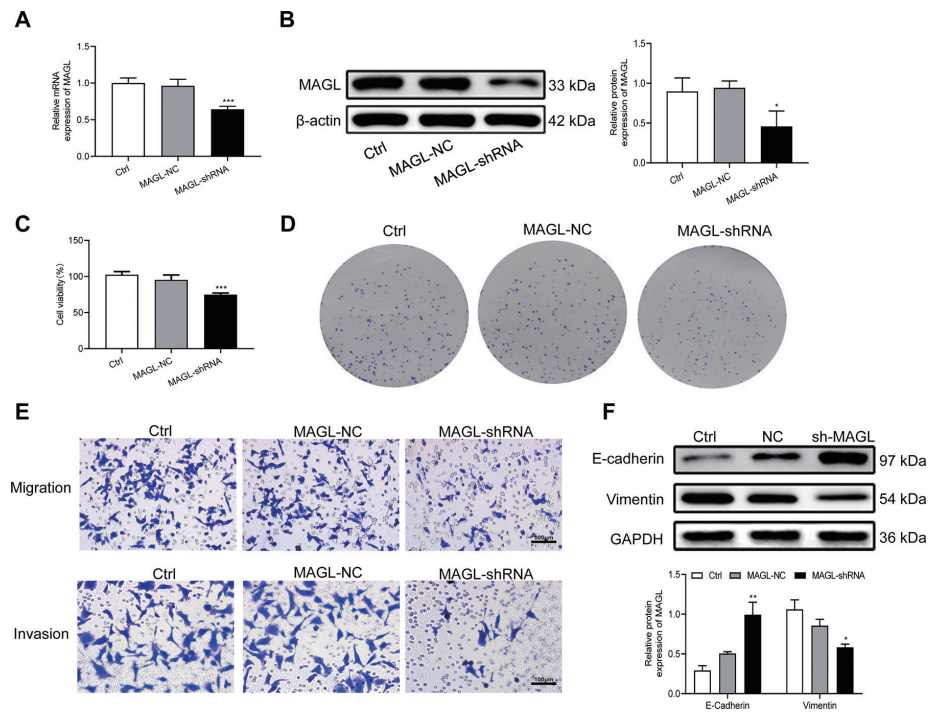

Fig. 2. MAGL silencing suppresses the proliferation, migration and invasion of osteosarcoma cells.
We constructed MG-63 cells with MAGL knockdown. (A) RT-PCR and (B) Western blot were respectively performed to determine the mRNA and protein expression of MAGL in osteosarcoma tissue and adjacent normal tissues from patients. MG-63 cell proliferation and colony formation were evaluated by (C) CCK-8 assay and (D) colony formation assay, respectively. (E) Transwell assay was utilized to detect cell migration and invasion of MG-63 cells. (F) Western blot analysis of epithelial-mesenchymal transition (EMT)-related proteins E-cadherin and vimentin. Data are shown as mean ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, compared with MAGL-NC group.

