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The lncRNA HOXA11-AS promotes glioma cell growth and metastasis by targeting miR-130a-5p/HMGB2

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Abstract. – OBJECTIVE: Long noncoding RNAs (lncRNAs) serve as important regulators of diverse types of cancer, including glioma. Nevertheless, their precise roles in cancers remain sufficiently unexplored.

PATIENTS AND METHODS: Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was used to determine the levels of HOMEOBOX A11 antisense RNA (HOXA11-AS) and miR-130a-5p in glioma tissues and cell lines. Short hairpin RNAs (shRNAs) targeting HOXA11-AS or pcDNA3.1 were transfected into cells via a vector encoding HOXA11-AS to decrease or increase the level of HOXA11-AS. Cell Counting Kit-8 (CCK-8), colony formation, wound healing, flow cytometry and transwell assays were applied to assess the role of HOXA11-AS in glioblastoma cell growth, apoptosis and aggressiveness. The expression of N-cadherin and E-cadherin was determined using immunofluorescence staining. The expression of high-mobility group protein B2 (HMGB2) was determined using Western blot analysis in vitro and immunohistochemistry (IHC) staining *in vivo*. The direct target of HOXA11-AS and miR-130a-5p was confirmed using the Luciferase reporter assay. Glioblastoma cells were subcutaneously implanted into nude mice to determine the role of HOXA11-AS in tumor growth *in vivo*.

RESULTS: In the current study, we demonstrated that the lncRNA HOXA11-AS was overexpressed in glioma. The overexpression of HOXA11-AS was correlated with advanced stages of glioma and poor prognosis. Downregulating HOXA11-AS expression significantly suppressed the proliferation, migration and invasion of glioma cells and increased their apoptosis. The growth of glioma cells *in vitro* was also suppressed by the downregulation of HOXA11-AS. Finally, we revealed that HOXA11-AS exerted its oncogenic effects by binding to miR-130a-5p, thereby neutralizing the suppressive effect of miR-130a-5p on HMGB2.

CONCLUSIONS: Our results demonstrate that HOXA11-AS regulates the growth and metastasis of glioma by targeting the miR-130a-5p-HMGB2 signaling axis.

Key Words:
LncRNA, HOXA11-AS, MiR-130a-5p, HMGB2, Glioma.

Introduction

Glioma is one of the most aggressive malignant brain cancers and accounts for nearly 80% of all malignant brain cancers[1-3]. The highly infiltrative growth characteristics of glioma make elusive targets for valid operative treatment and are associated with a high rate of relapse. Although advances in treatment options for neuronal oncology are being made, the prognosis of glioma is still unsatisfactory[4]. Hence, there is an urgent need to develop new strategies for the treatment of glioma. Recently, the roles of long noncoding RNAs (lncRNAs) in cancers have attracted sufficient attention[5]. LncRNAs are diverse and poorly conserved RNAs that constitute a large proportion of transcriptomes[6]. Although poorly described, lncRNAS have been proved to participate in cell determination, chromatin modification and RNA alternative splicing[7]. Furthermore, lncRNAs are involved in various brain functions as well as in the pathobiology of glioma[8,9]. Despite comprehensive analysis has indicated that lncRNAs are related to the histological subtypes of glioma and suggests that lncRNAs are potential promoters of glioma development, systematic investigations of the functions of lncRNAs have not been performed[10,11]. Another class of noncoding RNAs that plays a vital role in tumor progression is microRNAs. MicroRNAs control gene degradation or translational repression by binding to the 3'-UTRs of targeted genes[12]. Previous investigations[13-15]

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have proven that the aberrant expression of microRNAs is closely related to the development of glioma, as it regulates various cellular processes, such as cell proliferation, differentiation, apoptosis and metastasis. Moreover, substantive evidence reveals cross-regulation between lncRNAs and microRNAs. In this study, we demonstrated that HOXA11-AS was markedly overexpressed in glioma. Furthermore, downregulating the expression of HOXA11-AS markedly inhibited the growth, migration and invasion of glioma cells. We identified miR-130a-5p as the potential target of HOXA11-AS. HOXA11-AS directly bound to miR-130a-5p and prevented the inhibitory effect of miR-130a-5p on the expression of HMGB2. In conclusion, we identified that HOXA11-AS exerts its oncogenic functions in glioma partly by targeting the miR-130a-5p-HMGB2 signaling pathway.

Materials and Methods

Glioma Cell Lines and Tissues

Forty-three pairs of glioma tissues and corresponding normal brain samples were obtained from patients at the First Affiliated Hospital of Nanchang University from 2009 to 2016 (Nanchang, China). The experimental protocol was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University. Informed consent was obtained from all patients. Normal human astrocytes (NHA) were purchased from ScienCell Research Laboratories (ScienCell, Carlsbad, CA, USA) and cultured under Astrocyte Medium (ScienCell, Carlsbad, CA, USA). U251 and U87MG cells were purchased from Shanghai Institutes for Nanjing Cobioer Biotechnology Co., Ltd. (Nanjing, China) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Wisent, QC, Canada), 100 μg/ml streptomycin and 100 Ui/ml penicillin. All cells were cultured in 5% CO_2 at 37°C.

Cell Transfection

To increase HOXA11-AS expression, a HOXA11-AS complementary DNA (cDNA) was first cloned into the pcDNA3.1 vector (Genechem, Shanghai, China). The short hairpin RNA (shRNA) used for targeting HOXA11-AS as well as the miR-130a-5p mimics, miR-130a-5p inhibitor and control were synthesized by Genechem (Shanghai, China). U251 and U98MG cells were infected with lentivirus and transfected with shRNAs or miRNAs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) for 24 h.

Cell Counting Kit-8 (CCK-8) and Colony Formation Assays

The cells (3000 cells/well) were seeded into a 96-well plate. Cell proliferation was detected using the Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). Optical density (OD) values were measured at 450 nm. For the colony formation assay, cells were seeded into 6-well plates (1000 cells/well) and incubated for two weeks. Then, cell colonies were fixed using 75% alcohol and stained with 1% crystal violet. Cell colonies were counted in five randomly selected fields.

