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OIP5-AS1 promotes the progression of gastric cancer cells via the miR-153-3p/ZBTB2 axis

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Abstract. – OBJECTIVE: Gastric cancer (GC) remains a serious disease to human health with high mortality worldwide. Evolving evidence implied that long non-coding RNA Opa interacting protein 5-antisense RNA1 (OIP5-AS1) went in for the pathological progress of GC. Nevertheless, the potential molecular mechanism of OIP5-AS1 needed to be further investigated.

MATERIALS AND METHODS: Levels of OIP5-AS1, microRNA (miR)-153-3p, and zinc finger and BTB domain containing 2 (ZBTB2) were assessed using quantitative real-time polymerase chain reaction (qRT-PCR) or Western blot assays. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was implemented to detect cell proliferation *in vitro*. Cell apoptosis was evaluated by flow cytometry. Besides, transwell assay was conducted to examine cell migration and invasion in AGS and MKN45 cells. The interaction between miR-153-3p and OIP5-AS1 or ZBTB2 was validated utilizing dual-luciferase reporter assay. Lastly, the role of OIP5-AS1 in tumor growth was researched through adopting xenograft tumor model.

RESULTS: OIP5-AS1 and ZBTB2 were strongly higher in GC tissues than noncancerous samples. OIP5-AS1 silencing remarkably curbed cell proliferation, migration and invasion, and elevated cell apoptosis in both AGS and MKN45 cells. Functional analysis indicated that OIP5-AS1 regulated ZBTB2 expression via binding to miR-153-3p. Moreover, the role of miR-153-3p in cell growth and metastasis was abrogated by ZBTB2 overexpression. Above all, OIP5-AS1 could reduce the growth of xenograft tumor *in vivo*.

CONCLUSIONS: OIP5-AS1 exerted its role via miR-153-3p/ZBTB2 axis in the progression of GC cells. These findings might supply a biomarker for the diagnosis and therapy of GC clinically.

Key Words:
OIP5-AS1, MiR-153-3p, ZBTB2, GC.

Introduction

Gastric cancer (GC) is the fourth frequent malignancy with high mortality[1]. Although curative therapeutic approaches for early-stage GC patients, such as surgical resection, chemotherapy, and radiation, have been found and improved, numerous patients are inoperable at diagnosis. In addition, the recurrence and metastasis of the therapeutic GC patients also lead to a low five-year survival rate[2-4]. Besides, the median survival time is less than one year in advanced patients[5]. Following the development in the understanding of cancer pathogenesis, the prognosis has been successfully improved. However, the effective molecular-targeted therapy needs to be further investigated.

With the continuous progression of high-throughput genome and transcriptome sequencing, 75% genomes are transcribed into non-coding RNAs (ncRNAs)[6,7]. By definition, long non-coding RNAs (lncRNAs) belong to ncRNAs with over than 200 nucleotides in length. The ectopic expression of lncRNAs, such as the upregulation, deficiency, or mutant, could participate in plenty of complex diseases, including cancers[8,9]. Among them, Growth Arrest-Specific Transcript (GAS5) is a tumor suppressor in GC, and its detection contributes to cell proliferation and poor prognosis[10]. Originally, Opa interacting protein 5-antisense RNA1 (OIP5-AS1) was excavated in zebrafish[11] and it has been revealed to facilitate tumor growth and metastasis in diverse human cancers[12,13]. Many reports[14,15] also suggest that OIP5-AS1 is generally upregulated in multiple cancers. However, the potential function of OIP5-AS1 is still not completely understood.

Until now, microRNAs (miRNAs) have gained extreme interest in mediating the progression of human cancers[16]. They can modulate several pathophysiological processes and post-transcriptional gene downregulation[17]. In addition, the aberrant expression of miRNAs is connected with the pathogenesis and progression of cancers[18]. In particular, miR-203 constrains the radiosensitivity of nasopharyngeal carcinoma by targeting protein kinase B (AKT) signaling pathway[19]. Notably, miR-153-3p

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is characterized as a key factor in tumorigenesis with the low-expression in melanoma[20]; its overexpression can reduce cell growth and invasiveness in melanoma by regulating snail family transcriptional repressor 1 (SNAI1). Moreover, miR-153-3p contributes cell radiosensitivity by modifying B cell lymphoma-2 gene (BCL2) in glioma[21]. Zinc finger and BTB domain containing 2 (ZBTB2) belongs to POK family; it can regulate the p53 signaling pathway[22]. Earlier works imply that ZBTB2 participates in the progression and initiation of cancers.

In this study, we research the expression signatures of OIP5-AS1, miR-153-3p, and ZBTB2. Further, the regulatory network between miR-153-3p and OIP5-AS1 or ZBTB2 was also the aim of the present study.

Materials and Methods

Clinical Samples and Cell Culture

The matched tissues containing GC tissues (n=30) and adjacent noncancerous tissues (n=30) were donated by GC patients who underwent surgical resection at The Affiliated Huai'an Hospital of Xuzhou Medical University. The recruited patients did not receive any chemotherapy and radiotherapy before the surgical operation. This study was approved by the Ethics Committee of The Affiliated Huai'an Hospital of Xuzhou Medical University, and the written informed consent was given by the participators.

In regard to cell culture, we purchased AGS, MKN-45, and HS-746T cell lines from BeNa culture collection (Beijing, China), GC9811-P (marked as GC9811), and GES-1 cell lines were obtained from MINGZHOU Biotechnology Co., Ltd (Zhejiang, China). AGS cells were grown in Ham's F-12K medium (Thermo Fisher Scientific, Waltham, MA, USA), MKN45, and GC9811 cells were cultured with Roswell Park Memorial Institute-1640 (RPMI-1640; Thermo Fisher Scientific, Waltham, MA, USA). Besides, HS-746T and GES-1 cells, as the control of GC cell lines, were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA). The above mediums were generated as complete mediums *via* adding 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 1 × penicillin and streptomycin (containing 100 U/mL penicillin and 100 μg/mL streptomycin). The GC cells and its control were incubated in a moist air incubator with 5% CO_2 at 37 °C.

Transient Transfection

For OIP5-AS1 and ZBTB2 upregulation, we inserted the cDNA sequence of them into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA), thereby forming overexpression vectors (OIP5-AS1 and ZBTB2) and their control (NC and pcDNA). The miR-153-3p mimic (miR-153-3p), miR-153-3p inhibitor (anti-miR-153-3p), and small interference RNAs (siRNAs) for the knockdown of OIP5-AS1 (si-OIP5-AS1#1 and si-OIP5-AS1#2) expression were designed and obtained from GenePharma (Shanghai, China). Simultaneously, the negative controls of mimic (miR-NC), inhibitor (anti-miR-NC) and siRNA (si-NC) were also constructed in GenePharma (Shanghai, China). Subsequently, transfection was carried out by using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) following the standard specifications. Besides, short hairpin RNA (shRNA) against OIP5-AS1 (sh-OIP5-AS1) and its control (sh-NC) were used to construct the stably transfected GC cells *via* lentivirus-mediation.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Assay