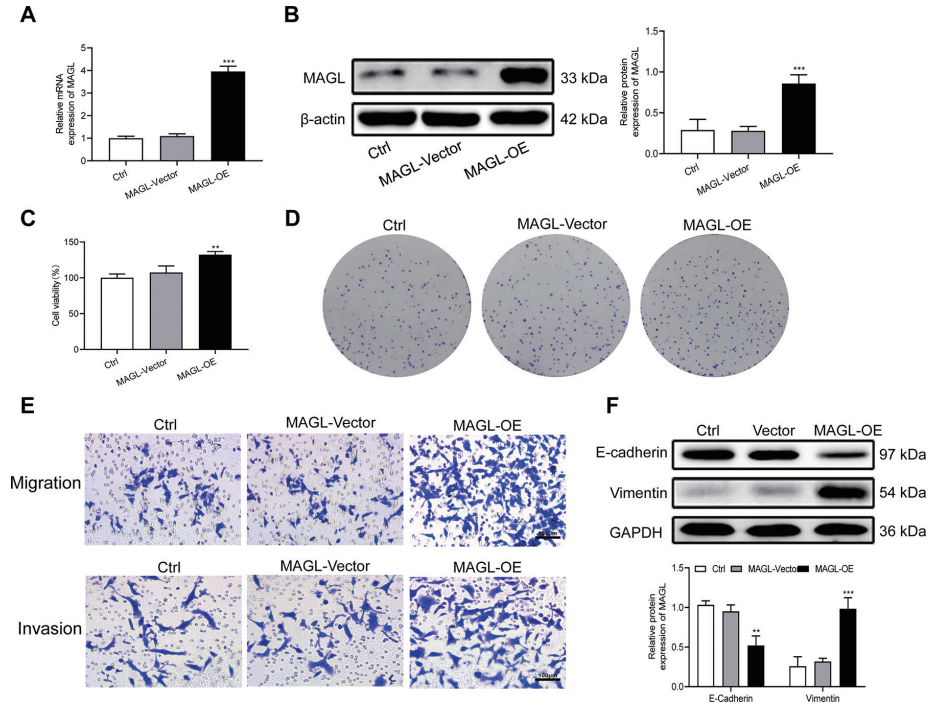

Fig. 3. MAGL overexpression promotes osteosarcoma cells growth and metastasis.
We constructed 143B cells with MAGL overexpression. (A) RT-PCR and (B) Western blot were respectively performed to determine the mRNA and protein expression of MAGL in osteosarcoma tissue and adjacent normal tissues from patients. 143B cell proliferation and colony formation were evaluated by (C) CCK-8 assay and (D) colony formation assay, respectively. (E) Transwell assay was utilized to detect cell migration and invasion of 143B cells. (F) Western blot analysis of E-cadherin and vimentin. Data are shown as mean ± SD (n = 3). $^{**}p < 0.01$ and $^{***}p < 0.001$, compared with MAGL-Vector group.

