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RESEARCH PAPER

LncRNA SOX2-OT regulates miR-192-5p/RAB2A axis and ERK pathway to promote glioblastoma cell growth

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ABSTRACT

Glioblastoma (GBM) is the most frequent tumor in the central nervous system. Long non-coding RNAs (lncRNAs) have been widely accepted as essential participators in cancer progression. Nonetheless, the specific role and mechanism of lncRNA SRY-box transcription factor 2 overlapping transcript (SOX2-OT) in GBM have not been studied. We evaluated expression levels of SOX2-OT, miR-192-5p and Ras-related protein Rab-2A (RAB2A) in GBM cells via qRT-PCR. To investigate the roles of SOX2-OT in GBM cells, CCK-8, JC-1, EdU, and western blot assays were performed. The connection among SOX2-OT, miR-192-5p and RAB2A in GBM cells was explored through pull down, luciferase reporter, and RIP assays. Western blot and qRT-PCR were employed to analyze the activity of extracellular-signal-regulated kinase (ERK) signaling pathway. SOX2-OT expression was higher in GBM cell lines than in normal cells. SOX2-OT knockdown repressed proliferation and promoted apoptosis of GBM cells. Mechanism assays revealed that SOX2-OT could sponge miR-192-5p. Moreover, RAB2A was certified to be the target gene of miR-192-5p. Overexpression of RAB2A reversed the repressive function of SOX2-OT knockdown on GBM cell growth. Furthermore, SOX2-OT activated ERK signaling pathway in GBM cells. SOX2-OT regulated miR-192-5p/RAB2A axis and ERK pathway to promote GBM cell growth.

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Introduction

Glioblastoma (GBM) is the most frequent tumor that occurs in the central nervous system. Its five-year overall survival rate is less than 10% [1]. Significant progress has been made in developing multimodal therapeutic methods for GBM, including temozolomide (TMZ)-based chemotherapy [2], immunotherapy [3], and surgical resection [4]. Despite these efforts, GBM prognosis remains dismal [5]. Consequently, to enhance GBM cure rates, we are in urgent need for a better understanding of molecular mechanisms in GBM.

More than 80% human genomes are transcribed to non-coding RNAs (ncRNAs) [6], in which long non-coding RNAs (lncRNAs) are a kind of ncRNA with a length no less than 200 nucleotides [7,8]. Increasing evidence has manifested that lncRNAs could impact cancer cell biology via multiple mechanisms [9,10]. For example, competitive endogenous RNA (ceRNA) network [11]. In this network, lncRNAs can regulate messenger RNA (mRNA) by competitively sponging microRNA (miRNA) [11,12]. More lncRNAs have been reported to participate in GBM progression in recent years [13–16]. SOX2-OT, short for SRY-box transcription factor 2 (SOX2) overlapping transcript, has been proven to promote osteosarcoma [17], cholangiocarcinoma [18], prostate cancer [19], and other malignancies. Nonetheless, the biological functions of SOX2-OT in GBM have not been identified yet.

MiRNAs consist of short ncRNAs with a length of 18–25 nucleotides [20]. MiRNAs can adjust gene expression by paring with target mRNAs [21,22]. MiR-192-5p has been proven to be an anticancer gene in different types of malignancies, such as bladder cancer [23] and lung cancer [24]. As an mRNA, Ras-related protein Rab-2A (RAB2A), can promote progression of numerous tumors including oral squamous cell carcinoma

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[25], breast cancer [26], and bladder cancer [27]. However, whether miR-192-5p could target RAB2A in GBM cells has not been researched yet.

This study investigated the connection among SOX2-OT, miR-192-5p and RAB2A in GBM cells for the first time. We employed a series of function and mechanism tests to determine whether SOX2-OT sponged miR-192-5p to upregulate RAB2A and further promoted GBM cell growth.

Materials and methods

Cell lines and cell culture

Human astrocyte (HA) and GBM cells (U251, A172, U87, T98G, and GL15) were purchased from Mingzhou Bio (Ningbo, China). Cells grew in Roswell Park Memorial Institute-1640 (RPMI1640; GIBCO, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, Germany) at 37°C with 5% CO_2.