Total RNA was extracted and purified from clinical samples and cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The separated RNA (1 μg) was reverse-transcribed to cDNA adopting ReverTra Ace kit (Toyobo, Osaka, Japan) according to the standard introductions. QRT-PCR was administrated with SYBR-Green PCR kit (TaKaRa, Dalian, China) following the manufacture's manuals in ABI 7900 system (Applied Biosystems, Foster City, CA, USA). Ultimately, the genes were normalized by glyceraldehyde-phosphate dehydrogenase (GAPDH) or U6 *via* the $2^{-\Delta\Delta Ct}$ method. The primers were listed: OIP5-AS1 (forward 5'-TGCGAAGATGGCGGAGTAAG-3' and reverse 5'-TAGTTCCTCTCCTCTGGCCG-3'); miR-153-3p (forward 5'-ACACTCCAGCTGGGTTGCATAGTCACAAA-3' and reverse 5'-CAGTGCGTGTCGTGGAGT-3'); ZBTB2 (forward 5'-GATCGGATCCGATTTGGCCAACCATGGA-3' and reverse 5'-GATCCTCGAGAGAAAGGCTCCCTGGCT-3'); GAPDH (forward 5'-CAGCTAGCCGCATCTTCTTTT-3' and reverse 5'-GTGACCAGGCGCCCAATAC-3'); U6 (forward 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3').

Cell Proliferation Assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) was employed to analyze the level of

cell proliferation in GC cells after transfection. The AGS and MKN45 cells were seeded into a 96-well plate for 24 h, then transfected with siRNA, pcDNA or miRNA. At 0 day, 1 day, 2 days, or 3 days post-transfection, MTT reagent (5 mg/mL with 20 μL) was supplemented into each well. After that, 100 μL dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was added into every well to dissolve the precipitate. The optical density of lysates was evaluated at 490 nm *via* a microplate reader.

Flow Cytometry Assay

The Annexin V-fluorescein isothiocyanate (FITC)/Propidium Iodide (PI) kit (KeyGen Biotechnology, Nanjing, China) was employed to stain the apoptotic cells. Firstly, AGS and MKN45 cells were trypsinized after transfection with vector or oligonucleotides for 36 h, and then, the transfected cells were stained with Annexin V-FITC and PI for 15 min darkly. In the end point, the apoptotic cells were analyzed utilizing FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA).

Transwell Assay

Cell migration assay was administrated using a transwell chamber (Corning, Corning, NY, USA). Firstly, the transfected GC cells with serum-free medium (~2×10^4 cells) were plated into the upper chamber. The lower chamber was filled with complete RPMI-1640 or F12-K medium. Later, the non-migrating cells were erased using a cotton swab, and the remained cells were stained by 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). Finally, the migrating cells were stained and observed under a light microscope. For cell invasion assay, the protocols were similar with cell migration assay except that the upper chamber was pre-coated Matrigel (Corning, NY, USA) prior to seeding cells.

Dual-Luciferase Reporter Assay

MiR-153-3p sequence containing the complementary domain with OIP5-AS1 or ZBTB2, the putative wildtype and the designed mutant of the binding sites were inserted into pmirGLO vector (Promega, Madison, WI, USA) to generate wildtype reporters (OIP5-AS1-WT and ZBTB2-WT) and mutant reporters (OIP5-AS1-MUT and ZBTB2-MUT). With the help of Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA), the above wildtype or mutant reporter was co-transfected with miR-153-3p or miR-NC into GC cells. At 2 days post-transfection, the relative activity was assessed using dual-luciferase reporter kit (Promega, Madison, WI, USA) in the light of the producer's instructions. All samples were run thrice.

Western Blot Assay

The process of Western blot was done according to the previous study[23]. Briefly, the separated proteins were transfected onto the polyvinylidene difluoride (PVDF; Millipore, Billerica, MA, USA). The blot was incubated with ZBTB2 (Abcam, Cambridge, MA, USA; ab117756, 1:8000) or GAPDH (Abcam, Cambridge, MA, USA; ab8245, 1:5000). Then, the corresponding second antibody was applied to combine the complex of protein-primary antibody. Finally, an enhanced chemiluminescence (ECL) kit (Millipore, MA, USA) could supply the reagent to appear the special signals.

Xenograft Tumor Model

Briefly, the male athymic nude mice (4-week-old) were purchased from Shanghai Laboratory Animal Center Co., Ltd. (Shanghai, China), and then were randomly divided into two groups (n=5/group), including treatment (sh-OIP5-AS1-mediation) and control (sh-NC-mediation) groups. The animal experiments were approved by Animal Ethics Committee of The Affiliated Huai'an Hospital of Xuzhou Medical University and were performed in accordance with the institutional guide for the care and use of laboratory animals. Firstly, the mice were injected with 200 μL phosphate buffer solution (PBS; Gibco, Rockville, MD, USA, containing 2×10^6 AGS cells) subcutaneously. Tumor volume was measured every 5 days for 6 times. Next, the tumor volume was calculated based on the formula: volume=length–width^2–0.5. After mice were sacrificed at 32 days post-injection, the xenograft tumors were fetched and weighed.

Statistical Analysis

The data were processed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and presented as the mean±standard deviation (SD). The Student's *t*-test (for two-group) or one-way ANOVA (for three or more groups) followed by Tukey's tests was used to analyze the statistically significant. It was deemed as the statistical difference when *p*-value was less than 0.05.

Results

Level of OIP5-AS1 Was Significantly Upregulated in GC Tissues and Cell Lines

To figure out the implication of OIP5-AS1 in GC, the expression level of OIP5-AS1 was measured. QRT-PCR analysis indicated that OIP56-AS1 level was evidently triggered in GC tissues in

comparison with matched para-cancerous tissues (Figure 1A). Also, we detected the expression tendency of OIP5-AS1 by qRT-PCR assay in GC cells, and the results presented that OIP5-AS1 was remarkably augmented in GC cell lines, AGS and MKN45 cells were selected for subsequent assays (Figure 1B). These data meant that OIP5-AS1 was upregulated in GC tissues, and the ectopic expression might be involved in the progression of GC.

OIP5-AS1 Detection Reduced Cell Proliferation, Migration, Invasion, and Reinforced Cell Apoptosis In Vitro

Considering that OIP5-AS1 was overexpressed in GC tissues and cell lines, we next explored the impacts of OIP5-AS1 detection on cell phenotypes *in vitro*. Firstly, vector of si-NC, si-OIP5-AS1#1, or si-OIP5-AS1#2 was transfected into AGS and MKN45 cells and it was found that the expression of OIP5-AS1 was efficiently blocked *via* transfection with si-OIP5-AS1#1 or si-OIP5-AS1#2 as compared with control. After comprehensive consideration, si-OIP5-AS1#1 was picked as the knockdown vector of OIP5-AS1 (Figure 2A). Notably, the deficiency of OIP5-AS1 could clearly repress cell proliferation in both AGS and MKN 45 cells (Figure 2B and 2C). In addition, cell apoptosis in both GC cells was notably expedited by OIP5-AS1 detection (Figure 2D). Simultaneously, transwell analysis manifested that compared with control, OIP1-AS1 silencing prominently constrained cell migration and invasion in GC cells (Figure 2E and 2F). The evidence demonstrated that OIP5-AS1 deficiency could suppress cell proliferation, migration, invasion, and promote cell apoptosis in GC cells.