Wound Healing Assay

Cells were cultured in a 6-well plate, and once they reached confluence, the cell monolayer was scratched using a 200 μl sterile pipette tip. Cell debris was removed using PBS, and the plates were cultured for 48 h. Cell gaps were photographed at 0 h and 48 h[16].

Transwell Invasion Assay

Glioma cells were cultured in the medium without FBS for 16 h. Next, 5×10^4 cells were plated into the upper chamber of the BD BioCoat tumor invasion system (BD Biosciences, Franklin Lakes, NJ, USA), and 20% FBS was then added to the lower chamber. After 24 h, glioma cells in the upper chamber were cleared, and the cells that had invaded the membrane were stained with crystal violet. The number of invaded glioma cells on the membrane was counted in five randomly selected fields[17].

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) Assay

RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Briefly, cDNA was synthesized using the Maxima First Strand cDNA Synthesis Kit (Sangon Biotech, Shanghai, China). qRT-PCR was conducted using the ABI 7500 RT-PCR system (Applied Biosystems, Foster City, CA, USA) with the SYBR Premix Ex Taq kit (Sangon Biotech, Shanghai, China). The primer sequences for qRT-PCR were as follows (forward and reverse, respectively): HOXA11-AS, 5'-TGCCAAGTTGTACTTACTACGTC-3' and 5'-GTTGGAGGAGTAGGAGTATGTA-3'; HMGB2, 5'-GCTCGTTATGACAGGGAGATG-3' and

5'-TTGCCCTTGGCACGGTATG-3'; E-cadherin, 5'-ATTTTTCCCTCGACACCCGAT-3' and 5'-TCCCAGGCGTAGACCAAGA-3'; N-cadherin, 5'-AGCTCCATTCCGACTTAGACA-3' and 5'-CAGCCTGAGCACGAAGAGTG-3'; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'- ATGGGACGATGCTGGTACTGA -3' and 5'-TGCTGACAACCTTGAGTGAAAT -3'.

Luciferase Reporter Assay

The wild-type (wt) or mutant (mut) 3'-UTR regions of HMGB2 and HOXA11-AS were cloned into the pmiRGLO Luciferase reporter vector (Promega, Madison, WI, USA). 293K cells were seeded into 24-well plates and transfected with pmiRGLO empty vector, pmiRGLO-HOXA11-AS-wt, pmiRGLO-HOXA11-AS-mut, pmiRGLO-HMGB2-wt or pmiRGLO-HMGB2-mut utilizing the Effectene transfection reagent (Qiagen, Hilden, Germany) or miRNA-130-5p mimics. After 24 h, both the Firefly Luciferase activity and Renilla Luciferase activity were measured using the Dual-Luciferase assay system (Promega, Madison, WI, USA). The Firefly Luciferase activity was normalized to that of Renilla Luciferase as an internal control.

Immunofluorescence Staining

Cells were fixed with 4% paraformaldehyde for 30 min and permeabilized with a 5% blocking solution. The cells were then incubated with an anti-N-cadherin antibody or an anti-E-cadherin antibody overnight at 4°C. Following a Phosphate-Buffered Saline (PBS) wash, the cells were incubated with a goat anti-rabbit IgG DyLight 488 antibody for 2 h. Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Images were captured with a fluorescence microscope (IX71, Olympus Corporation, Tokyo, Japan).

Immunoblotting Assay

Total proteins from cells were extracted using a lysis buffer (Roche, Basel, Switzerland) and 25 μg of protein were separated by 8% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred onto polyvinylidene difluoride (PVDF) membranes, which were subsequently incubated with antibodies (HMGB2 or GAPDH). The PVDF membranes were incubated with horseradish peroxidase-conjugated secondary antibodies. Bands were detected using an electrochemiluminescence (ECL) system (Pierce, Waltham, MA, USA).

Apoptosis Assay

Cell apoptosis was detected using the Annexin V- Fluorescein Isothiocyanate (FITC) apoptosis detection kit (Beyotime Biotechnology, Shanghai, China). Cells were trypsinized, collected and then stained with Annexin V-FITC and propidium iodide (PI) for 15 min. Cell apoptosis was analyzed immediately on the BD FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Transplanted Tumor Model

All animal experiments were approved by the Animal Care and Use Committee (ACUC) of the First Affiliated Hospital of Nanchang University (Nanchang, China). For survival analysis, 5×10^5 glioblastoma cells were subcutaneously injected into nude mice. After five weeks, the animals were sacrificed, and the tumors were collected. The expression of HMGB2 in the tumor tissues was detected by immunohistochemistry.

Statistical Analysis

GraphPad Prism (La Jolla, CA, USA) was used for data analysis. Data are expressed as the mean ± standard deviation (SD). Student's *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was utilized to analyze the differences between the two groups. $p < 0.05$ was considered statistically significant.

Results

HOXA11-AS is Upregulated in Glioma

To identify potential lncRNAs that are aberrantly expressed in glioma, the expression patterns of miRNAs between normal and glioma tissues were compared using the GEO data set GSE104267. The heat map generated by differentially expressed genes revealed that HOXA11-AS was remarkably upregulated in glioma (Figure 1A). Moreover, the level of HOXA11-AS was detected in 43 pairs of glioma tissues and noncancerous tissues *via* qRT-PCR and found to be remarkably lower in glioma tissues than in noncancerous tissues (Figure 1B). In addition, statistical analysis of glioma tissues suggested that the level of HOXA11-AS was closely related to the stage of glioma advancement (Figure 1C). The expression of HOXA11-AS was also measured in normal human astrocytes (NHA) and four glioma cell lines (Figure 1D). Finally, the Kaplan-Meier survival analysis suggested that the lower level of HOXA11-AS was related to poor survival (Figure

