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miR-425-5p decreases LncRNA MALAT1 and TUG1 expressions and suppresses tumorigenesis in osteosarcoma *via* Wnt/β-catenin signaling pathway

Guohui Yang[a], Chi Zhang[b], Nan Wang[a], Juwu Chen[a,*]

[a] Department of Emergency Surgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, PR China
[b] Department of Orthopedics, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, PR China

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ABSTRACT

Multiple miRNAs have been recognized as critical regulators in osteosarcoma (OS) carcinogenesis. miR-425-5p was demonstrated to be downregulated in the serum of OS patients. However, the detailed roles of miR-425-5p in OS progression and its underlying molecular mechanism are far from being addressed. In our study, the reduced expression of miR-425-5p was observed in OS tissues and cells. Functional analyses showed that miR-425-5p overexpression suppressed OS cell proliferation, invasion and migration *in vitro*. Moreover, miR-425-5p upregulation decreased the expressions of MALAT1 and TUG1 in OS cells *via* directly binding them. miR-425-5p upregulation strikingly abrogated the activation of Wnt/β-catenin signaling pathway induced by MALAT1 and TUG1 overexpression in OS cells. Finally, we validated that miR-425-5p hindered OS tumor growth, and suppressed MALAT1 and TUG1 expressions and the Wnt/β-catenin signaling pathway *in vivo*. Our findings concluded that miR-425-5p suppressed the tumorigenesis of OS *via* decreasing MALAT1 and TUG1 expressions through inactivation of the Wnt/β-catenin signaling pathway, contributing to a better understanding of the molecular mechanism of the tumorigenesis of OS.

1. Introduction

Osteosarcoma (OS) is one of the most frequent primary malignancies of the bone in children and young adults, with extraordinarily high incidence and mortality rate (Biazzo et al., 2017). OS is characterized by high degree of local invasion and distant metastasis, causing a relatively poor response to OS therapy including surgical resection combined with chemotherapy and radiotherapy (Bielack et al., 2016). Despite great improvements in multi-modal therapeutics, the prognosis for OS patients is still unsatisfactory when related to OS metastasis and 5-year survival rate of OS patients with metastasis is as low as 30% (Allison et al., 2012; Coventon, 2017). Thus, it is imperative to elucidate the molecular mechanisms underlying OS carcinogenesis and investigate potential therapeutic agents to improve the therapy efficiency of OS.

In recent decades, increasing studies have focused on the roles of microRNAs (miRNAs) in the pathogenesis and carcinogenesis of malignancies (Farazi et al., 2013). miRNAs are a group of endogenous small noncoding RNAs with approximately 20–25 nucleotides in length, which usually imperfectly target the 3′untranslated region (3′UTR) of mRNA to induce mRNA degradation or translational inhibition (Eichhorn et al., 2014). Convincing evidence has suggested that miRNAs functions in a broad array of biological processes, including cell growth, apoptosis, invasion, metastasis and tumorigenesis (Yates et al., 2013). Meanwhile, previous studies have proposed that the dysregulated and dysfunctional miRNAs are frequently observed in different cancers and boost cancer development and progression by acting as oncogenes or tumor suppressors (Cai et al., 2009). Particularly, multiple miRNAs have been recognized as critical regulators in OS carcinogenesis, and may serve as independent biomarkers for prognosis and therapeutic targets for OS patients (Namlos et al., 2012). For example, reduced miR-505 expression is significantly correlated with poorer clinical prognosis in patients with OS and miR-505 can repress proliferation, migration and invasion of OS cells *via* suppressing high-mobility group box 1 (HMGB1) (Liu et al., 2018). miR-365 acts as a tumor suppressor to inhibit cell proliferation, invasion and migration in OS partially by targeting cysteine-rich angiogenic inducer 61 (CYR61) expression (Xu et al., 2018a,b). miR-411 upregulation has been reported to boost OS cell proliferation and migration *via* constraining metastasis suppressor protein 1 (MTSS1) expression (Xu et al.,

* Corresponding author at: No. 1 Jianshe East Road, Zhengzhou, 450052, Henan, PR China.
E-mail address: ZZUeasy@163.com (J. Chen).

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2018a,b). Among these miRNAs, miR-425-5p, located in human chromosome 3, has been found to be abnormally expressed in various tumors (Ueda et al., 2010; Liu et al., 2015). More notably, miR-425-5p has been demonstrated to be downregulated in the serum of OS patients and may be used as a diagnostic biomarker for OS (Li et al., 2015). However, the detailed roles of miR-425-5p in OS progression and its underlying molecular mechanism are far from being addressed.

Therefore, here, we investigated the expression, roles and potential mechanisms of miR-425-5p in OS to provide a promising target for OS treatment.

2. Materials and methods

2.1. Patients and tissue samples

Forty-three paired OS tissues and matched adjacent normal tissues were harvested from patients who underwent surgical resection at the First Affiliated Hospital of Zhengzhou University between May 2016 and October 2017. All OS patients enrolled in this study did not receive any chemotherapy or radiotherapy before surgery. All protocol was approved by the Research Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China) and written informed consent was obtained from all participants.

2.2. Cell culture and transfection

Human OS cell lines (MG63, U2OS, SaOS-2, 143B, and HOS), human normal osteoblastic cell line hFOB1.19, and HEK293 T cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). OS cell lines and hFOB1.19 cells were maintained in RPMI 1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37 °C in a humidified atmosphere containing 5% CO_2. HEK293 T cells were cultured in DMEM medium (Invitrogen, Carlsbad, CA, USA).

miR-425-5p agomir (miR-425-5p) and its matched control (miR-Ctrl), miR-425-5p antagomir (anti-miR-425-5p) and antagomir control (anti-miR-Ctrl), miR-425-5p overexpression lentivirus (Lv-miR-425-5p) and the matched control lentivirus (Lv-miR-Ctrl) were all gained from GenePharma Co., Ltd. (Shanghai, China). To overexpress MALAT1 or TUG1 expression, the full-length sequence of MALAT1 or TUG1 was synthesized and cloned into pcDNA3.1 vector (Invitrogen) to produce pcDNA-MALAT1 or pcDNA-TUG1, with pcDNA empty vector as the control (pcDNA). MG63 and U2OS cells were seeded in 6-well culture plates at a dose of 1×10^5 cells/well and transfected with miRNAs or pcDNAs using Lipofectamine 2000 (Invitrogen) when cell confluence was ˜80%. Following 48 h of transfection, cells were collected for further analyses.