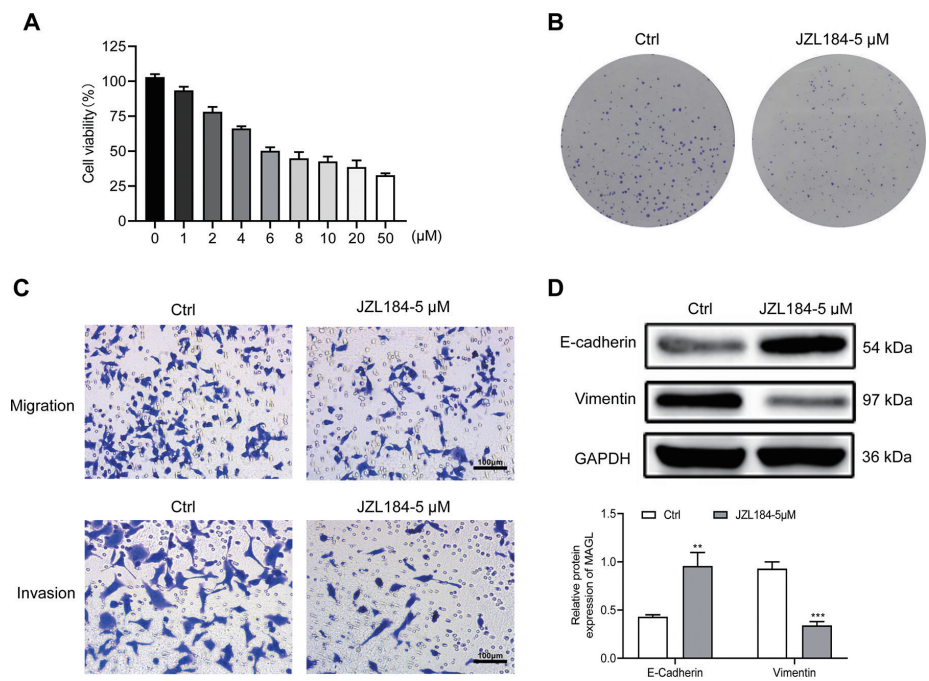

Fig. 4. JZL184 inhibits the metastatic potential of MG-63 cells.
The effect of MAGL inhibitor JZL184 on MG-63 cell proliferation and colony formation were evaluated using (A) CCK-8 and (B) colony formation assays, respectively. (C) Transwell assay was utilized to detect 143B cell migration and invasion. (D) Western blot analysis of E-cadherin and vimentin in MG-63 cells treated with JZL184. Data are shown as mean ± SD (n = 3). $^{**}p < 0.01$ and $^{***}p < 0.001$, compared with control group.

Modulation of MAGL affects sensitivity of cisplatin-resistant MG-63 cells in vitro and in vivo

To determine whether MAGL is related to the sensitivity of osteosarcoma cell lines to cisplatin, the expression of MAGL in cisplatin-resistant MG-63 (MG-63/R) cells was detected by Western blot. Compared with MG-63 cells, MAGL expression increased 2.19-fold in MG-63/R cells ($p < 0.01$) (Fig. 5A). In addition, treatment of MG-63/R cells with cisplatin together with JZL184 significantly decreased cell viability, clone formation and metastatic ability (Fig. 5B-D). Meanwhile, ROS production was significantly enhanced after cisplatin treatment ($p < 0.001$), and further increased after the addition of JZL184 ($p < 0.0001$) (Fig. 5E).

On the basis of our *in vitro* studies, we finally examined the *in vivo* function of MAGL in MG-63/R xenograft model. Compared with cisplatin group, significant inhibition of the growth of xenograft tumors was noticed after the combined treatment of cisplatin and JZL184 (Fig. 6A-C). These results suggested that inhibition of MAGL increased the sensitivity of osteosarcoma to cisplatin, and JZL184 could re-sensitize MG-63/R cells to cisplatin by promoting the production of ROS.

Discussion

Recently, the involvement of MAGL in cancer progress via regulating fatty acid networks enriched in oncogenic signaling lipids to promote migration, invasion, sur-