Cell transfection

Small hairpin RNAs (shRNAs) against SOX2-OT (sh-SOX2-OT#1 and sh-SOX2-OT#2), their negative control (NC; sh-NC), miR-192-5p mimics/inhibitor, NC mimics/inhibitor, pcDNA3.1/RAB2A, and pcDNA3.1/NC were synthesized in GenePharma Co., Ltd. (Shanghai, China). Cells were all transfected with the help of Lipofectamine 3000 produced by Invitrogen (Carlsbad, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA extraction was performed with TRIZOL (Invitrogen). The complementary DNA (cDNA) was synthesized by means of the iScriptTM Reverse Transcription Supermix (Bio-Rad, USA). QPCR analysis was achieved using SYBR qPCR Mix (Takara, China) on ABI PRISM7500 system (Applied Biosystems, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the internal reference for SOX2-OT and RAB2A. U6 functioned as the internal reference for miR-192-5p. $2^{-\Delta\Delta Ct}$ method was utilized to evaluate the expression levels.

Cell counting kit-8 (CCK-8) assay

Transfected U87 and U251 cells were incubated in 96-well plates at room temperature with 5% CO_2. 10 ml of CCK-8 reagent (Yeasen Biotech, China) was injected to each well at different hours. After 2-hour incubation, optical density (OD) values were quantified using BioTek Elx800 Absorbance Microplate Reader (USA).

5-Ethynyl-2′-deoxyuridine (EdU) assay

The GBM cell lines were incubated with 50 μM EdU reagent (RiboBio, China), and stained using 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, Germany) dye solution. EdU positive cells were quantified using fluorescence microscope produced by Leica Microsystems (Germany).

JC-1 mitochondrial membrane potential assay

Transfected U87 and U251 cell lines were cultured in a 24-well dish, and incubated at 37°C with 5% CO_2 for 24 hours. Cells were washed by phosphate-buffered saline (PBS) twice. 0.5 ml of diluted JC-1 solution (Abbkine Scientific Co., Ltd., USA) was added. After being washed with PBS twice, cells were counted with the help of the fluorescence microscope.

Western blot assay

Bradford Protein Assay (Bio-Rad, USA) was used to extract and assess proteins from GBM cells. Proteins were isolated with the application of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to Polyvinylidene difluoride filter (PVDF; Sangon Biotech, China) membranes by means of iBlot Dry Blotting Transfer System (Life Technologies Corporation, USA). The membranes were incubated with primary antibodies (Abcam) for one night at 4°C. Then membranes were incubated with secondary antibodies for 2 hours. Protein bands were eventually visualized by means of enhanced chemiluminescence detection reagent (EMD Millipore, Germany), and quantified by ImageJ (version 1.8, NIH). GAPDH was the internal control.

Nuclear and cytoplasmic fraction assay

Cellular localization of SOX2-OT in U87 and U251 cells was identified using PARIS Kit (Thermo Fisher Scientific, USA). U6 was the internal control in nuclei and GAPDH acted as cytoplasmic control.

Fluorescence in situ hybridization (FISH) assay

SOX2-OT probes were synthesized by RiboBio (Guangzhou, China). To detect probe signals, a FISH Kit (RiboBio, China) was utilized. U87 and U251 cells were treated with Hoechst dye solution (Beyotime, China). The fluorescence microscope (Leica, Germany) was used to capture images.

Pull down assay

Five potential miRNAs were biotinylated by Biotin RNA labeling mix (Roche, USA). Sequentially, cell lysates, biotin-coupled RNA complexes, and streptavidin-coated magnetic beads were cultured together. Finally, qRT-PCR was performed to assess RNA enrichment.

Bioinformatics analysis

GEPIA 2 (http://gepia2.cancer-pku.cn/#analysis) was used to obtain the boxplot of SOX2-OT expression in GBM tissue samples. StarBase (http://starBase.sysu.edu.cn/index.php) and LncBase (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=lncbasev2%2Findex-predicted) were used to screen out potential miRNAs for SOX2-OT. To screen out mRNAs that miR-192-5p might target in GBM cells, starBase were utilized. StarBase database was also employed to project binding sites of miR-192-5p and SOX2-OT as well as miR-192-5p and RAB2A.