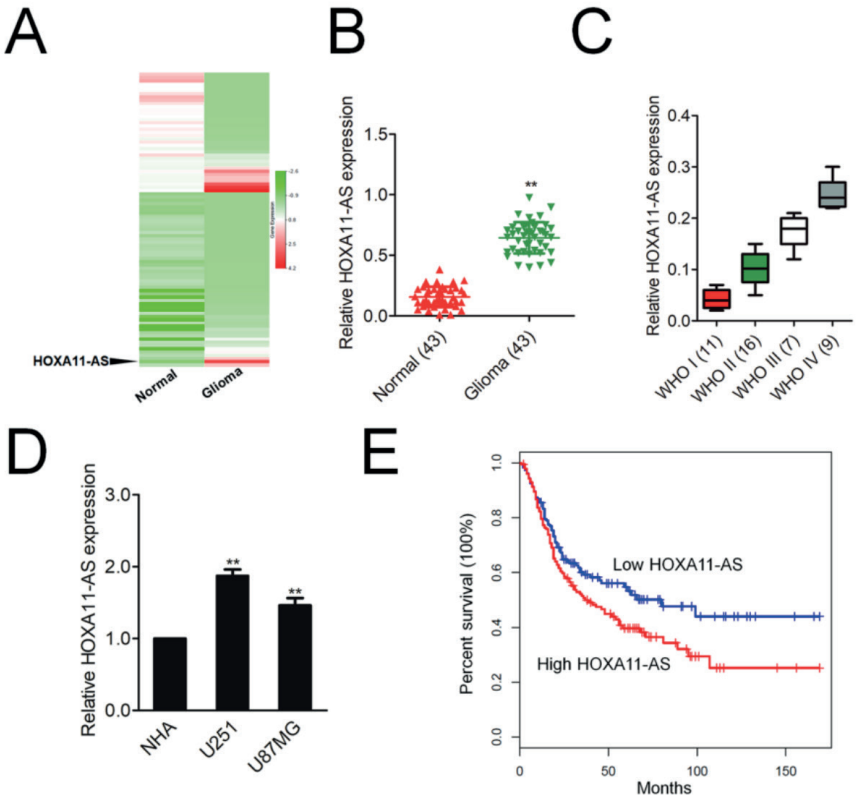

Figure 1. HOXA11-AS was down-expressed in glioma tissues. ***A,*** Microarray analysis of lncRNAs dysexpression in glioma tissues corresponding normal tissues. ***B,*** The expression of HOXA11-AS in glioma and matched normal tissues was analyzed by using qRT-PCR. $^{**}p < 0.01$ compared to normal. ***C,*** The level of HOXA11-AS in patients of different stages was detected via qRT-PCR. ***D,*** The expression of HOXA11-AS in glioma cell lines was analyzed by using qRT-PCR assay. $^{**}p < 0.01$ compared to NHA. ***E,*** Kaplan-Meier survival curves of patients with different level of HOXA11-AS.

1E). These results suggest that HOXA11-AS might serve as a suppressor of glioma.

HOXA11-AS Affects the Viability of Glioma Cells

To explore whether HOXA11-AS is involved in gliomagenesis, a short hairpin RNA (shRNA) targeting HOXA11-AS (sh-HOXA11-AS) was transfected into the glioma cell lines U87MG and U251. The transfection efficiency was determined by qRT-PCR (Figure 2A). Then, CCK-8 assays were employed to assess the impact of HOXA11-AS on the viability of glioma cells. Cell proliferation was significantly suppressed when HOXA11-AS was downregulated (Figure 2B). Consistently, the colony formation of U87MG and U251 cells was suppressed by shHOXA11-AS (Figure 2C). To explore whether the inhibitory effect of HOXA11-AS on cell proliferation was a result of sh-HOXA11-AS-induced apoptosis, a flow cytometry assay (Annexin V and PI staining) was conducted. As shown in Figure 2D, the apoptosis was significantly increased when HOXA11-AS was downregulated in glioma cells. Altogether, these findings suggest that the downregulation of HOXA11-AS inhibits the proliferative ability of glioma cells and induces apoptosis.

Downregulating the Expression of HOXA11-AS Inhibits the Migration and Invasion of Glioma Cells

To explore whether HOXA11-AS contributes to glioma cell migration and invasion, the wound healing and transwell assays were applied to both U87MG and U251 cells. As shown in Figure 3A-3B, the migration and invasion abilities of cells that were transfected with sh-HOXA11-AS were significantly inhibited when compared to that of control cells. Given that the downregulated expression of HOXA11-AS inhibited the migration and invasion of glioma cells, we next explored the impact of HOXA11-AS on the epithelial-mesen-

chymal transition (EMT) process of glioma cell. The mRNA levels of EMT markers were detected by qRT-PCR. We found that the expression of the epithelial marker E-cadherin was significantly higher in HOXA11-AS knockdown cells than in control cells, whereas the expression of the mesenchymal marker N-cadherin was markedly decreased (Figure 3C). Immunofluorescence assays also consistently demonstrated that HOXA11-AS knockdown increased the protein expression of E-cadherin and inhibited N-cadherin expression in glioma cells (Figure 3D).

Reciprocal Repression Exists Between HOXA11-AS and MiR-130a-5p

Next, the miRanda and starBase 2.0 online analysis tools were used to predict four potential miRNA targets of HOXA11-AS. We assessed the levels of these miRNAs in glioma cells that were transfected with sh-HOXA11-AS; among these miRNAs analyzed, miR-130a-5p was the most upregulated (Figure 4A). Meanwhile, when miR-130a-5p was transfected into U87MG and U251 cells, the level of HOXA11-AS was markedly downregulated. Consistently, the miR-130a-5p inhibitor significantly increased the expression of HOXA11-AS (Figure 4B), which suggests a reciprocally repressive relationship between miR-130a-5p and HOXA11-AS. Utilizing target prediction analysis, the binding sites between miR-130a-5p and HOXA11-AS were found and are shown in Figure 4C. To prove this relationship, we constructed Luciferase reporters containing wild-type (wt) or mut HOXA11-AS and then per-