MiR-153-3p Was a Target of OIP5-AS1

As mentioned above, we investigated the candidate targets of OIP5-AS1. The putative binding sites between OIP5-AS1 and miR-153-3p predicted by starBase v3.0 were shown in Figure 3A. Meanwhile, the Luciferase activity of OIP5-AS1-WT was strikingly decreased by miR-153-3p mimic, while miR-153-3p mimic had no significant effect on the Luciferase activity of mutant reporter in both AGS and MKN45 cells (Figure 3B and 3C). Afterwards, we exposed whether OIP5-AS1 could regulate the level of miR-153-3p; qRT-PCR analysis confirmed that the overexpression of OIP5-AS1 clearly impeded miR-153-3p level, but miR-153-3p level was apparently boosted under OIP5-AS1 silencing in AGS and MKN45 cells (Figure 3D and 3E). In addition, the level of miR-153-3p was examined, and an effective lower expression of miR-153-3p was observed in GC tissues and cell lines compared with corresponding controls (Figure 3F and 3G). Besides, we also found that miR-153-3p level was passively correlated with OIP5-AS1 level (Figure 3H). These data suggested that OIP5-AS1 was a sponge of miR-153-3p.

ZBTB2 Was Directly Targeted by MiR-153-3p

Given the above introductions, we further searched for the possible targets of miR-153-3p. TargetScan predicted that ZBTB2 was one of the putative targets of miR-153-3p (Figure 4A). Subsequently, Dual-Luciferase reporter assay was performed to verify whether miR-153-3p could target ZBTB2, and the results exhibited that miR-153-3p could reduce the Luciferase activi-

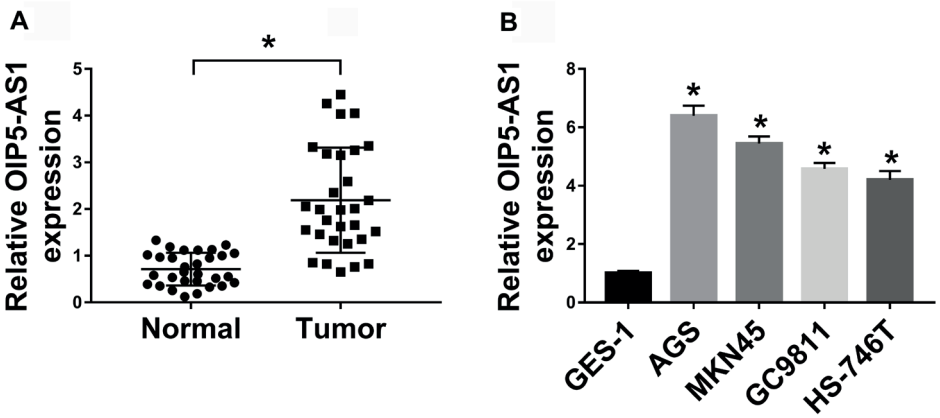

Figure 1. The level of OIP5-AS1 was significantly upregulated in GC tissues and cell lines. **A,** Relative expression level of OIP5-AS1 in clinical samples. **B,** Relative expression level of OIP5-AS1 in GC cell lines and normal cells. *$p<0.05$.

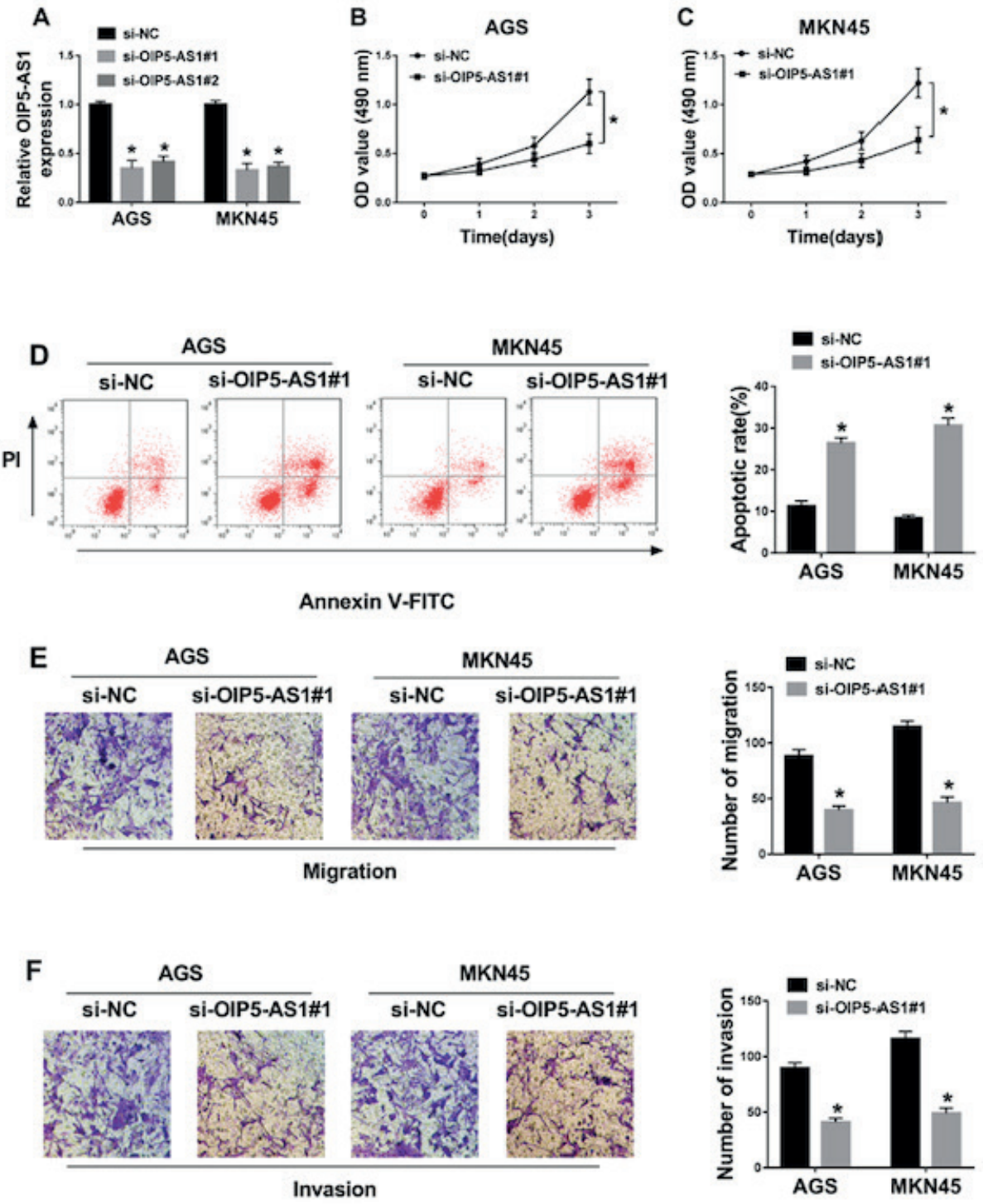

Figure 2. OIP5-AS1 detection reduced cell proliferation, migration, invasion, and reinforced cell apoptosis *in vitro*. AGS and MKN45 cells were transfected with si-NC, si-OIP5-AS1#1, or si-OIP5-AS1#2, respectively. **A,** The knockdown efficiency of each vector in OIP5-AS1 levels. **B,** and **C,** Effect of OIP5-AS1 silencing on proliferation of GC cell lines. **D,** Level of cell apoptosis after knockdown of OIP5-AS1 *in vitro*. **E,** and **F,** The role of OIP1-AS1 deficiency in migration and invasion of AGS and MKN45 cells (magnification ×100). **p*<0.05.