RNA immunoprecipitation (RIP) assay

Magna RIP Kit (Millipore, USA) was employed to perform RIP experiment. RIP lysis buffer was used to lyse U87 and U251 cell line. Cell lysates were then incubated with magnetic beads combining with anti-Argonaute 2 (anti-Ago2; Cell Signaling Technology, USA) or anti-immunoglobulin G (IgG) (Abcam, USA). RNA abundances were evaluated via qRT-PCR.

Luciferase reporter assay

The mutated (MUT) and wild-type (WT) SOX2-OT were respectively cloned into pmirGLO (Promega Corporation, USA). The synthesized pmirGLO vectors were co-transfected with miR-192-5p mimics or NC mimics into U87 and U251 cells by means of Lipofectamine 3000 (Invitrogen, CA). After 48-hour transfection, luciferase kit (Promega, Madison, WI, USA) was utilized to evaluate relative luciferase activity. Renilla was set as luciferase internal control.

Statistical analysis

Each experiment was conducted at least for three times, and its results were exhibited as mean ± standard deviation (SD). Statistical Product and Service Solutions (SPSS) version 20.0 (IBM, USA) was used to perform data analysis. If there were two groups, data differences were evaluated utilizing student's t-test. One-way analysis of variance (ANOVA) or two-way ANOVA was applied when there were more than two groups. Data discrepancies were defined as significant only when p values were less than 0.05.

Results

SOX2-OT is overexpressed and its knockdown inhibits proliferation and accelerates apoptosis in GBM cells

As we failed to collect enough clinical samples to investigate the expression of SOX2-OT in GBM tissues, we utilized online database GEPIA 2. The searched boxplot confirmed that SOX2-OT was prominently upregulated in GBM tissues in comparison of the corresponding normal tissues (Figure 1(a)). GBM cells (including U251, A172, U87, T98G, and GL15) and HA cells were used to investigate roles of SOX2-OT in GBM. The following qRT-PCR manifested that SOX2-OT expression was higher in GBM cells (especially in U87 and U251) than in normal HA cells (Figure 1(b)).