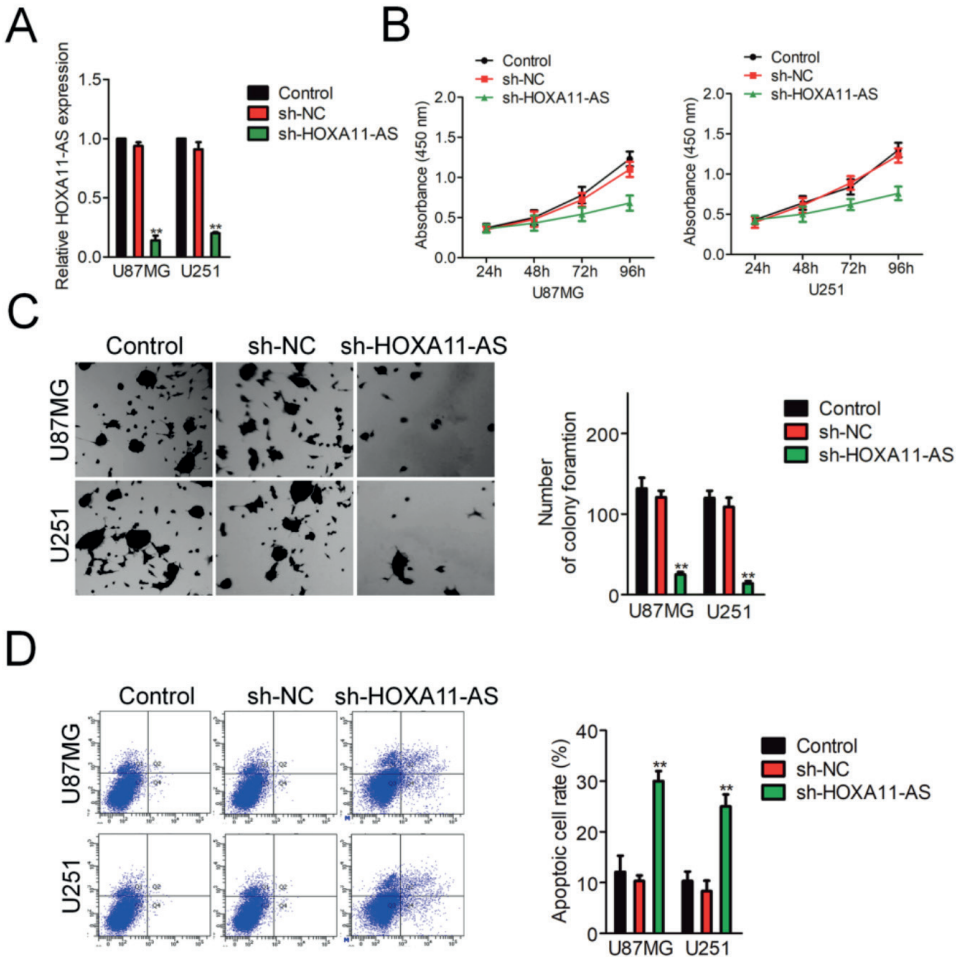

Figure 2. Effects of HOXA11-AS on the proliferation and apoptosis of glioma cells. ***A,*** U87MG and U251 cells were transfected with sh-HOXA11-AS and the expression of HOXA11-AS was determined by qRT-PCR assay. ***B,*** U87MG and U251 cell was transfected with sh-HOXA11-AS. The growth of the infected cell was detected by using CCK-8 assay. ***C,*** Colony formation. ***D,*** Apoptosis of cell was determined by with Annexin V and PI staining followed by flow cytometry assay. $^{**}p < 0.01$ compared with control.

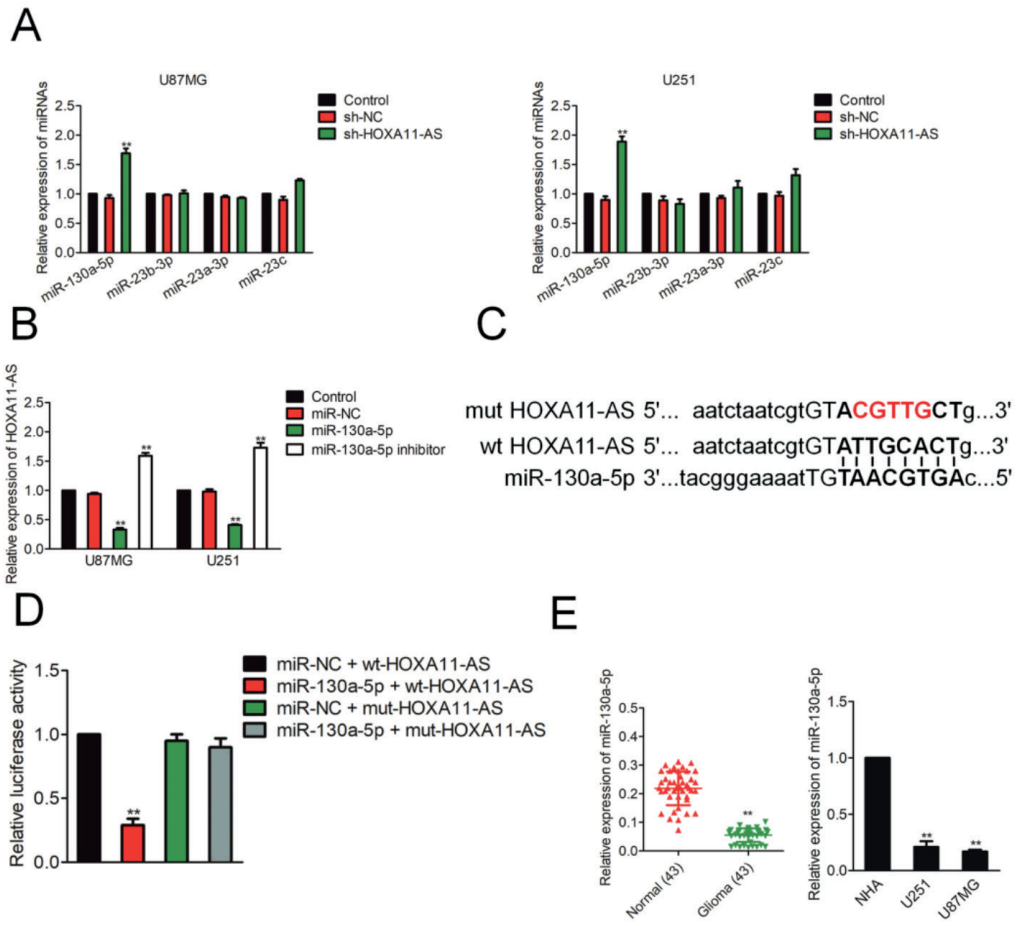

Figure 3. Effects of HOXA11-AS on the migration, invasion and EMT of glioma cell. ***A,*** U251 and U87MG cells were transfected with sh-HOXA11-AS. Cell migration was determined by wound healing assay. ***B,*** Cell invasion was determined by transwell assay. ***C,*** The mRNA levels of E-cadherin and N-cadherin were measured by qRT-PCR assay. ***D,*** The protein expressions of E-cadherin and N-cadherin were detected by immunofluorescence staining. $^{**}p < 0.01$ compared with control.