ty of ZBTB2-WT, whereas had no evident role in mutant Luciferase activity in both AGS and MKN45 cells (Figure 4B and 4C). Next, we investigated the function of miR-153-3p in ZBTB2 level. qRT-PCR and Western blot analyses displayed that the introduction of miR-153-3p mimic could specially retard the mRNA and protein levels of ZBTB2, but the role of miR-153-3p inhibitor in regulating mRNA and protein levels of ZBTB2 was opposite with miR-153-3p mimic in GC cells (Figure 4D-4G). Furthermore, the expression level of ZBTB2 was strongly intensified in GC tissues and cell lines (Figure 4H-4K). Expectantly, an inverse correlation between ZBTB2 expression and miR-153-3p level was analyzed by qRT-PCR (Figure 4L). The above evidence proved that miR-153-3p could directly target ZBTB2.

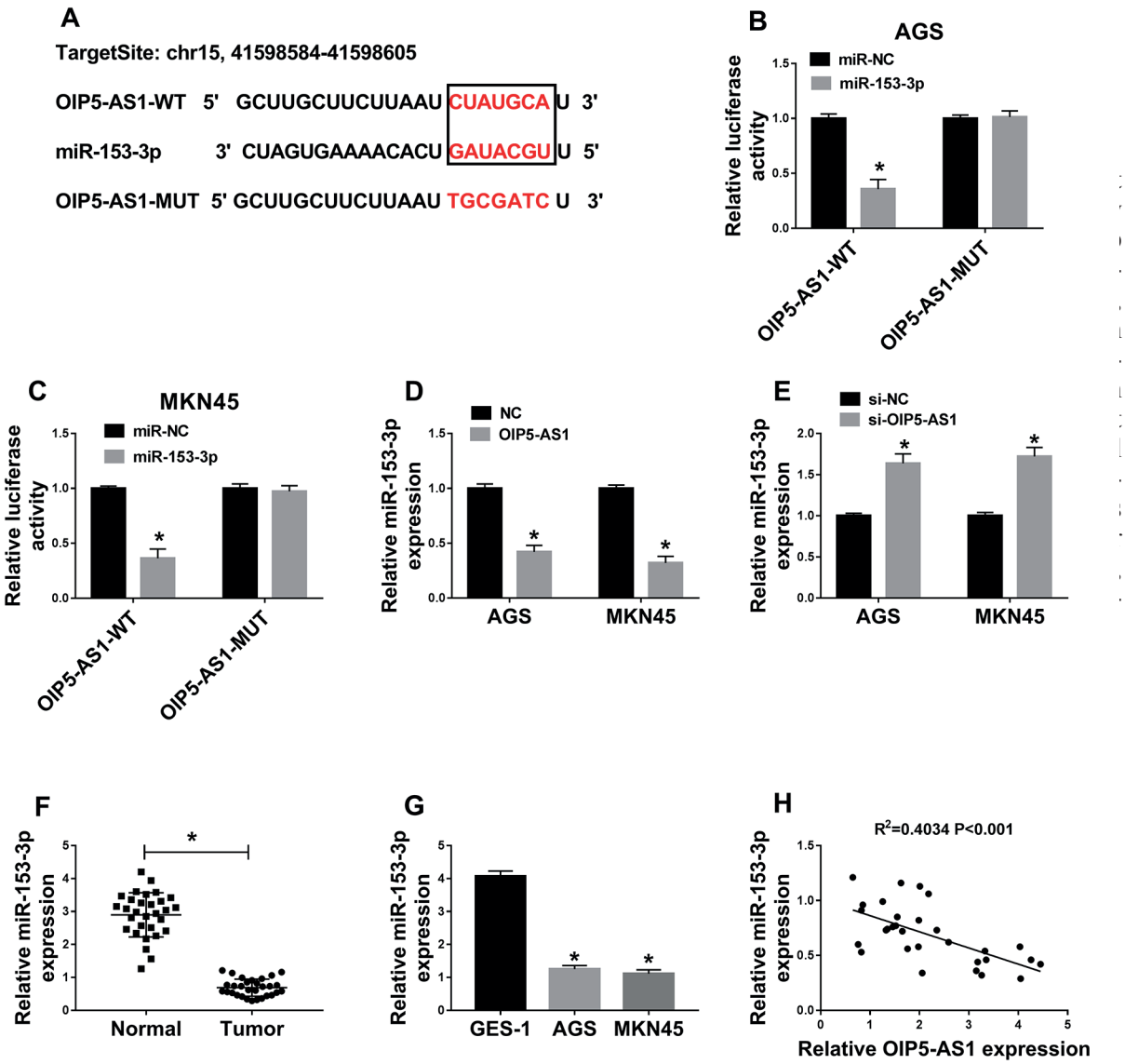

Figure 3. MiR-153-3p was a target of OIP5-AS1. **A,** The predictive binding sites between OIP5-AS1 and miR-153-3p. **B,** and **C,** Dual-Luciferase reporter analysis for the interrelation between miR-153-3p and OIP5-AS1 in AGS and MKN45 cells. **D,** and **E,** QRT-PCR analysis for the expression level of miR-153-3p under OIP5-AS1 overexpression or detection in GC cells. **F,** and **G,** Level of miR-153-3p in tissues (GC tissues and adjacent noncancerous tissues) and cell lines (GES-1, AGS and MKN45). **H,** QRT-PCR analysis for the correlation between miR-153-3p level and OIP5-AS1 level. *p<0.05.