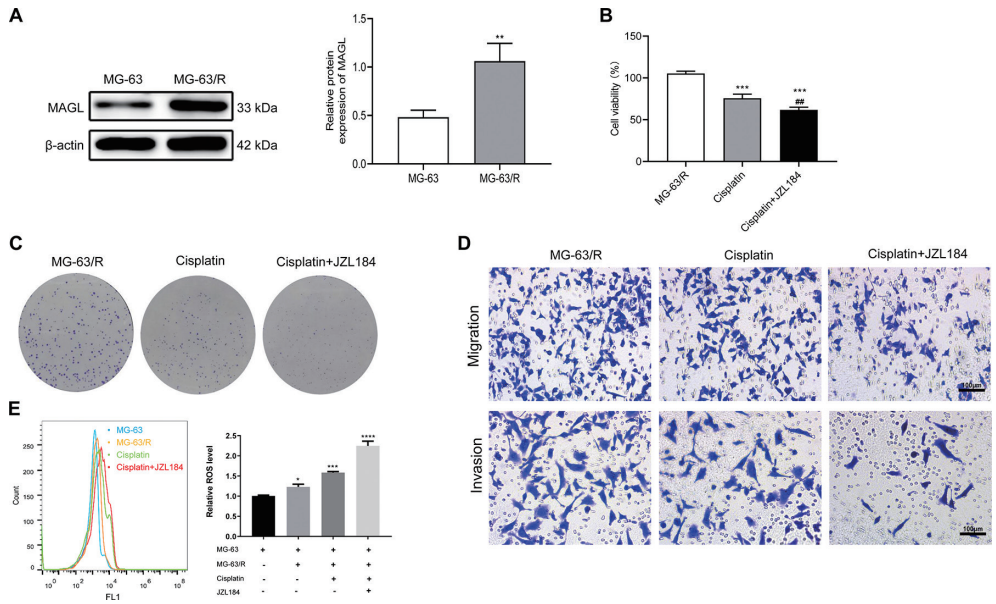

Fig. 5. JZL184 affects sensitivity of cisplatin-resistant MG-63 (MG-63/R) cells.
Obvious upregulation of MAGL was found in MG-63/R cells compared with MG-63 cells by using (A) Western blot. Data are shown as mean ± SD (n = 3). $^{**}p < 0.01$, compared with MG-63 cells. The function of JZL184 on MG-63/R cell proliferation and colony formation were evaluated using (B) CCK-8 and (C) colony formation assays, respectively. Data are shown as mean ± SD (n = 3). $^{***}p < 0.001$, compared with MG-63/R group; $^{\#\#}p < 0.01$, compared with MG-63/R + cisplatin group. (D) Transwell assay was adopted to evaluate the cell migration and invasion of MG-63/R cells. (E) The role of JZL184 in ROS generation was determined by ROS experiment. Data are shown as mean ± SD (n = 3). $^{*}p < 0.05$, $^{***}p < 0.001$ and $^{****}p < 0.0001$, compared with MG-63 cells.

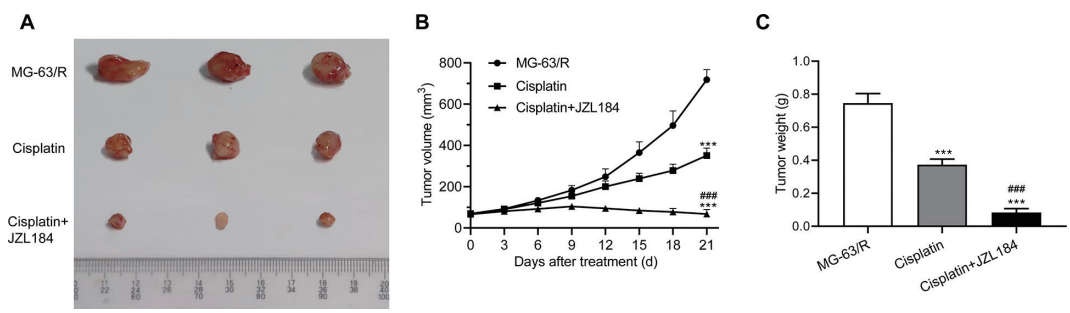

Fig. 6. Effect of JZL184 D on tumor growth in MG-63/R xenograft tumor growth.
(A) Tumor morphology, (B) tumor volume (C) and tumor weight in nude mice after treatment with cisplatin and JZL184. Data are shown as mean ± SD (n = 3). $^{***}p < 0.001$, compared with MG-63/R group; $^{\#\#\#}p < 0.001$, compared with MG-63/R + cisplatin group.

vival and tumor growth (Nomura et al. 2010). Studies have demonstrated that MAGL is highly expressed in aggressive human cancer cells and primary tumors, including malignant melanoma, endometrial cancer and colorectal cancer (Baba et al. 2017; Pagano et al. 2017; Li et al. 2019). Consistently, the expression of MAGL was significantly increased in both human osteosarcoma cell lines (MG-63, Saos2, 143B and U2OS) and osteosarcoma tissues in this study. Genetic or pharmacological inhibition of MAGL exhibits anti-cancer functions. To identify the role of MAGL overexpression in osteosarcoma cells, we constructed 143B cells with MAGL overexpression. Results showed that upregulated MAGL expression significantly facilitated 143B cell viability and clone formation. To our knowledge, metastasis is the leading cause of organ failure and even cancer-related death, and its cascade consists of many immune cells that exert pro-metastatic or anti-metastatic efficacy (Zhang et al. 2019). To find out the function of MAGL in osteosarcoma, the invasion and migration of 143B cells were evaluated, and showed that MAGL overexpression increased 143B cell migration and invasion.