2.3. Quantitative real-time PCR

Total RNAs from the tissue and cultured cell samples were isolated using TRIzol reagent (Invitrogen). The first strand of cDNA was synthesized from 1 μg total RNAs using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). miR-425-5p expression was determined by mirVanaTM qRT-PCR miRNA Detection Kit (Ambion, Austin, TX, USA), with U6 small nuclear RNA (snRNA) as an endogenous control. MALAT1 and TUG1 expressions were determined by using SYBR Premix Ex Taq II (Takara, Shiga, Japan), with β-actin as an internal control. All PCR reactions were performed on the StepOnePlus Real-Time PCR System (Applied Biosystems). The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. For semi-quantitative PCR analyses, agarose gel electrophoresis was performed to visualize the expressions of MALAT1 and TUG1 and the gel was observed using an UV imaging system. The primer sequences used were listed as follows: miR-425-5p, forward: 5′-TGCGGAATGACACGATCACTCCCG-3′, reverse: 5′−CCAGTGCAGGGTCCGAGGT-3′; MALAT1, forward: 5′-AAAGCAAGGTCTCCCCACAAG-3′, reverse: 5′-GGTCTGTGCTAGATCAAAAGGCA-3′; TUG1, forward: 5′-TAGCAGTTCCCCAATCCTTG-3′, reverse: 5′-CACAAATTCCCATCATTCCC-3′; U6, forward: 5′-TGCGGGTGCTCGCTTCGGCAGC-3′, reverse: 5′−CCAGTGCAGGGTCCGAGGT-3′; β-actin, forward: 5′-CAGAGCCTCGCCTTTGCC-3′, reverse: 5′-GTCGCCCACATAGGAATC-3′.

2.4. Western blot analyses

Total proteins were extracted from tissues or the cultured transfected MG63 and U2OS cells using RIPA lysis buffer (Beyotime, Shanghai, China) and the concentrations of proteins in different samples were quantified by a BCA protein assay kit (Tiangen, Beijing, China). Subsequently, 20 μg of protein samples per lane were loaded on a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Following blocked with 5% non-fat milk for 1 h, the membranes were immunoblotted with the indicated primary antibodies overnight at 4 °C including proliferating cell nuclear antigen (PCNA; Abcam, Cambridge, UK), matrix metalloproteinase (MMP)-2 (Cell Signaling Technology, Beverly, MA, USA), MMP-7 (Cell Signaling Technology), CyclinD1 (Abcam), c-Myc (Abcam), β-catenin (Cell Signaling Technology) and β-actin (Abcam) as an internal control. After washed with TBST, the membranes were incubated with the corresponding horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology) for 1 h at room temperature. The protein bands were detected with enhanced chemiluminescence detection kit (GE Healthcare, Pittsburgh, PA, USA) and the images were analyzed using Quantity One (Bio-Rad, Hercules, CA, USA).

2.5. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) assays

Cell proliferation was assessed by MTT assays. In brief, the MG63 and U2OS cells transfected with miR-425-5p or miR-Ctrl were seeded into 96-well plates with 3×10^3 cells/well and routinely incubated for 1, 2, and 3 days, respectively. Then, 20 μl of MTT reagent (5 mg/ml; Sigma-Aldrich, St. Louis, MO, USA) was added into each well and incubated for another 4 h at 37 °C. Subsequently, the cultural supernatant was discarded and 150 μL of dimethylsulfoxide (DMSO; Sigma-Aldrich) was supplemented to dissolve the formazan crystals for 10 min. The optical density at 490 nm was recorded on the Spectramax M5 microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA).

2.6. Scratch wound healing and transwell invasion assays

For determining the migratory capability of cells, the scratch wound healing assays were performed. In brief, the transfected cells were seeded into 6-well plates at the density of 5×10^5 cells/well. When the cells were grown to ˜80% confluence, the uniform wounds on the monolayer cells were generated by using a 10 μL pipette tip. Wound healing gaps of cells were imaged at 0 and 48 h at × 40 magnification.

Cell invasion was evaluated using Transwell insert chambers (8 μm pore size, Corning Inc., Corning, NY, USA). 100 μL of 2% Matrigel (BD Biosciences, San Jose, CA, USA) was applied to pre-coate the upper chamber. 1×10^5 MG63 and U2OS cells transfected with miR-425-5p or miR-Ctrl were resuspended in 200 μl of serum-free media in the upper chamber. Complete culture medium (600 μL) with 10% FBS was added to the lower chamber as a chemoattractant. After 48 h of incubation at 37 °C, the cells which had invaded to the lower chambers were fixed with methanol and stained with 0.1% crystal violet. The invasive cells were then imaged at ×200 magnification and counted.

2.7. Luciferase reporter assays

The fragments of MALAT1 or TUG1 containing the predicted binding sites of miR-425-5p were amplified by PCR and separately cloned into the *Kpn*I and *Bgl*II sites of pGL3 luciferase reporter vector (Promega, Madison, WI, USA), namely MALAT1-WT and TUG1-WT. In order to confirm the binding specificity, mutation of the binding sites were performed and the recombinant plasmids were named as MALAT1-MUT and TUG1-MUT. For luciferase reporter assays, 5×10^4 HEK293T cells were plated in the 48-well plates and cotransfected with the constructed luciferase plasmids and miR-425-5p or miR-Ctrl using Lipofectamine 2000 (Invitrogen). At 48 h posttransfection, cells were harvested and Renilla and firefly luciferase activities were detected using Dual-Luciferase Reporter Assay System (Promega). Renilla luciferase activity was used as the normalization.

2.8. Construction of xenograft mouse models

The animal experiment protocol was approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Zhengzhou University and performed based on the NIH guidelines for the care and use of laboratory animals. A total of 22 male BALB/c nude mice (aged 4–6 weeks, 15–20 g weight) were purchased from the Experimental Animal Center of Zhengzhou University. The MG63 cells stably transfected with Lv-miR-425-5p or Lv-miR-Ctrl (5×10^6) were suspended in 200 µl of PBS and injected subcutaneously into the flank of each nude mouse. Volumes of xenograft tumors were estimated every week for 6 weeks. After 6 weeks of injection, the mice were sacrificed and weighed, and the tumor tissues were dissected and weighed. In addition, the resected tumor tissues were subjected to qRT-PCR and western blot analyses.