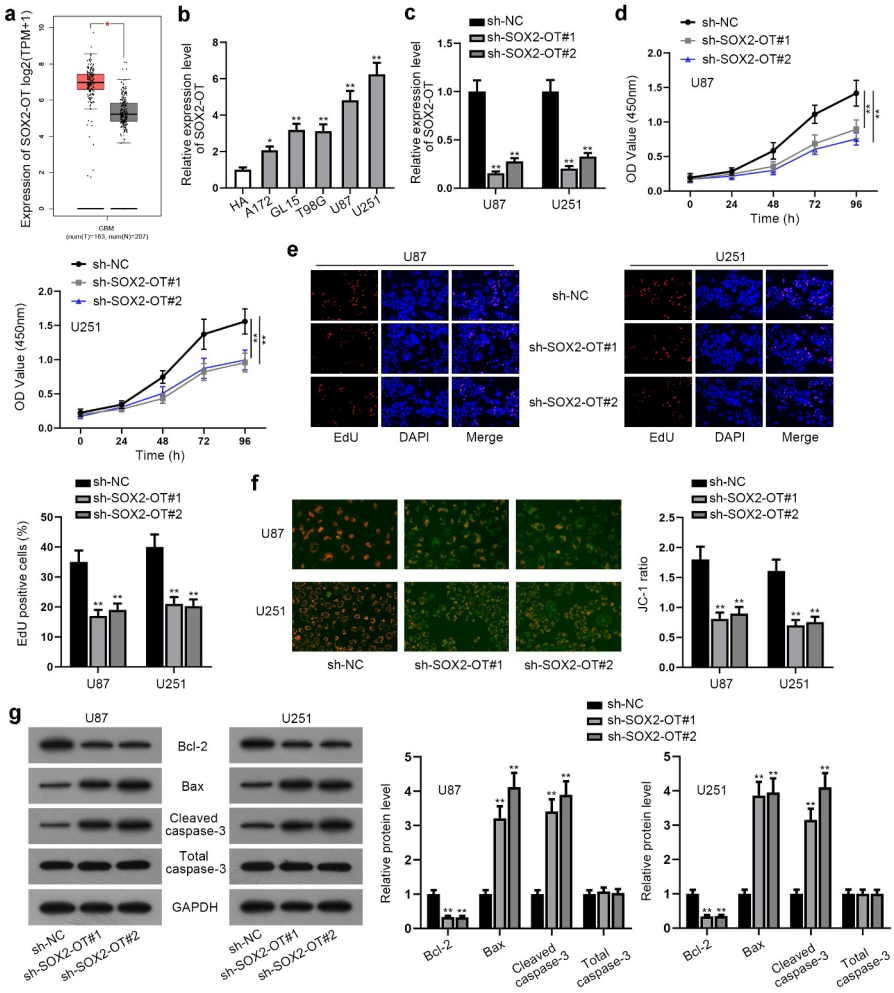

Figure 1. SOX2-OT is overexpressed in GBM cells and its knockdown inhibits proliferation and accelerates apoptosis of GBM cells. A. GEPIA 2 boxplot displayed SOX2-OT expression in GBM tissue samples and normal tissues. B. qRT-PCR was performed to measure SOX2-OT expression in GBM cells (U251, A172, U87, T98G, and GL15) and normal cells (HA). C. qRT-PCR was implemented to examine knockdown efficiency of sh-SOX2-OT#1/2. D, E. CCK-8 and EdU experiments were utilized to analyze proliferation abilities of transfected GBM cells. F. JC-1 experiment was conducted to assess apoptosis of transfected U87 and U251 cells. G. Western blot was applied to evaluate protein level of Cleaved caspase-3, Bax, Total caspase-3, and Bcl2 and GAPDH was the internal control. *P < 0.05, **P < 0.01.

Therefore, SOX2-OT expression in U87 and U251 cells was knocked down in cells by the treatment of sh-SOX2-OT#1 and sh-SOX2-OT#2 (Figure 1(c)). CCK-8 and EdU experiments illustrated that SOX2-OT depletion suppressed cell proliferation (Figure 1(d) and Figure 1(e)). JC-1 experiment revealed that GBM cell apoptosis was increased after SOX2-OT was cut down (figure 1(f)). Based on western blot, protein level of Bcl2 was descended while that of Cleaved caspase-3 and Bax were ascended, implying that SOX2-OTinhibition induced apoptosis of GBM cells (Figure 1(g)). In summary, SOX2-OT is overexpressed and its knockdown inhibits proliferation and accelerates apoptosis in GBM cells.

SOX2-OT binds with miR-192-5p in GBM cells

After exploring the biological function of SOX2-OT in GBM cells, we delved into the regulatory mechanism of SOX2-OT. We applied nuclear and cytoplasmic fraction assay and FISH assay to determine SOX2-OT distribution in GBM cells. Results elucidated that SOX2-OT was largely localized in cytoplasmic GBM cells (Figure 2(a) and Figure 2(b)). Based on current knowledge,