formed Luciferase assays. As shown in Figure 4D, miR-130a-5p lost its suppressive effects upon mutation of the binding sites. Next, we measured the level of miR-130a-5p in 133 glioma tissues and glioma cells (U87MG and U251). The qRT-PCR assay indicated that miR-130a-5p was markedly downregulated in the glioma tissues compared with that in adjacent normal tissues and downregulated in glioma cells compared to that in normal astrocytes (Figure 4E). These findings support the existence of a negative correlation between HOXA11-AS and miR-130a-5p in glioma.

HOXA11-AS Compensates the Negative Effects of MiR-130a-5p on Glioma Cells

To investigate whether miR-130a-5p reverses the effects of downregulated HOXA11-AS in glioma cells, we assessed the effects of miR-130a-5p on glioma cell growth, migration and invasion. The viability and colony formation ability of U87MG cells transfected with miR-130a-5p were markedly inhibited (Figure 5A-5B). Meanwhile, apoptosis was greatly increased upon miR-130-5p transfection (Figure 5C). In addition, the upregulation of miR-130a-5p suppressed the migration and invasion of glioma cells as well as alternated the expression of EMT-related genes (Figure 5D-5F). Importantly, when HOXA11-AS was overexpressed in glioma cells that were transfected with miR-130a-5p mimics, the inhibitory impact of miR-130a-5p was significantly reversed (Figure 5A-5F). Similar results were observed in U251 glioma cells (data not shown), which prove the suppressive role of HOXA11-AS on the functions of miR-130a-5p.

HOXA11-AS Regulates the Expression of HMGB2

By utilizing the online bioinformatics analysis tools starBase 2.0, RNA hybrid and miRanda, we identified that the HMGB2 gene is the target of miR-130a-5p (Figure 6A). To elucidate whether the HMGB2 gene is the functional target of miR-130a-5p, Luciferase reporter assays were applied. As shown in Figure 6B, the Luciferase activity of wild-type HMGB2 was significantly inhibited when miR-130a-5p was transfected into 293K cells, whereas the Luciferase activity of mutant HMGB2 was not inhibited. Based on the above results, we assumed that HOXA11-AS regulated the expression of HMGB2 *via* miR-130a-5p as a competing endogenous RNA (ceRNA). Next, we investigated whether the level of HMGB2 is controlled by HOXA11-AS. Immunoblotting analysis indicated that downregulating HOXA11-AS markedly decreased the level of HMGB2 in U87MG cells, whereas the overexpression of HOXA11-AS increased the expression of HMGB2 (Figure 6C). In addition, after HOXA11-AS was transfected into U87MG cells, its impact on HMGB2 was neutralized by miR-130a-5p (Figure 6D). Finally, we knocked down HOXA11-AS in U87MG cells. The decreased level of HMGB2 induced by the downregulation of HOXA11-AS was attenuated by miR-130a-5p inhibitor transfection (Figure 6E). These findings suggest that the oncogenic role of HOXA11-AS is partly dependent on the miR-130a-5p-HMGB2 axis.