2.9. Statistical analyses

The experimental results were presented as mean ± standard errors (SD). All statistical data were analyzed by GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). The significance of differences between two or more groups was evaluated using Student's *t*-test or one-way analysis of variance (ANOVA). P values < 0.05 were considered as statistically significant.

3. Results

3.1. miR-425-5p was downregulated in OS tissues

To address the potential significance of miR-425-5p in OS, the expression of miR-425-5p in 43 paired OS tissues and adjacent matched tissues was detected by qRT-PCR. As shown in Fig. 1A and B, miR-425-5p expression was significantly lower in OS tissues than that in normal controls, suggesting that miR-425-5p might play crucial roles in OS progression.

3.2. miR-425-5p upregulation constrained proliferation of OS cells

Consistently with the above result, a significant drop in miR-425-5p expression was also detected in OS cell lines (MG63, U2OS, SaOS-2, 143B, and HOS) when compared with normal osteoblastic cell line hFOB1.19, especially in MG63 and U2OS cells (Fig. 2A). Thus, MG63 and U2OS cells were selected for next experiments. To evaluate the roles of miR-425-5p in OS malignant phenotype, miR-425-5p agomir was transiently transfected into MG63 and U2OS cells to upregulate miR-425-5p expression. As expected, miR-425-5p levels were markedly elevated in miR-425-5p-transfected group as compared to miR-Ctrl group in both MG63 and U2OS cells (Fig. 2B). The subsequent MTT assays revealed that cell proliferation at days 2 and 3 was drastically impeded following miR-425-5p overexpression with respect to miR-Ctrl group in MG63 and U2OS cells (Fig. 2C). Further, the expression change of PCNA, an important indicator of cell proliferation, was estimated in MG63 and U2OS cells after treatment with miR-425-5p by western blot. As displayed in Fig. 2D, transfection with miR-425-5p led to a remarkable decrease of PCNA protein level in MG63 and U2OS cells in comparison with miR-Ctrl-transfected group. Collectively, these data demonstrated that overexpression of miR-425-5p suppressed proliferation of OS cells.

3.3. Ectopic expression of miR-425-5p inhibited OS cell migration and invasion

Next, scratch wound healing and Transwell invasion assays demonstrated that the relative rate of wound closure and number of invasive cells was greatly declined in MG63 and U2OS cells transfected with miR-425-5p (Fig. 3A and B). As we know, MMPs including MMP-2 and MMP-7 mediate extracellular matrix (ECM) degradation which plays crucial roles in cell invasion and metastasis (Kessenbrock et al., 2010). In accordance with the results from scratch wound healing and invasion assays, introduction of miR-425-5p obviously decreased expression of MMP-2 and MMP-7 in MG63 and U2OS cells (Fig. 3C) in comparison with control group. Together, these results suggested that enforced expression of miR-425-5p alleviated migration and invasion of OS cells.

3.4. miR-425-5p suppressed the expression of MALAT1 and TUG1 in OS cells

To figure out the molecular mechanism of the regulatory role of miR-425-5p in OS development, bioinformatics analyses were conducted. These results showed that MALAT1 and TUG1, two well-known oncogenes in OS (Cai et al., 2016; Yun-Bo et al., 2016), both contained the probable complementary binding sites on the miR-425-5p sequence, as shown in Fig. 4A. Moreover, in OS tissues a negative relationship between miR-425-5p level and MALAT1 or TUG1 level was observed

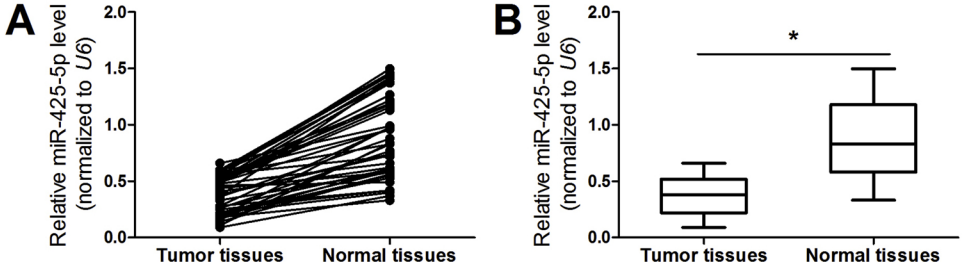

Fig. 1. The expression profile of miR-425-5p in OS tissues. (A and B) The expression of miR-425-5p was measured by qRT-PCR assays in 43 paired OS tissues and adjacent normal tissues. *$P < 0.05$.