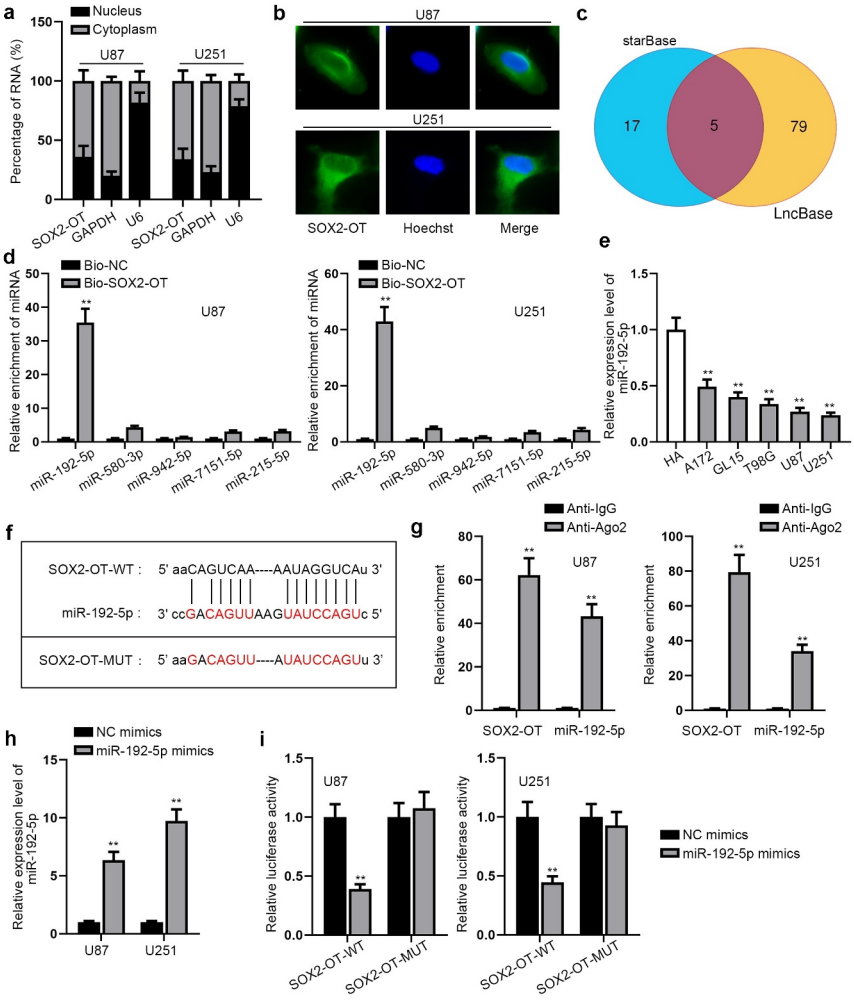

Figure 2. SOX2-OT binds with miR-192-5p in GBM cells.

A. SOX2-OT distribution in U87 and U251 cells was illustrated by nuclear and cytoplasmic fraction assay. B. Data from FISH experiment verified cytoplasmic localization of SOX2-OT in U87 and U251 cells. C. Venn diagram was drawn to screen out qualified miRNAs for SOX2-OT. D. Enrichment of five candidate miRNAs in Bio-SOX2-OT was detected in RNA pull down assay. E. qRT-PCR was carried out to evaluate miR-192-5p expression in U251, HA, A172, U87, T98G and GL15 cell lines. F. Binding sites of miR-192-5p and SOX2-OT in starBase were exhibited. G. RIP experiment was conducted to quantify enrichment of miR-192-5p and SOX2-OT in anti-IgG and anti-Ago2. H. Overexpression efficiency of miR-192-5p mimics was determined using qRT-PCR. I. Luciferase activity of SOX2-OT-WT and SOX2-OT-MUT in U87 and U251 cells was detected through luciferase reporter experiment. **P < 0.01.

increasing lncRNAs have been identified to bind with microRNA (miRNA) as a ceRNA to regulate gene expression of protein-coding mRNAs [11,28]. In this study, we primarily conjectured that SOX2-OT might function as a ceRNA in GBM cells. With the prediction of LncBase (threshold: 0.95) and starBase websites, we screened out five potential miRNAs that possibly had the binding capability with SOX2-OT, namely miR-192-5p, miR-580-3p, miR-942-5p, miR-7151-5p, and miR-215-5p (Figure 2(c)). Compared with other candidate miRNAs in RNA pull down assay, miR-192-5p was notably pulled down by Bio-SOX2-OT in U87 and U251 cells, for which miR-192-5p was decided to involve in the following investigation (Figure 2(d)). Subsequently, expression of miR-192-5p in normal HA cells and GBM cells was measured by qRT-PCR. As shown in Figure 2(e), miR-192-5p showed lower expression in GBM cells than in normal cells. StarBase website identified binding sites of miR-192-5p and SOX2-OT (figure 2(f)). RIP assay manifested that the enrichment of miR-192-5p and SOX2-OT in anti-Ago2 protein group was significantly higher than that in anti-IgG (Figure 2(g)). The valid overexpression effect of miR-192-5p mimics in U87 and U251 cells was

examined through qRT-PCR (Figure 2(h)). It was testified by luciferase reporter assay that miR-192-5p mimics lowered relative luciferase activity of SOX2-OT-WT in U87 and U251 cells (Figure 2(i)). In brief, SOX2-OT combines with miR-192-5p in GBM cell lines.