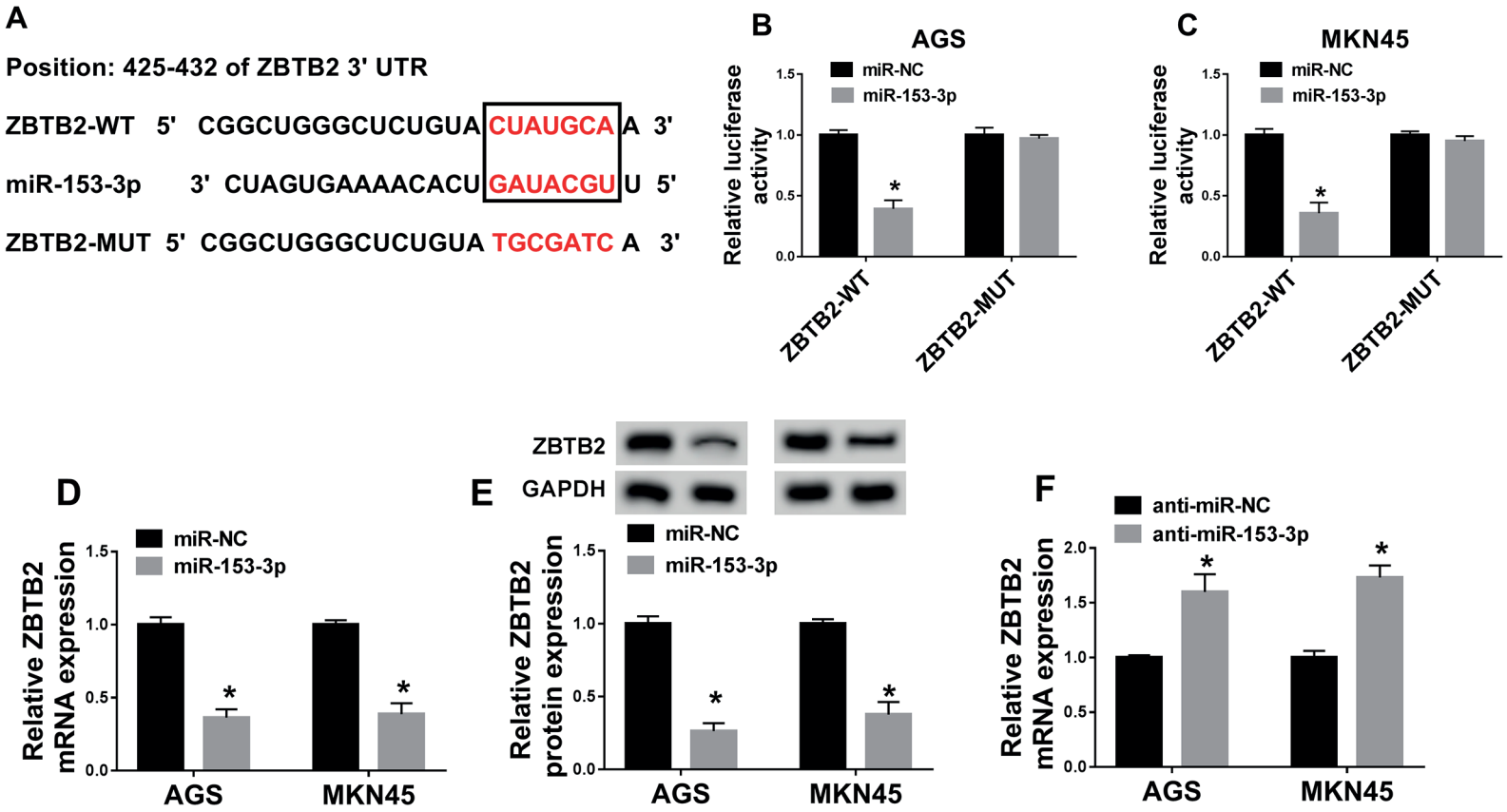

Figure 4. ZBTB2 was directly targeted by miR-153-3p. **A,** The complementary sequences between miR-153-3p and ZBTB2. **B,** and **C,** Dual-Luciferase reporter analysis for the luciferase activity of ZBTB2-WT or ZBTB2-MUT in GC cells under miR-153-3p overexpression. **D-G,** QRT-PCR and western blot analyses for the mRNA and protein levels of ZBTB2 under miR-153-3p overexpression or detection in AGS and MKN45 cells. **H-K,** Expression levels of ZBTB2 in GC tissues and cell lines at the aspects of mRNA and protein. **L,** QRT-PCR analysis for the correlation between miR-153-3p level and ZBTB2 level. *$p<0.05$.

Figure continued

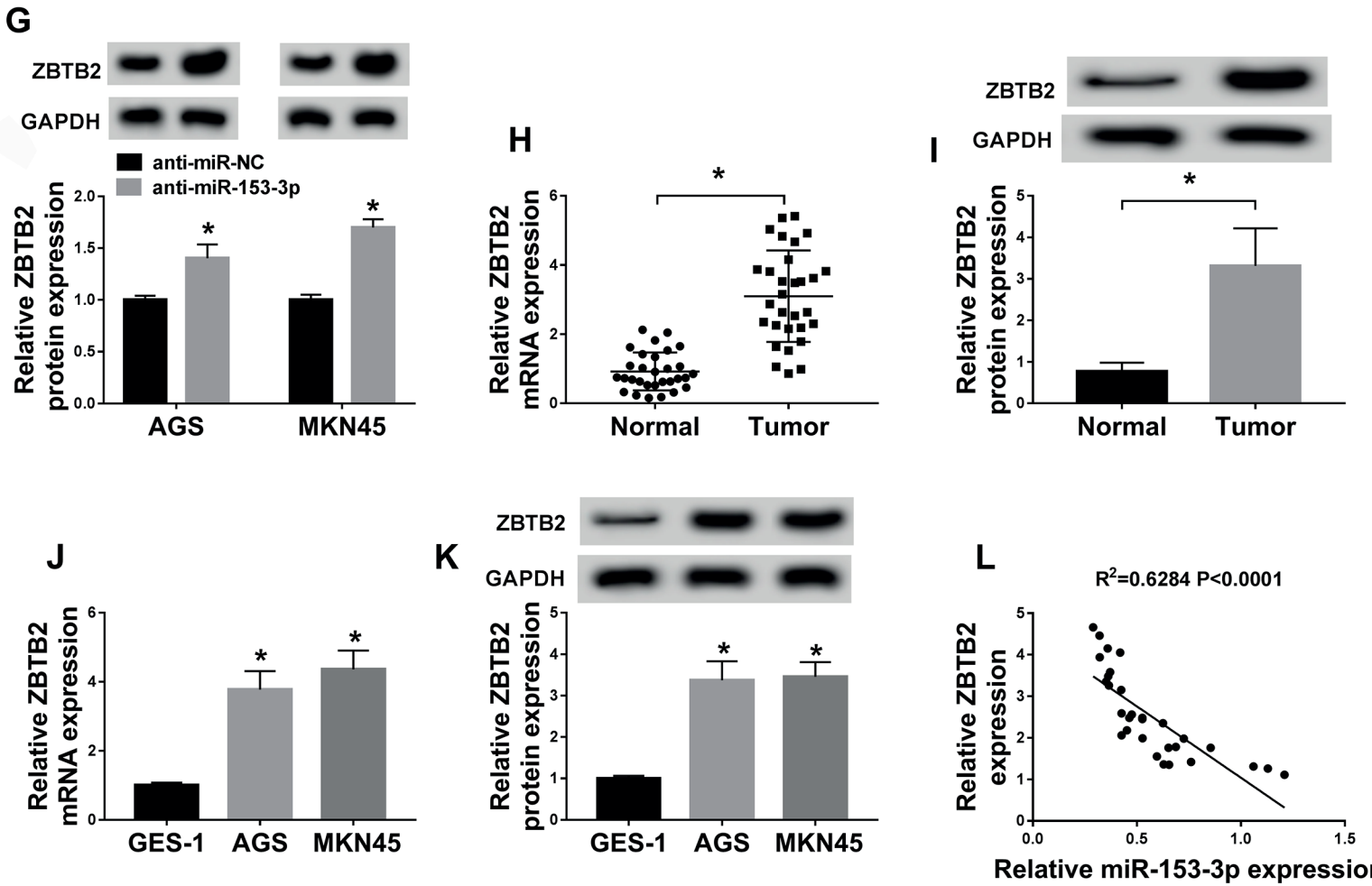

Figure 4 ***(Continued).*** ZBTB2 was directly targeted by miR-153-3p. **A,** The complementary sequences between miR-153-3p and ZBTB2. **B,** and **C,** Dual-Luciferase reporter analysis for the luciferase activity of ZBTB2-WT or ZBTB2-MUT in GC cells under miR-153-3p overexpression. **D-G,** QRT-PCR and western blot analyses for the mRNA and protein levels of ZBTB2 under miR-153-3p overexpression or detection in AGS and MKN45 cells. **H-K,** Expression levels of ZBTB2 in GC tissues and cell lines at the aspects of mRNA and protein. **L,** QRT-PCR analysis for the correlation between miR-153-3p level and ZBTB2 level. **p*<0.05.