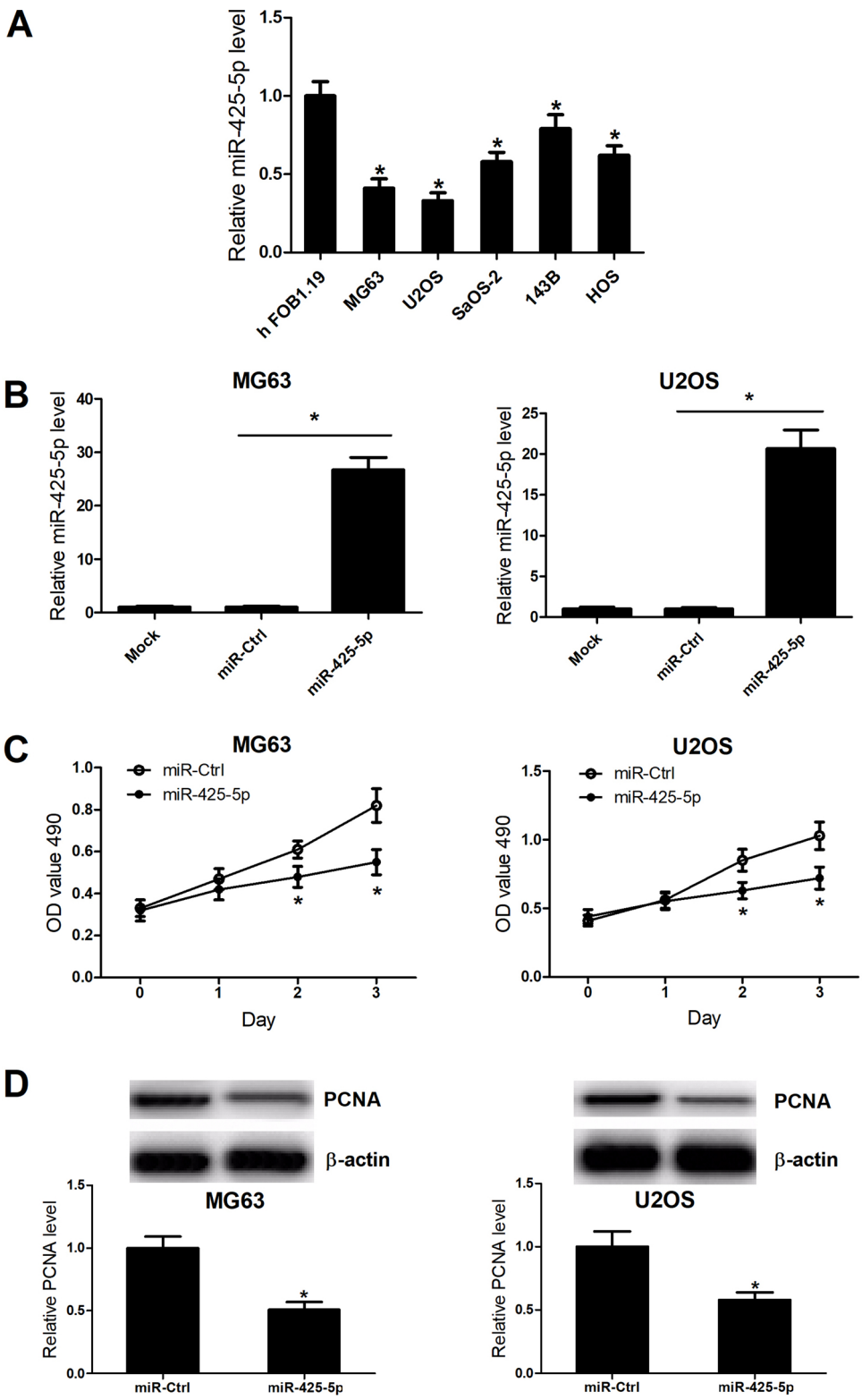

Fig. 2. The effects of miR-425-5p overexpression on OS cell proliferation. (A) miR-425-5p levels in OS cell lines (MG63, U2OS, SaOS-2, 143B, and HOS) and normal osteoblastic cell line hFOB1.19 was examined by qRT-PCR. (B) The expression of miR-425-5p in MG63 and U2OS cells after transfection with miR-425-5p or miR-Ctrl was evaluated by qRT-PCR. (C) MTT assays were performed to assess cell proliferation at days 1, 2, and 3 in MG63 and U2OS cells introduced with miR-425-5p or miR-Ctrl. (D) The protein level of PCNA in miR-425-5p- or miR-Ctrl-treated MG63 and U2OS cells was analyzed by western blot. *P < 0.05.

(Fig. 4B and C). Additionally, we confirmed that MALAT1 and TUG1 expression was both aberrantly upregulated in MG63 and U2OS cells compared with hFOB1.19 cells (Fig. 4D and E). Further, for purpose of validating the interaction between MALAT1, TUG1 and miR-425-5p, luciferase reporter assays were carried out. The results exhibited that the enhanced expression of miR-425-5p remarkably decreased the luciferase activities of MALAT1-WT and TUG1-WT in HEK293T cells, but showed no significant effect on MALAT1-MUT and TUG1-MUT (Fig. 4F