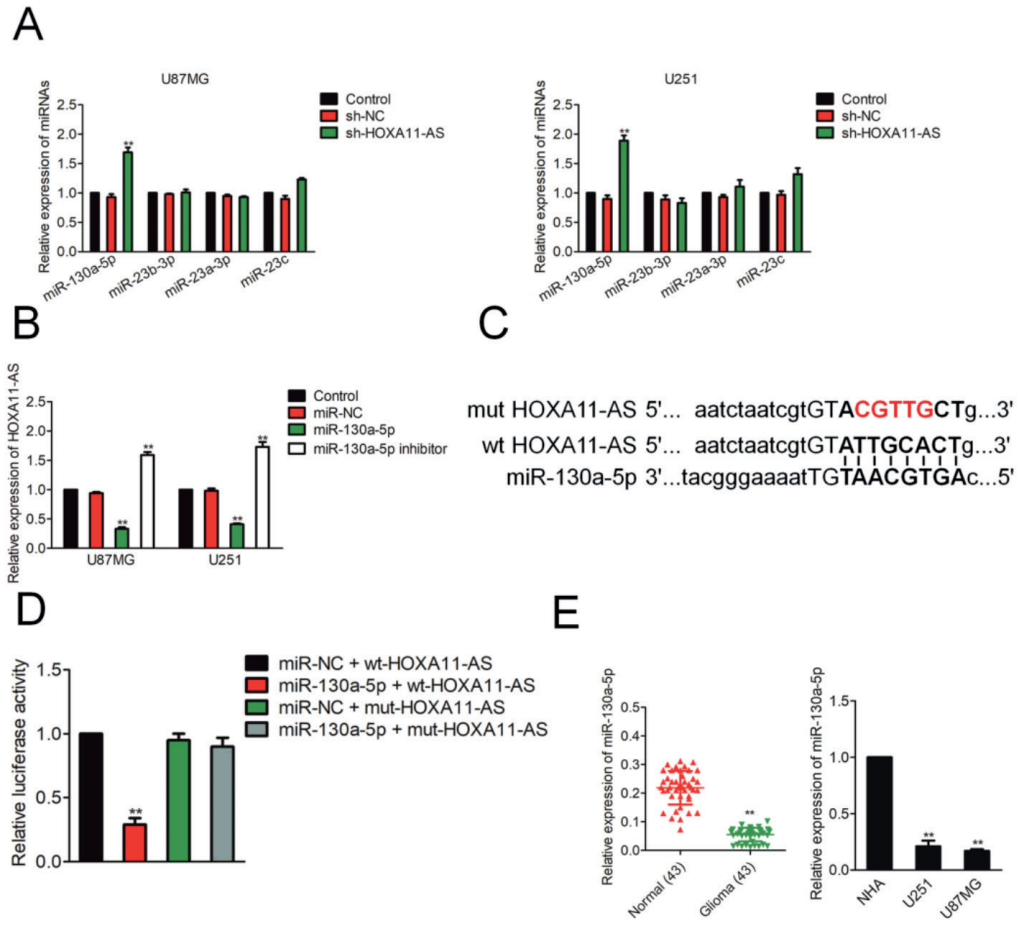

Figure 4. MiR-130a-5p is the target of HOXA11-AS. ***A,*** Glioma cells were transfected with sh-HOXA11-AS. The levels of four potential miRNAs were determined by qRT-PCR. ***B,*** Glioma cells were transfected with miR-130a-5p or inhibitor. The levels of HOXA11-AS were detected by qRT-PCR. ***C,*** The binding sites between HOXA11-AS and miR-130a-5p were predicted. $^{**}p < 0.01$ compared with control. ***D,*** Luciferase reporter assay. $^{**}p < 0.01$ compared with miR-NC + wt-HOXA11-AS. ***E,*** The expression of miR-130a-5p in glioma tissues and two glioma cell lines were determined by qRT-PCR. $^{**}p < 0.01$ compared with normal or NHA.

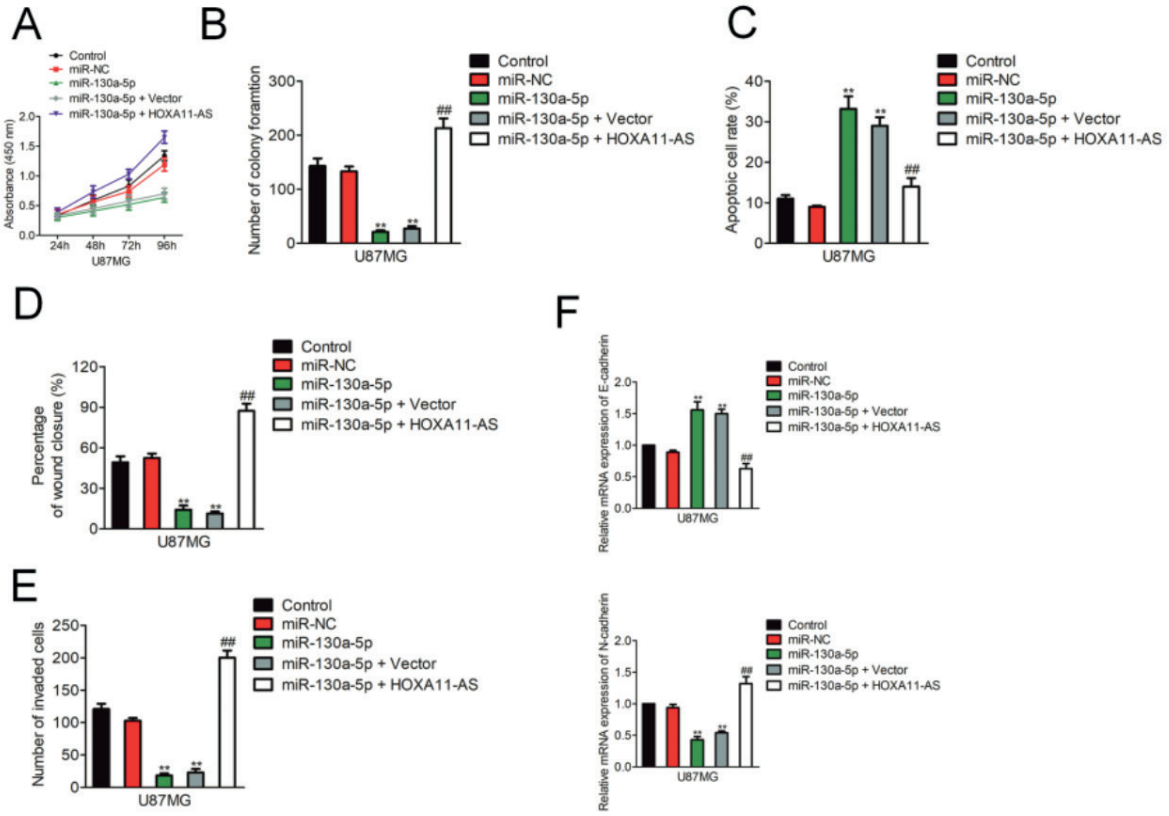

Figure 5. The suppressive roles of HOXA11-AS on the functions of miR-130a-5p. ***A,*** Glioma cells were transfected with miR-130a-5p alone or together with HOXA11-AS. ***B,*** Colony formation assay. ***C,*** Apoptosis were determined by using Annexin V and PI staining. ***D,*** The migration of cell was detected by the wound healing assay. ***E,*** Invasion of glioma cells was detected by transwell assay. ***F,*** U87MG glioma cell was transfected with miR-130a-5p. The expression of E-cadherin and N-cadherin was measured by qRT-PCR assay. $^{**}p < 0.01$ compared with control; $^{\#\#}p < 0.01$ compared with miR-130a-5p.