Effect of MiR-153-3p on Cell Behaviors Was Abolished by ZBTB2 Upregulation in GC Cells

To seek whether ZBTB2 was related to the regulation of miR-153-3p on cell behaviors, miR-NC, miR-153-3p, miR-153-3p+pcDNA or miR-153-3p+ZBTB2 was introduced into AGS and MKN45 cells. Firstly, the level of ZBTB2 was determined, and qRT-PCR and Western blot analyses implied that the mRNA and protein levels of ZBTB2, conspicuously weakened by miR-153-3p mimic, were recovered after co-transfection with ZBTB2 in GC cells (Figure 5A-5D). Through MTT assay, we discovered that the inhibiting effect of miR-153-3p mimic on cell proliferation was rescued *via* co-transfecting with ZBTB2 in AGS and MKN45 cells (Figure 5E and 5F). Moreover, miR-153-3p caused the acceleration of cell apoptosis, and such a result could be partially abolished by the ectopic upregulation of ZBTB2 in both GC cells (Figure 5G and 5H). Synchronously, the reintroduction of ZBTB2 could relieve the repressive effects of miR-153-3p mimic on the capacities of cell migration and invasion in both AGS and MKN45 cells (Figure 5I-5L). In sum, the overexpression of ZBTB2 could abrogate the effects of miR-153-3p mimic on cell behaviors in GC cells.

ZBTB2 Was Co-Regulated by OIP5-AS1 and MiR-153-3p

In order to expound the regulatory mechanism between ZBTB2 and miR-153-3p or OIP5-AS1, OIP5-AS1 alone or OIP5-AS1 plus miR-153-3p was introduced into AGS and MKN45 cells. qRT-PCR analysis elucidated that the promoting effect of OIP5-AS1 transfection on ZBTB2 mRNA level was overturned *via* co-introduction of miR-153-3p in AGS and MKN45 cells (Figure 6A and 6B). Also, the similar phenomenon was observed at the protein level of ZBTB2 (Figure 6C and 6D). These descriptions confirmed that OIP5-AS1 and miR-153-3p could co-regulate ZBTB2 level in both GC cells.

OIP5-AS1 Silencing Suppressed Tumor Growth In Vivo

AGS cells with OIP5-AS1 detection or control were injected into nude mice subcutaneously. In line with *in vitro* analysis, the tumor volume and weight of the excised tumor were particularly lower in treatment (lentivirus-mediated sh-OIP5-AS1) group as compared with negative control (Figure 7A and 7B). Moreover, an effective lower level of OIP5-AS1 was observed in sh-OIP5-AS1-mediated group (Figure 7C). Knockdown of OIP5-AS1 could markedly elevate miR-153-3p level in xenograft tumors (Figure 7D). Apart from that, the mRNA and protein levels of ZBTB2 were effectively restrained in transplanted tumors (Figure 7E and 7F). In brief, OIP5-AS1 deficiency could lead to the decrease of tumor growth *in vivo*.

Discussion

The study supplied evidence that OIP5-AS1 and ZBTB2 were expressed at high levels, but miR-153-3p was expressed at a low level in GC tissues. The similar expression patterns of OIP5-AS1, miR-153-3p, and ZBTB2 was also observed in GC cell lines (AGS and MKN45). Besides, the knockdown of OIP5-AS1 could strengthen cell apoptosis, retard proliferation, migration, and invasion in AGS and MKN45 cells.

There is a wide consensus that lncRNAs are associated with the malignant progression of numerous tumorigenesis[24-26]. However, the regulatory mechanisms of lncRNAs are extremely complicated as compared with the well-documented RNAs. We have indicated that lncRNAs are implicated in DNA, RNA and so on, thereby modulating the local gene expression[27]. LncRNAs are involved in a series of biological processes, including transcriptional regulation, post-transcriptional regulation chromatin interaction, and epigenetic regulation[28-30]. Firstly, we explored that OIP5-AS1 was aberrantly expressed in GC tissues and cell lines as compared with the match controls. Therefore, we hypothesized that OIP5-AS1 was strictly involved in the pathogenesis of GC. Subsequent assays presented that OIP5-AS1 deficiency particularly hindered cell growth, showing as the repression of proliferation, migration, invasion, and the acceleration of cell apoptosis in both GC cells. This means that OIP5-AS1 acted as an oncogenic gene in GC progression. This conclusion was in line with previous evidence[12,31]. Especially, OIP5-AS1 could facilitate the tumorigenesis of osteosarcoma *via* miR-223[14]. All the data confirmed that OIP5-AS1 could mediate the growth and metastasis in GC progression. However, whether OIP5-AS1 exerted its role *via* sponging miRNAs was the subsequent target.

Tay et al[32] have indicated that lncRNAs can function as sponge of miRNAs to regulate mRNA genes. Thus, we hypothesized that OIP1-AS1 regulated the progression of GC cells *via* targeting several miRNAs. Then, starBase v3.0 predic-