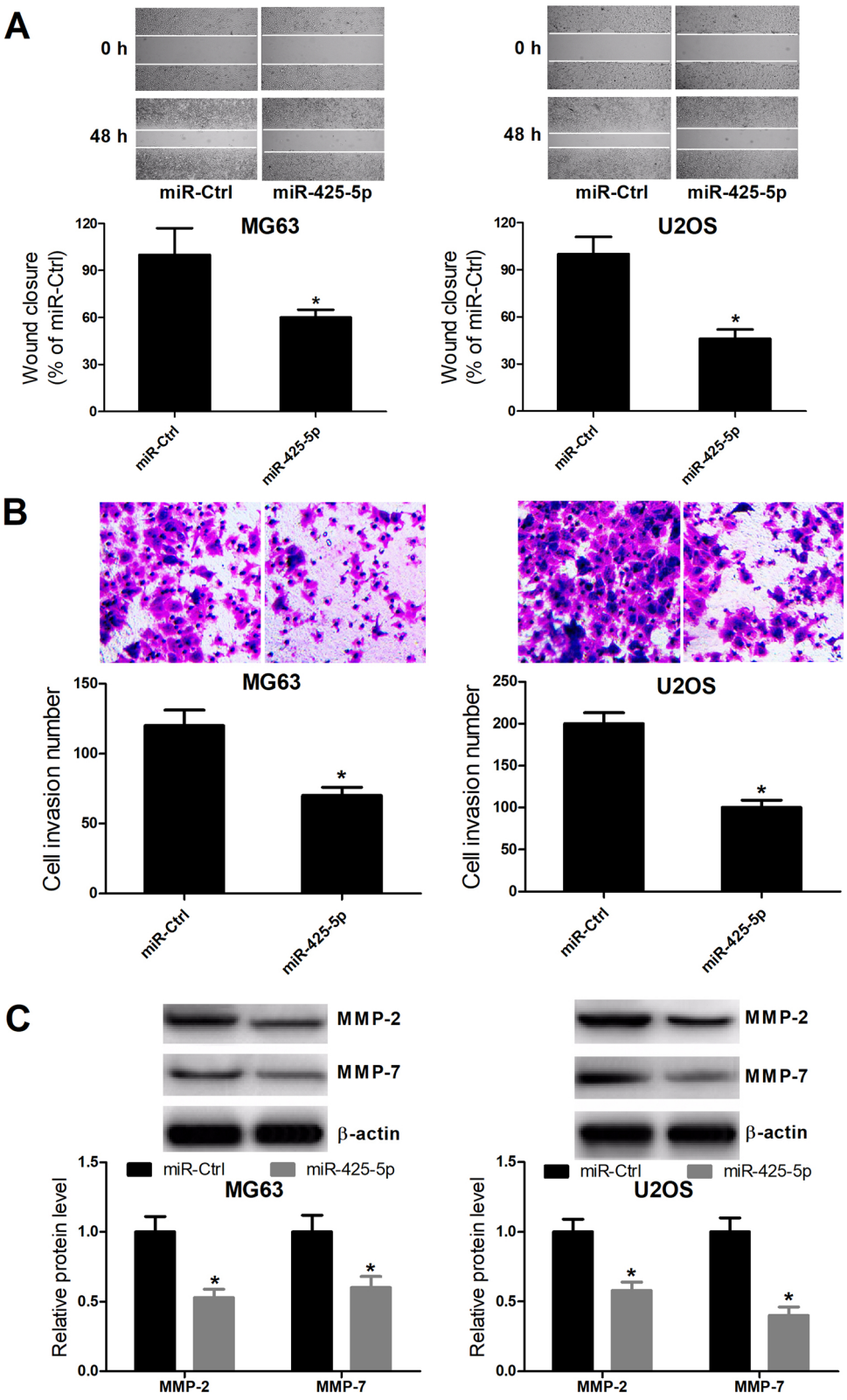

Fig. 3. The effects of miR-425-5p overexpression on OS cell migration and invasion. MG63 and U2OS cells were transfected with miR-425-5p or miR-Ctrl and incubated for 48 h. (A and B) Scratch wound healing and invasion assays were conducted to estimate cell migratory and invasive abilities of the treated MG63 and U2OS cells. (C) Western blot was carried out to detect the protein levels of MMP-2 and MMP-7 in the transfected MG63 and U2OS cells. *$P < 0.05$.

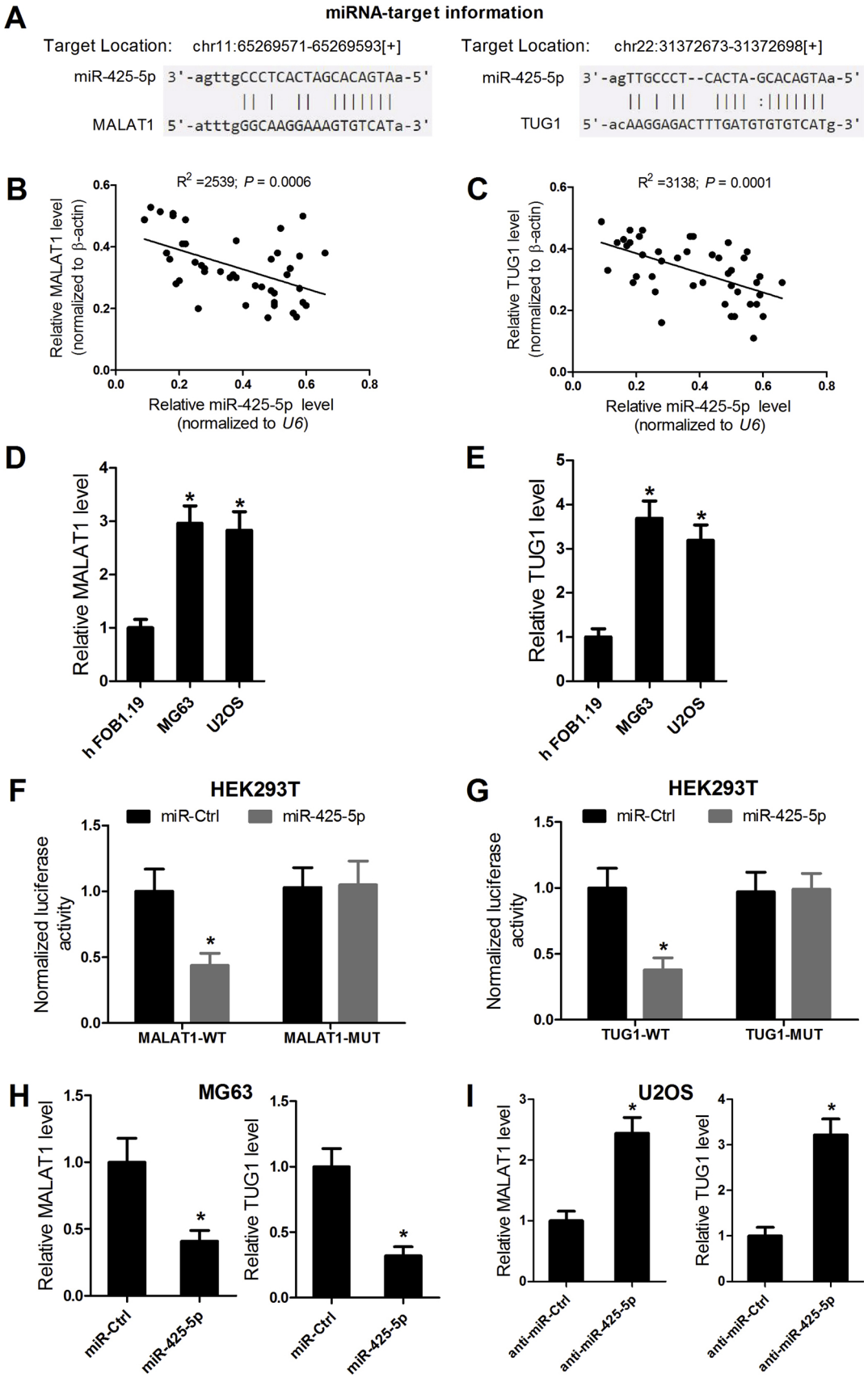

Fig. 4. The interaction between MALAT1, TUG1 and miR-425-5p in OS cells. (A) The predicted binding sites between MALAT1, TUG1 and miR-425-5p. (B and C) A negative relationship between miR-425-5p and MALAT1 or TUG1 in OS tissues. (D and E) The expression of MALAT1 and TUG1 in MG63, U2OS, and hFOB1.19 cells was analyzed by qRT-PCR. (F and G) The relative luciferase activity was determined in MG63 and U2OS cells after cotransfection with constructed luciferase reporter plasmids containing the wild-type or mutated MALAT1 or TUG1 sequences and miR-425-5p or miR-Ctrl. (H and I) The expression of MALAT1 and TUG1 in MG63 and U2OS cells transfected with miR-425-5p, anti-miR-425-5p, or corresponding controls was determined by qRT-PCR. *$P < 0.05$.