Downregulation of HOXA11-AS in Combination with Upregulation of MiR-130a-5p Suppresses Glioma Cell Tumor Growth In vivo

To investigate the role of HOXA11-AS and miR-130a-5p in the tumorigenic capability of glioma cells *in vivo*, U87MG cells transfected with sh-HOXA11-AS were subcutaneously implanted into nude mice. As shown in Figure 7A, the tumor growth of glioma cells was significantly inhibited by the downregulation of HOXA11-AS, and the tumor weight was markedly decreased. Similar results were obtained in the xenograft model when miR-130a-5p was transfected into U87MG cells. Most strikingly, tumors comprising U87MG cells cotransfected with sh-HOXA11-AS and miR-130a-5p exhibited the smallest tumor volume and weight, supporting the overlaid effects of downregulating HOXA11-AS and overexpressing miR-130a-5p (Figure 7A-7C). Next, we performed immunohistochemistry analysis of tumor tissues with an antibody against HMGB2. Compared to the higher HMGB2 immunostaining detected in tumor tissue formed by the cells transfected with sh-NC, the sh-HOXA11-AS- or miR-130a-5p-transfected groups exhibited lower levels of HMGB2. As expected, cells cotransfected with sh-HOXA11-AS and miR-130a-5p exhibited the lowest expression of HMGB2 (Figure 7D), indicating the involvement of HOXA11-AS/miR-130a-5p/HMGB2 signaling in the growth of glioma.

Discussion

Glioma is a primary malignant carcinoma of the central nervous system[10]. Recently, investigations into malignant cancer progression have mainly focused on protein-coding genes, while the functions of lncRNAs remain unexplored[18].

Microarray expression profiling analysis demonstrated that HOXA11-AS was significantly overexpressed in glioma tissue compared with that in the corresponding peritumoral tissue. In addition, the level of HOXA11-AS was related to the stages of glioma and inversely correlated with the prognosis of patients. The experimental downregulation of HOXA11-AS significantly suppressed the proliferation, colony formation, migration and invasion abilities of glioma cells while increasing their apoptosis. The tumor growth of glioma cells was also inhibited *in vivo*. We further demonstrated that HOXA11-AS competitively binds miR-130a-5p, which inhibits the suppressive effect of miR-130a-5p on HMGB2 expression. Noncoding RNAs play a crucial role in various biological processes, including cell growth, cell differentiation, cell apoptosis and stress responses[19]. Recently, numerous investigations have revealed the precise underlying mechanism of lncRNAs in tumorigenesis and glioma progression[20,21]. For example, the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) suppresses the proliferation and invasion of glioma cells *via* targeting the extracellular-regulated protein kinase/mitogen-activated protein kinase (ERK/MAPK) signaling axis and activation of matrix metalloproteinase (MMP)[22]. In addition, the lncRNA differentiation-antagonizing nonprotein-coding RNA (DANCR) serves as a competing endogenous RNA to regulate the expression of Ras-related protein Rab-1A (RAB1A) by sponging miR-634 in glioma[23]. Recently, HOXA11-AS was proven to promote gastric cancer cell migration and invasion *in vitro*, but it also pro-

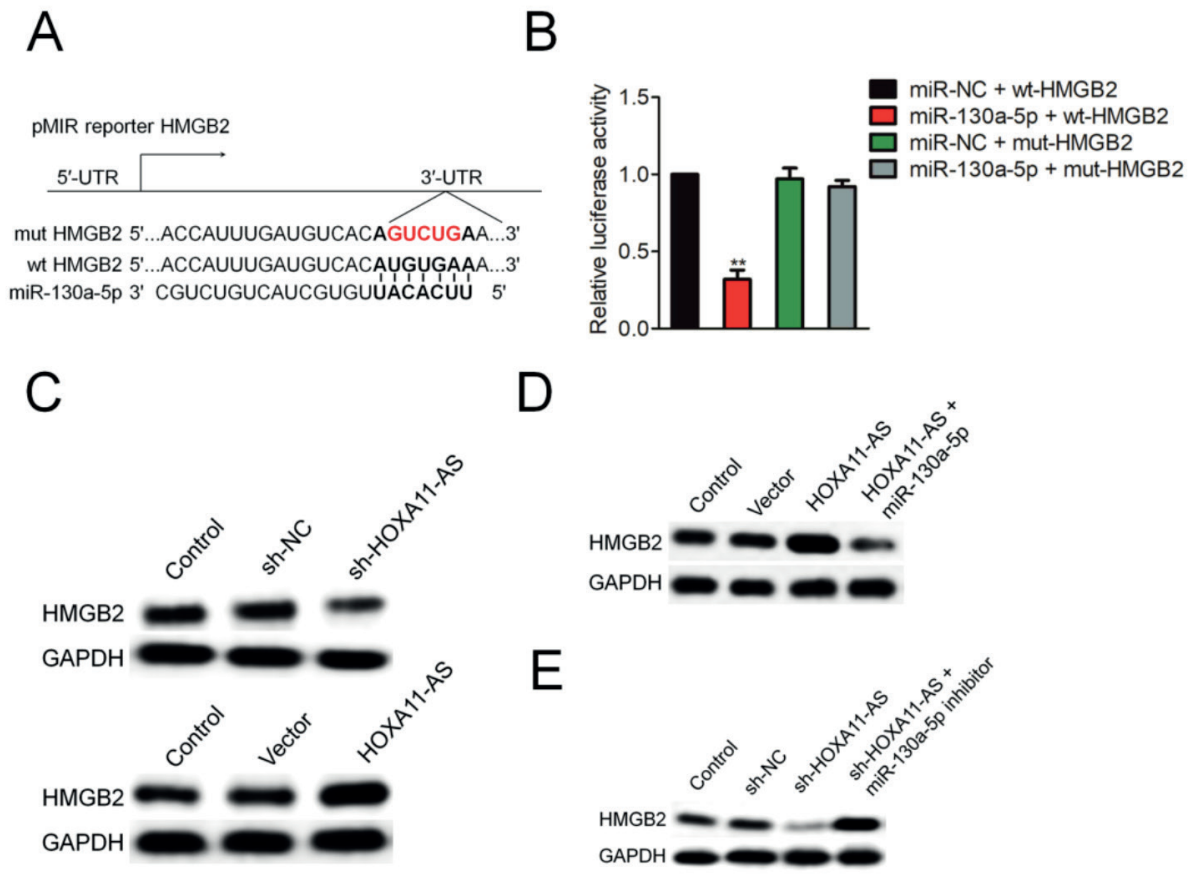

Figure 6. HOXA11-AS regulates the expression of HMGB2 through miR-130a-5p. ***A,*** The binding sites between HMGB2 and miR-130a-5p were shown. ***B,*** Luciferase reporter assay. $^{**}p < 0.01$ compared with miR-NC + wt-HMGB2. ***C,*** U87MG cell was transfected with sh-HOXA11-AS or HOXA11-AS. The expression of HMGB2 was measured by immunoblotting. ***D,*** U87MG cell was transfected with HOXA11-AS alone or cotransfected HOXA11-AS together with miR-130a-5p. The protein expression of HMGB2 was measured by Western blotting. ***E,*** U87MG cell was transfected with sh-HOXA11-AS alone or together with miR-130a-5p inhibitor. The level of HMGB2 was measured by immunoblotting assay.