MAGL overexpression in osteosarcoma cells indicated that MAGL knockdown or inhibition may be a potential therapy for osteosarcoma. Ye et al. (2011) suggested that silencing of MAGL diminished tumor growth and volume of colorectal cancer *in vivo*. MAGL knockdown causes G1 phase arrest of endometrial cancer cells (Li et al. 2019). In the present research, we constructed MG-63 cells with MAGL knockdown, and noticed that inhibition of MAGL activity with shRNA remarkably restrained MG-63 cell viability, migration and invasion. A study conducted by Marino et al. (2019) has pointed out that the MAGL inhibitor JZL184 can restrain bone tumor growth, metastasis and ectopic bone formation in osteosarcoma models, and also impede cachexia in metastatic osteosarcoma and prolong the survival in mice. As expected, we found that JZL184 significantly impeded the proliferation and metastasis of MG-63 cells, confirming the involvement of MAGL in the progression of osteosarcoma.

Understanding the mechanism of metastasis is crucial to identifying new therapeutic targets. EMT drives the migration and invasion of cancer cells, in which E-cadherin downregulation controlled by EMT-mediated protein snail plays important role in the process of EMT (Abouhashem et al. 2016). E-cadherin is a transmembrane adhesion protein that regulates cell-cell adhesion and stimulates anti-growth signals through interactions with β-catenin in cytoplasm (Abouhashem et al. 2016). It has been reported that patients with E-cadherin loss were more likely to experience tumor progression by recurrence and metastasis, accompanied by shorten overall survival (Kroepil et al. 2013). Besides, inhibition of EMT by upregulating E-cadherin and downregulating vimentin repressed tumor growth and invasion (Mishra et al. 2020). In agreement with Mishra et al. (2020), the present study revealed a significant inverse relationship between E-cadherin and vimentin expression in osteosarcoma cells.

MAGL expression was correlated with EMT proteins including E-cadherin, vimentin and Snail. Hu et al. (2014) reported that overexpression of MAGL enhanced Snail and vimentin expression, but decreased E-cadherin expression in nasopharyngeal carcinoma, which was consistent with our data in osteosarcoma. Further, protein expression of E-cadherin and vimentin were altered by pharmacological inhibition (JZL184) or knockdown of MAGL, revealing that decreased tumor cell proliferation and metastatic ability were attributed to E-cadherin upregulation and vimentin downregulation with subsequent induction of EMT.

Another finding of this study was that JZL184 led to increased sensitivity to cisplatin in MG-63/R cells. Cisplatin is one of the most effective chemotherapeutic agents, which generates DNA intra-strand crosslinks between adjacent purines by forming bivalent adducts with nucleophilic sites on purines in DNA, thus eliciting anti-cancer functions (Qu et al. 2015). However, chemoresistance is a typical challenge in the effective therapy of cancers, while efforts to enhance the curative efficacy of chemotherapy drugs have provided limited results. Han and Tang (2016) indicated that Saos2-lung cells with CXCR1 overexpression were more resistant to cisplatin than their parental Saos2 cells, whereas, CXCR1 knockdown improved the cisplatin sensitivity of osteosarcoma cells. As a selective and effective inhibitor of MAGL, JZL184 can significantly decrease the hydrolytic activity of MAGL (Ma et al. 2016). We hypothesized that MAGL inhibition might improve the sensitivity of osteosarcoma to cisplatin. To verify the speculation, MG-63/R cells were treated with JZL184 and MG-63/R xenograft model was also established in nude mice. *In vitro* and *in vivo* experimental results revealed that treatment of MG-63 cells with cisplatin plus JZL184 obviously restrained cell viability, clone formation, metastatic ability and tumor growth, and also promoted ROS production.