SOX2-OT serves as the sponge of miR-192-5p to facilitate GBM cell growth

With the intention of validating that SOX2-OT sponged miR-192-5p to promote GBM progression, we carried out rescue experiments in U87 cells. U87 cells were co-transfected with sh-SOX2-OT#1+ miR-192-5p inhibitor for further research. CCK-8 and EdU experiments uncovered that transfection with sh-SOX2-OT#1+ miR-192-5p inhibitor enhanced GBM cell proliferation ability which were repressed before by sh-SOX2-OT#1 (Figure 3(a) and Figure 3(b)). In JC-1 experiment, the promoted cell apoptosis led by SOX2-OT depletion was decreased after additional treatment with miR-192-5p inhibitor (Figure 3(c)). Western blot analysis suggested that sh-SOX2-OT#1+ miR-192-5p inhibitor transfection repressed GBM cell apoptosis which was enhanced before by SOX2-OT down-regulation (Figure 3(d)). Given the above findings, it is concluded that SOX2-OT facilitates GBM cell growth by acting as a sponge of miR-192-5p in U87 cells.

RAB2A functions as a target gene for miR-192-5p in GBM cells

In search for the target gene of miR-192-5p in GBM cells, we utilized starBase database under the specific condition (CLIP-Data ≥ 5). According to the intersection of miRmap, microT, miRanda, PicTar and TargetScan database, we found 4 possible mRNAs, namely RAB2A, BLCAP, WNK1 and PKP4 (Figure 4(a)). For further screening, qRT-PCR was conducted on these mRNAs and RAB2A showed the highest expression in GBM cell lines (Figure 4(b)). In Figure 4(c), the binding sites of RAB2A and miR-192-5p predicted from starBase were exhibited. The results of RIP experiment indicated that SOX2-OT, RAB2A, and miR-192-5p were abundantly enriched by anti-Ago2, one of the RNA-induced silencing complex (RISC) components, implying they co-existed in the same RISC complex (Figure 4(d)). The previous study unveils that an Ago protein within the RISC complex could bind to a miRNA, allowing the target mRNA to be silenced [29]. Herein, we performed qRT-PCR to evaluate the influence of overexpressing miR-192-5p on RAB2A expression and found that miR-192-5p overexpression inhibited RAB2A expression, suggesting their negative correlation (Figure 4(e)). For further verification, miR-192-5p mimics lowered luciferase activity of RAB2A-WT in U87 and U251 cells, indicating that RAB2A

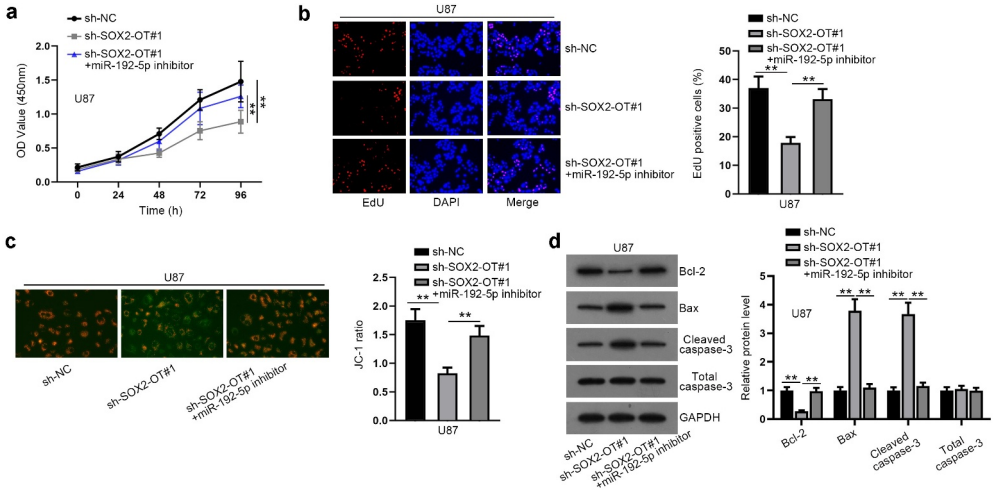

Figure 3. SOX2-OT facilitates GBM cell malignant behaviors via binding with miR-192-5p.