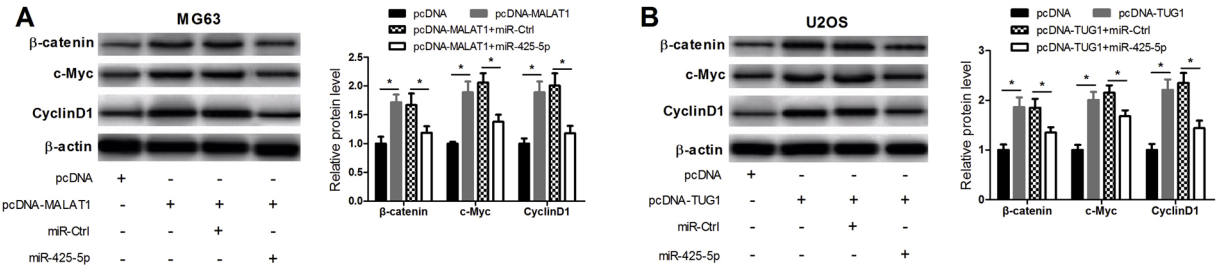

Fig. 5. The effects of MALAT1 and TUG1 or combined with miR-425-5p on the Wnt/β-catenin signaling pathway in OS cells. (A and B) MG63 and U2OS cells were transfected with pcDNA, pcDNA-MALAT1, pcDNA-TUG1, or along with miR-425-5p or miR-Ctrl and western blot was then applied to detect the protein levels of β-catenin, CyclinD1 and c-Myc). *$P < 0.05$.

and G). To evaluate the regulatory effects of miR-425-5p on MALAT1 and TUG1 expression, MG63 and U2OS cells were introduced with miR-425-5p agomir, anti-miR-425-5p, or the corresponding controls. qRT-PCR results disclosed that MALAT1 and TUG1 expression was reduced in miR-425-5p-transfected MG63 cells (Fig. 4H), but prominently increased in anti-miR-425-5p-introduced U2OS cells (Fig. 4I). Therefore, we concluded that miR-425-5p could suppress the expression of MALAT1 and TUG1 in OS cells.

3.5. miR-425-5p upregulation alleviated MALAT1 and TUG1-mediated activation of the Wnt/β-catenin signaling pathway in OS cells

It is believed that the abnormal activation of Wnt/β-catenin signaling pathway has intimate connection with the occurrence and tumorigenesis of OS (Cai et al., 2014). Therefore, we analyzed the effects of MALAT1 and TUG1, or combined with miR-425-5p on the Wnt/β-catenin signaling pathway in OS cells. As exhibited in Fig. 5A and B, the enforced expression of MALAT1 and TUG1 conspicuously increased the expression of β-catenin, CyclinD1 and c-Myc in MG63 and U2OS cells, suggesting that MALAT1 and TUG1 activated the Wnt/β-catenin signaling pathway in OS cells. However, upregulation of miR-425-5p strikingly abrogated the activation of Wnt/β-catenin signaling pathway induced by MALAT1 and TUG1 in OS cells. These data suggested that miR-425-5p could reverse MALAT1 and TUG1-mediated activation of the Wnt/β-catenin signaling pathway in OS cells.

3.6. miR-425-5p inhibited expression of MALAT1 and TUG1 as well as the Wnt/β-catenin signaling pathway in Lv-miR-425-5p MG63 cells

As shown in Fig. 6A, MG63 cells were stably transfected with Lv-miR-425-5p or Lv-miR-Ctrl to overexpress miR-425-5p. Moreover, MALAT1 (Fig. 6B) and TUG1 (Fig. 6C) expression was significantly decreased in Lv-miR-425-5p MG63 cells. miR-425-5p overexpression significantly suppressed the expression of β-catenin, CyclinD1 and c-Myc in MG63 cells (Fig. 6D).