In conclusion, the present study indicates that MAGL inhibition suppresses the proliferation, clone formation, invasion and migration of osteosarcoma cells *in vitro* by regulating EMT-related proteins (E-cadherin and vimentin). Besides, MAGL inhibitor JZL184 can re-sensitize MG-63/R cells to cisplatin treatment. These findings support the MAGL as a promising therapeutic target for osteosarcoma, while JZL184 might be a novel potential agent for osteosarcoma patients who are resistant to cisplatin.

Author Contributions

Xiaokang Gong and Xin Zheng: study conceptualization, supervision, and manuscript revision; Yang Huang and Weihai Song: experiment performance and data analysis; Gang Chen and Tao Chen: data collection and manuscript preparation.

Conflict of Interest

The authors declare no conflict of interest.

References

Abouhashem, N.S., Ibrahim, D.A. & Mohamed, A.M. (2016) Prognostic implications of epithelial to mesenchymal transition related proteins (E-cadherin, Snail) and hypoxia inducible factor 1alpha in endometrioid endometrial carcinoma. *Ann. Diagn. Pathol.*, **22**, 1-11.

Baba, Y., Funakoshi, T., Mori, M., Emoto, K., Masugi, Y., Ekmekcioglu, S., Amagai, M. & Tanese, K. (2017) Expression of monoacylglycerol lipase as a marker of tumour invasion and progression in malignant melanoma. *J. Eur. Acad. Dermatol. Venereol.*, **31**, 2038-2045.

Carbonetti, G., Wilpshaar, T., Kroonen, J., Studholme, K., Converso, C., d'Oelsnitz, S. & Kaczocha, M. (2019) FABP5 coordinates lipid signaling that promotes prostate cancer metastasis. *Sci. Rep.*, **9**, 18944.

Corre, I., Verrecchia, F., Crenn, V., Redini, F. & Trichet, V. (2020) The osteosarcoma microenvironment: a complex but targetable ecosystem. *Cells*, **9**, 976.

Gil-Ordonez, A., Martin-Fontecha, M., Ortega-Gutierrez, S. & Lopez-Rodriguez, M.L. (2018) Monoacylglycerol lipase (MAGL) as a promising therapeutic target. *Biochem. Pharmacol.*, **157**, 18-32.

Han, X. & Tang, T. (2016) CXCR1 knockdown improves the sensitivity of osteosarcoma to cisplatin. *Journal of Orthopaedic Translation*, **7**, 87.

Hu, W.R., Lian, Y.F., Peng, L.X., Lei, J.J., Deng, C.C., Xu, M., Feng, Q.S., Chen, L.Z., Bei, J.X. & Zeng, Y.X. (2014) Monoacylglycerol lipase promotes metastases in nasopharyngeal carcinoma. *Int. J. Clin. Exp. Pathol.*, **7**, 3704-3713.

Kroepil, F., Fluegen, G., Vallbohmer, D., Baldus, S.E., Dizdar, L., Raffel, A.M., Hafner, D., Stoecklein, N.H. & Knoefel, W.T. (2013) Snail1 expression in colorectal cancer and its correlation with clinical and pathological parameters. *BMC Cancer*, **13**, 145.

Li, X., Gao, S., Li, W., Liu, Z., Shi, Z., Qiu, C. & Jiang, J. (2019) Effect of monoacylglycerol lipase on the tumor growth in endometrial cancer. *J. Obstet. Gynaecol. Res.*, **45**, 2043-2054.

Luo, H., Wang, P., Ye, H., Shi, J., Dai, L., Wang, X., Song, C., Zhang, J. & Li, J. (2020) Serum-derived microRNAs as prognostic biomarkers in osteosarcoma: a meta-analysis. *Front. Genet.*, **11**, 789.