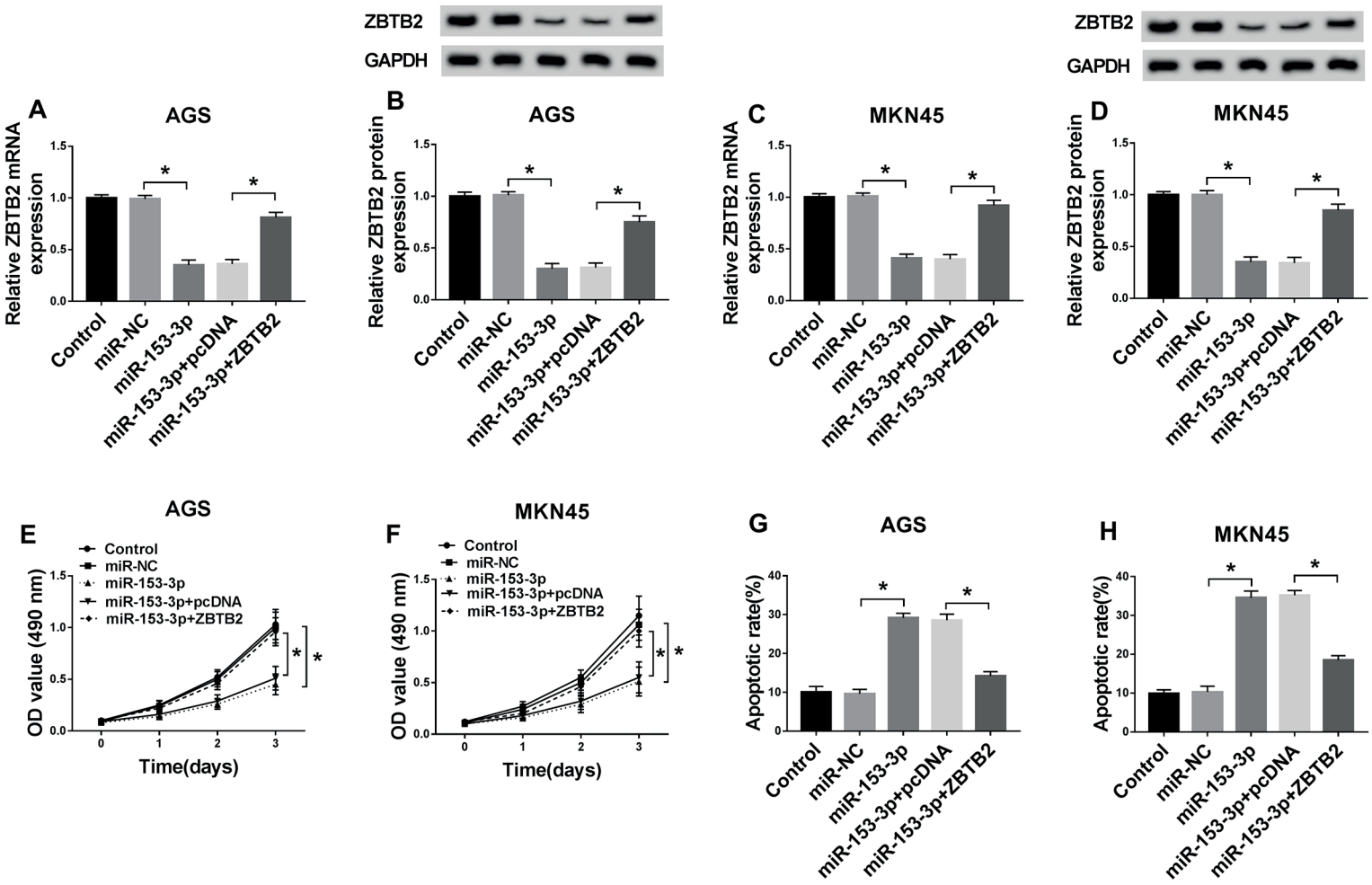

Figure 5. Effect of miR-153-3p on cell behaviors was abolished by ZBTB2 upregulation in GC cells. MiR-NC, miR-153-3p, miR-153-3p+pcDNA or miR-153-3p+ZBTB2 was introduced into AGS and MKN45 cells, respectively. **A-D,** QRT-PCR and Western blot analyses for the mRNA and protein levels of ZBTB2 in AGS and MKN45 cells. **E,** and **F,** The capacity of cell proliferation in GC cells. **G,** and **H,** Roles of miR-153-3p and ZBTB2 in the apoptosis of AGS and MKN45 cells. **I-L,** Transwell analysis for the abilities of cell migration and invasion *in vitro*. *$p<0.05$.

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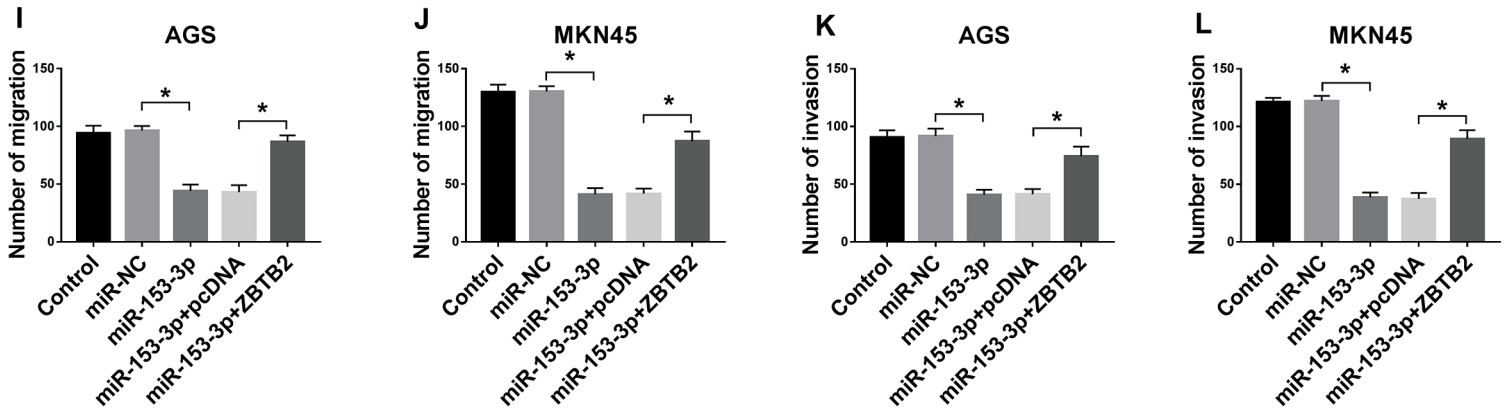

Figure 5 ***(Continued).*** **I-L,** Transwell analysis for the abilities of cell migration and invasion *in vitro.* **p*<0.05.

ulator of reprogramming (ROR), which mediated cisplatin resistance of osteosarcoma by inhibiting proliferation, migration, and invasion[33]. Besides, miR-153-3p also acted as the target gene of cancer susceptibility candidate 15 (CASC15) to modify the development of breast cancer cells[34]. In fact, the targets gene of miR-153-3p were predicted and investigated subsequently.

As mention above, we predicted the common fragments between miR-153-3p and the mRNAs. The results found that ZBTB2 was a potential target of miR-153-3p. By the results of Dual-Luciferase reporter assay, we elucidated that miR-153-3p could directly target ZBTB2. The rescue assays manifested that the effect of miR-153-3p mimic on cell behaviors was regained after reintroduction of ZBTB2 in AGS and MKN45 cells. ZBTB2 has been exposed to modulate the differentiation of embryonic stem cells[35] and accelerate the progression of glioblastoma[36] and GC[37]. In the pres-