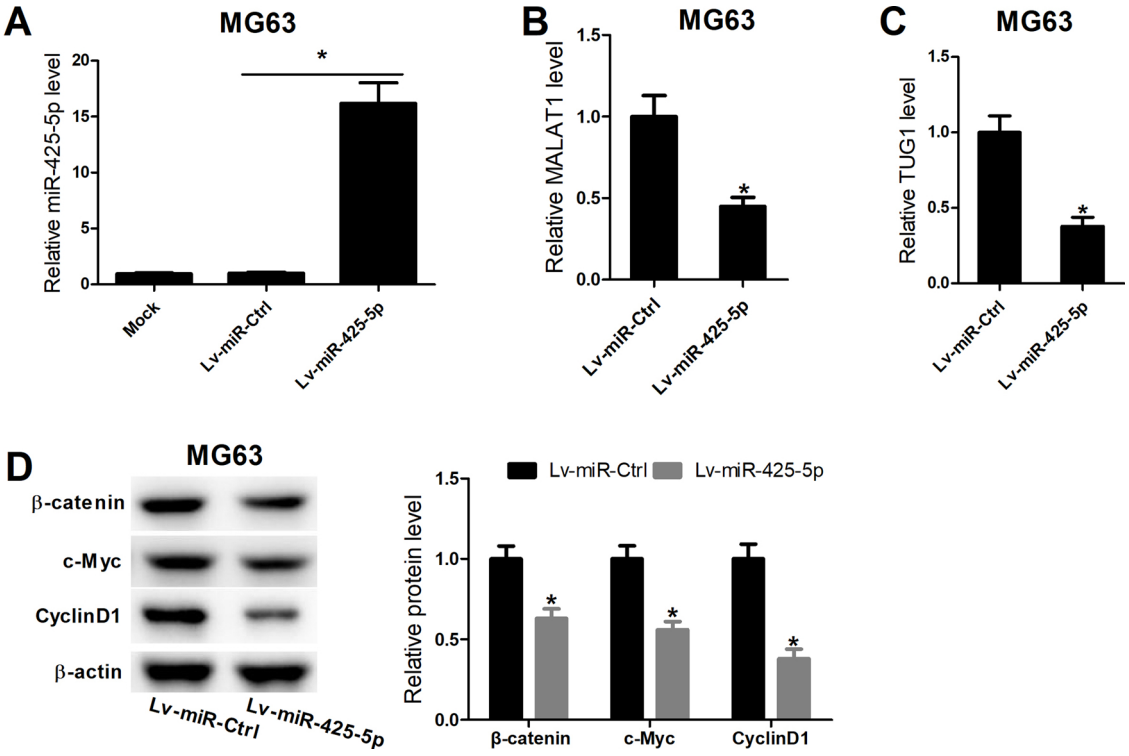

Fig. 6. The effects of miR-425-5p on expression of MALAT1 and TUG1 as well as the Wnt/β-catenin signaling pathway in Lv-miR-425-5p MG63 cells. (A) qRT-PCR analyses of miR-425-5p expression in Lv-miR-425-5p MG63 cells. qRT-PCR analyses of MALAT1 (B) and TUG1 (C) expression in Lv-miR-425-5p MG63 cells. (D) Western blot analyses of the protein levels of β-catenin, CyclinD1 and c-Myc) in Lv-miR-425-5p MG63 cells. *$P < 0.05$.

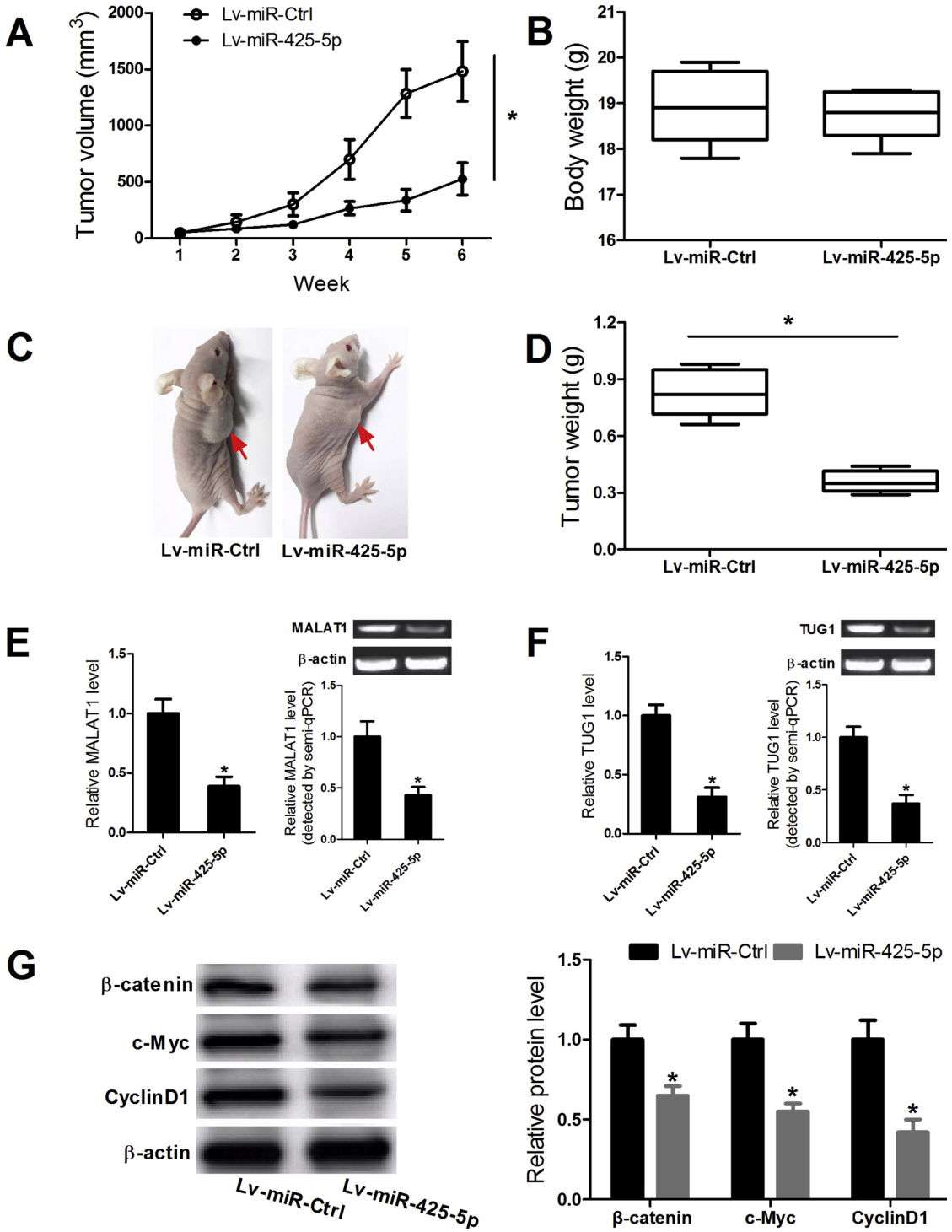

Fig. 7. The effects of miR-425-5p on OS tumor growth *in vivo* and the underlying mechanism. MG63 cells stably transfected with Lv-miR-425-5p or Lv-miR-Ctrl were injected subcutaneously into the flank of each nude mouse. (A) The tumor volume was estimated every week for 6 weeks. (B) After 6 weeks of injection, the mice were sacrificed and weighed. (C) Photographs of representative xenograft tumors in nude mouse. (D) Tumor tissues were dissected and weighed. (E and F) qRT-PCR and semi-quantitative PCR analyses were used to evaluate the expression of MALAT1 and TUG1 in the resected tumor tissues. (G) The protein levels of β-catenin, CyclinD1 and c-Myc in the resected tumor tissues were determined by western blot. *$P < 0.05$.