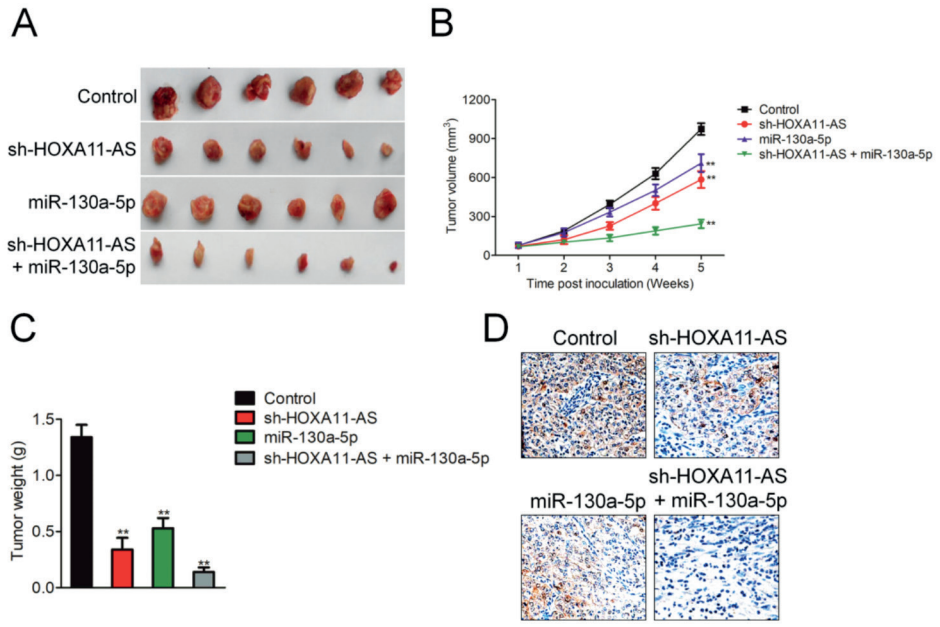

Figure 7. Effects of HOXA11-AS and miR-130a-5p on the growth of glioma cell *in vivo*. ***A,*** U87MG cells were transfected with sh-HOXA11-AS, miR-130a-5p or both and were subcutaneously implanted into nude mice. Representative xenograft tumors after 35 days. ***B,*** Growth curves of xenograft tumors. ***C,*** Tumor weight. ***D,*** Immunohistochemistry staining analysis of the expression of HMGB2 in the xenograft tumor. $^{**}p < 0.01$ compared with control.

motes gastric cancer cell metastasis *in vivo* by regulating β-catenin and Krüppel-like Factor 2 (KLF2)[24]. HOXA11-AS was expressed at relatively higher levels in breast cancer tissue than in adjacent tissue. The overexpression of HOXA11-AS increases the invasion and metastasis of breast cancer cells affecting EMT[25]. Nevertheless, the detailed mechanisms of HOXA11-AS functions in glioma are far from clarified. Utilizing target prediction databases (starBase and Targetscan), we found that miR-130a-5p has binding sites on HOXA11-AS[26]. MiR-130a has been characterized as a biomarker of several cancers[27-29]. For example, miR-130a is remarkably overexpressed in colon and gastric carcinoma[30]. MiRNA-130a promotes colorectal cancer cell proliferation and migration by targeting *Drosophila* mothers against decapentaplegic family member 4 (SMAD4)[31]. Our results suggested the existence of reciprocal repression between HOXA11-AS and miR-130a-5p. The overexpression of miR-130a-5p significantly inhibited the growth, migration and invasion of glioma cells and led to increased apoptosis. However, all these effects of miR-130a-5p were attenuated by HOXA11-AS overexpression. These findings indicated that miR-130a-5p might be involved in HOXA11-AS-regulated gliomagenesis. We also proved that downregulating the expression of HOXA11-AS markedly inhibited the expression of high-mobility group box 2 (HMGB2) in glioma cells. HMGB is ubiquitous and is the second most abundant proteins in humans[32]. Both HMGB1 and HMGB2 are highly conserved and have similar biological functions[33]. HMGB2 has been demonstrated to be involved in several diseases, such as sepsis, arthritis and cancer. The overexpression of HMGB2 has been identified in a multitude of human cancers, including pancreatic cancer, leukemia, hepatocellular carcinoma and breast cancer[34,35]. The overexpression of HMGB2 is closely related to the development and tumor-induced angiogenesis of bladder carcinoma[36]. The siRNA-mediated silencing of HMGB2 increased the sensitivity of head and neck squamous cell carcinoma (HNSCC) cell lines to cisplatin and 5-fluorouracil (5-FU)[37]. Furthermore, the inhibition of HMGB2 has been demonstrated to suppress the migration and invasion of various cancers[38]. Using miRanda and starBase2.0, we identified HMGB2 as the direct target of miR-130a-5p. In addition, the promotional effect of HOXA11-AS on the expression of HMGB2 was reversed by a miR-130a-5p inhibi-

tor, which suggested that HOXA11-AS regulates HMGB2 as a ceRNA.

Conclusions

We showed that HOXA11-AS, which serves as an oncogenic lncRNA, exerts its functions in the growth and metastasis of glioma *via* regulating the miR-130a-5p-HMGB2 axis. These results will provide a new perspective for therapeutic intervention for glioma.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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