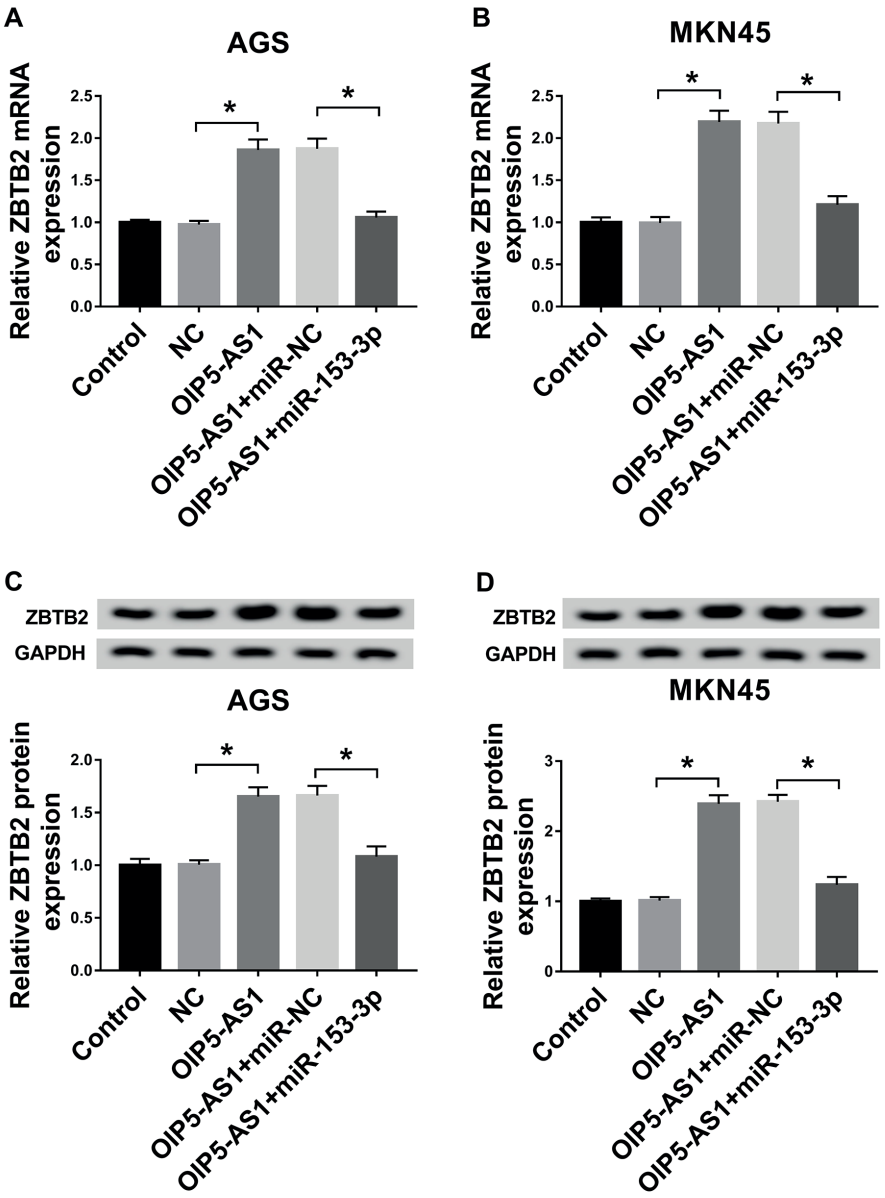

Figure 6. ZBTB2 was co-regulated by OIP5-AS1 and miR-153-3p. AGS and MKN45 cells were transfected with NC, OIP5-AS1, OIP5-AS1+miR-NC or OIP5-AS1+miR-153-3p, respectively. **A,** and **B,** QRT-PCR analysis for the mRNA level of ZBTB2 in GC cells. **C,** and **D,** Impacts of OIP5-AS1 and miR-153-3p on the protein level of ZBTB2 in AGS and MKN45 cells. *$p<0.05$.

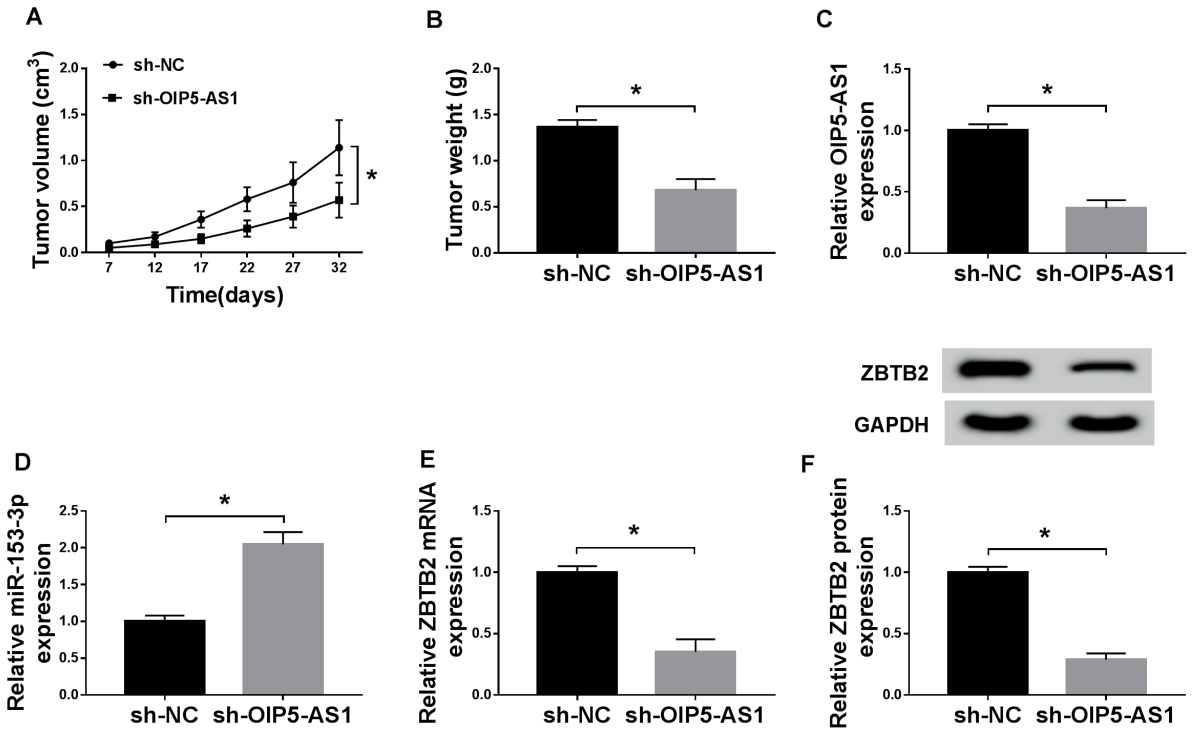

Figure 7. OIP5-AS1 silencing suppressed tumor growth *in vivo*. **A,** Differences in tumor volumes between two groups. **B,** The tumor weights of xenograft tissues. **C-E,** Relative expression levels of OIP5-AS1, miR-153-3p, and ZBTB2 in excised tissues. **F,** Western blot analysis for the protein level of ZBTB2 in the GC xenograft tumors. *$p<0.05$.

ent study, we shed light on the oncogenic role of ZBTB2 in GC progression, which was in keeping with previous studies[37].

Conclusions

Taken together, the expression patterns of OIP5-AS1, miR-153-3p, and ZBTB2 were expounded, exhibiting as the high expression levels of OIP5-AS1 and ZBTB2, and the low level of miR-153-3p in GC tissues and cell lines. Additionally, OIP5-AS1 was a sponge of miR-153-3p to isolate ZBTB2. Functionally, OIP5-AS1 silencing could significantly enhance cell apoptosis, and hamper proliferation, migration, as well as invasion *in vitro*. Apart from that, we also clarified that knockdown of OIP5-AS1 could distinctly hinder the growth of xenograft tumor *via* miR-153-3p/ZBTB2 axis. However, the precise role of OIP5-AS1 needed to be further highlighted.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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