3.7. miR-425-5p hindered OS tumor growth in vivo via decreasing MALAT1 and TUG1 expression by inactivation of the Wnt/β-catenin signaling pathway

Further, to confirm tumor suppressor function and mechanisms of miR-425-5p in OS tumorigenesis *in vivo*, MG63 cells stably transfected with Lv-miR-425-5p or Lv-miR-Ctrl were injected subcutaneously into the flank of each nude mouse to establish xenograft OS mouse models. Our results discovered that the enhanced expression of miR-425-5p significantly blocked the growth of MG63 xenografts in comparison with control group (Fig. 7A). We found that the body weight of nude mice showed no significant difference between Lv-miR-425-5p group and control group (Fig. 7B), but the tumors originating from Lv-miR-425-5p-transfected MG63 cells showed lower size and weight than

those treated with Lv-miR-Ctrl (Fig. 7C and D). Moreover, qRT-PCR and semi-quantitative PCR analyses discovered that miR-425-5p overexpression dramatically repressed the expression of MALAT1 and TUG1 in xenograft tumor tissues as compared with control group (Fig. 7E and F). Furthermore, the protein levels of β-catenin, CyclinD1 and c-Myc in the miR-425-5p-overexpressing tumors were markedly reduced in comparison with control group (Fig. 7G). Taken together, these results indicated that miR-425-5p hindered OS tumor growth *in vivo via* decreasing MALAT1 and TUG1 expression and inactivation of the Wnt/β-catenin signaling pathway.

4. Discussion

To date, growing evidence has implied that dysregulated miRNAs are involved in the tumorigenesis and progression of various tumors, including OS (Kushlinskii et al., 2016). Hence, a better understanding of the complex mechanisms underlying the involvement of specific miRNAs in OS carcinogenesis may provide novel opportunities for the diagnosis and treatment of OS. miRNAs, especially, miR-425-5p have been previously reported to be frequently dysregulated in numerous cancers. However, studies have expounded the contradictory roles of miR-425-5p in different types of tumors. For example, the increased miR-425-5p has been found in gastric cancer (GC) tissues and cells, and contributes to the invasion and migration of GC through a mechanism involving Cylindromatosis (CYLD) (Yan et al., 2017). The upregulated miR-425-5p has been reported to facilitate invasion and metastasis in hepatocellular carcinoma through suppressor of cancer cell invasion (SCAI)-mediated dysregulation of multiple signaling pathways (Fang et al., 2017). In contrast, a study has showed that miR-425 expression is demonstrated to be reduced in nasopharyngeal carcinoma tissues and cells, and miR-425 overexpression inhibits viability and invasion of NPC cells by targeting hepatoma-derived growth factor (HDGF) (Zhu et al., 2018). Notably, it is previously revealed that miR-425-5p is downregulated in OS patients (Li et al., 2015). Our results showed that a significant drop in miR-425-5p expression was detected in OS tissues and cells. Moreover, our study first provided evidence that restoration of miR-425-5p expression dampened proliferation, invasion and migration of OS cells *in vitro* and inhibited tumor growth *in vivo*, suggesting that miR-425-5p served as a tumor suppressor in OS.

LncRNAs, a group of non-protein encoding RNA molecules longer than 200 nucleotides, are well-documented to play vital roles in diverse cellular processes, such as tumor growth, metastasis and especially to cancer development and progression (Huarte, 2015). Much evidence has suggested that aberrantly expressed lncRNAs take part in the development of various cancers including OS (Li et al., 2016). MALAT1, located on chromosome 11q13, is initially confirmed as a tumor promoter in non-small cell lung cancer due to its role in elevating metastasis and invasion of cells (Ji et al., 2003). TUG1, a 7.1-kb lncRNA mapped to 22q12.2, is firstly reported to be upregulated in response to taurine treatment in retinal development (Young et al., 2005). Subsequently, the dysregulated MALAT1 and TUG1 have been confirmed to be closely associated with the progression of OS by functioning as oncogenes (Li et al., 2017b). MALAT1 expression is found to be increased in primary OS tissues and cells and promote OS cell proliferation through suppressing miR-205 and activating mothers against decapentaplegic homolog 4 (SMAD4) function (Li et al., 2017a). Moreover, TUG1 has been exhibited to be upregulated in OS tissues and cells, facilitate proliferation, and suppress apoptosis by regulating the miR-132-3p/sex determining region Y-box 4 (SOX4) (Li et al., 2018). In our study, we demonstrated that MALAT1 and TUG1 were targets of miR-425-5p and miR-425-5p upregulation inhibited the expression of MALAT1 and TUG1 in OS *in vitro* and *in vivo*.

The Wnt/β-catenin signaling pathway is well known to play important regulatory roles in a broad range of cellular processes, such as cell proliferation, invasion and tumorigenesis (Logan and Nusse, 2004). A previous study has convincingly demonstrated that abnormally activating the Wnt/β-catenin signaling pathway can enhance cancer development (Greer et al., 2013). Particularly, suppressing the activation of the Wnt/β-catenin signaling pathway has been proved to inhibit OS cell proliferation, metastasis and tumorigenesis (Rossini et al., 2013; Cai et al., 2014). Moreover, a prior study has reported that targeting the Wnt/β-catenin signaling pathway has been considered as a novel therapeutic approach for OS (Jin et al., 2013). Notably, previous studies have showed that MALAT1 activates the Wnt/β-catenin signaling pathway in tongue cancer (Liang et al., 2017a) and TUG1 knockdown suppresses the activation of Wnt/β-catenin signaling pathway in oral squamous cell carcinoma (Liang et al., 2017b). In the present study, we showed that MALAT1 and TUG1 increased the levels of β-catenin, CyclinD1 and c-Myc in MG63 and U2OS cells. However, upregulation of miR-425-5p strikingly abrogated the activation of Wnt/β-catenin signaling pathway induced by either MALAT1 or TUG1 in OS cells. Moreover, we found that miR-425-5p impeded the Wnt/β-catenin signaling pathway in OS *in vivo*. Collectively, all above results suggested that miR-425-5p suppressed the tumorigenesis of OS *in vitro* and *in vivo* by decreasing MALAT1 and TUG1 expression *via* inactivating the Wnt/β-catenin signaling pathway.

In conclusion, our study revealed that miR-425-5p expression was downregulated in OS tissues and cells. Moreover, we first provided evidence that miR-425-5p overexpression suppressed proliferation, invasion and migration of OS cells, as well as inhibited tumor growth *via* decreasing MALAT1 and TUG1 expression through dampening the Wnt/β-catenin signaling pathway, contributing to a better understanding of the molecular mechanism of the tumorigenesis of OS. Therefore, these foundings implicated that miR-425-5p may be a potential target for the treatment of OS.

Conflict of interest

All authors have declared that they have no competing interests.

Acknowledgements

No.

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