Ma, M., Bai, J., Ling, Y., Chang, W., Xie, G., Li, R., Wang, G. & Tao, K. (2016) Monoacylglycerol lipase inhibitor JZL184 regulates apoptosis and migration of colorectal cancer cells. *Mol. Med. Rep.*, **13**, 2850-2856.

Marino, S., de Ridder, D., Bishop, R.T., Renema, N., Ponzetti, M., Sophocleous, A., Capulli, M., Aljeffery, A., Carrasco, G., Gens, M.D., Khogeer, A., Ralston, S.H., Gertsch, J., Lamoureux, F., Heymann, D., et al. (2019) Paradoxical effects of JZL184, an inhibitor of monoacylglycerol lipase, on bone remodelling in healthy and cancer-bearing mice. *EBioMedicine*, **44**, 452-466.

Mishra, R., Nathani, S., Varshney, R., Sircar, D. & Roy, P. (2020) Berberine reverses epithelial-mesenchymal transition and modulates histone methylation in osteosarcoma cells. *Mol. Biol. Rep.*, **47**, 8499-8511.

Natarajan, A., Ramachandran, B., Gopisetty, G., Jayavelu, S., Sundersingh, S. & Rajkumar, T. (2021) Pioglitazone modulates doxorubicin resistance in a in vivo model of drug resistant osteosarcoma xenograft. *Naunyn Schmiedebergs Arch. Pharmacol.*, **394**, 361-371.

Nomura, D.K., Lombardi, D.P., Chang, J.W., Niessen, S., Ward, A.M., Long, J.Z., Hoover, H.H. & Cravatt, B.F. (2011) Monoacylglycerol lipase exerts dual control over endocannabinoid and fatty acid pathways to support prostate cancer. *Chem. Biol.*, **18**, 846-856.

Nomura, D.K., Long, J.Z., Niessen, S., Hoover, H.S., Ng, S.W. & Cravatt, B.F. (2010) Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. *Cell*, **140**, 49-61.

Pagano, E., Borrelli, F., Orlando, P., Romano, B., Monti, M., Morbidelli, L., Aviello, G., Imperatore, R., Capasso, R., Piscitelli, F., Buono, L., Di Marzo, V. & Izzo, A.A. (2017) Pharmacological inhibition of MAGL attenuates experimental colon carcinogenesis. *Pharmacol. Res.*, **119**, 227-236.

Poli, G., Lapillo, M., Jha, V., Mouawad, N., Caligiuri, I., Macchia, M., Minutolo, F., Rizzolio, F., Tuccinardi, T. & Granchi, C. (2019) Computationally driven discovery of phenyl (piperazin-1-yl)methanone derivatives as reversible monoacylglycerol lipase (MAGL) inhibitors. *J. Enzyme Inhib. Med. Chem.*, **34**, 589-596.

Qu, Y., Xia, P., Zhang, S., Pan, S. & Zhao, J. (2015) Silencing XIAP suppresses osteosarcoma cell growth, and enhances the sensitivity of osteosarcoma cells to doxorubicin and cisplatin. *Oncol. Rep.*, **33**, 1177-1184.

Ye, L., Zhang, B., Seviour, E.G., Tao, K.X., Liu, X.H., Ling, Y., Chen, J.Y. & Wang, G.B. (2011) Monoacylglycerol lipase (MAGL) knockdown inhibits tumor cells growth in colorectal cancer. *Cancer Lett.*, **307**, 6-17.

Zhang, P., Zhai, Y., Cai, Y., Zhao, Y. & Li, Y. (2019) Nanomedicine-based immunotherapy for the treatment of cancer metastasis. *Adv. Mater.*, **31**, e1904156.

Zhao, A., Liu, W., Cui, X., Wang, N., Wang, Y., Sun, L., Xue, H., Wu, L., Cui, S., Yang, Y. & Bai, R. (2021) lncRNA TUSC7 inhibits osteosarcoma progression through the miR181a/RASSF6 axis. *Int. J. Mol. Med.*, **47**, 583-594.