A, B. CCK-8 and EdU assays were done to assess proliferation of GBM cells transfected with sh-NC, sh-SOX2-OT#1 or sh-SOX2-OT#1 + miR-192-5p inhibitor. C. The results of JC-1 assay manifested GBM cell apoptosis in different transfection groups. D. Protein levels of Bcl2, Cleaved caspase-3, Bax, and Total caspase-3 were evaluated in western blot. **P < 0.01.

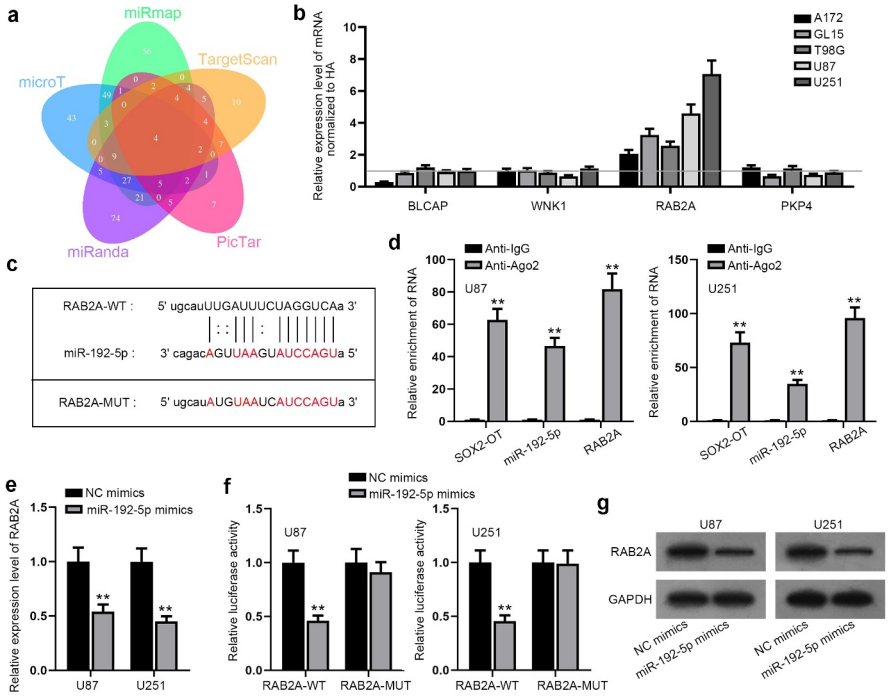

Figure 4. RAB2A functions as a target gene of miR-192-5p in GBM cells.

A. Venn diagram was utilized to uncover potential mRNAs for miR-192-5p. B. Expression levels of candidate mRNAs in GBM and normal cells were examined via qRT-PCR. C. The binding sites of RAB2A and miR-192-5p from starBase were exhibited. D. RIP assay was implemented to illustrate enrichment of SOX2-OT, miR-192-5p, and RAB2A in anti-IgG and anti-Ago2 groups. E. Data of qRT-PCR reflected RAB2A expression in cells transfected with NC mimics or miR-192-5p mimics. F. Luciferase activity of RAB2A-MUT and RAB2A-WT in U87 and U251 cells were detected. G. Western blot was performed to examine protein expression of RAB2A after treatment of miR-192-5p mimics. **P < 0.01.

could act as a target gene for miR-192-5p in GBM cell lines (figure 4(f)). Western blots displayed that the protein level of RAB2A was lessened after the treatment of miR-192-5p mimics (Figure 4(g)). To conclude, miR-192-5p targeted RAB2A in GBM cells.

SOX2-OT/miR-192-5p/RAB2A axis participates in regulation of GBM cell malignant behaviors

To certify the specific regulatory relationship among SOX2-OT, miR-192-5p and RAB2A expression in GBM cells, rescue assays were employed. At the very beginning, qRT-PCR experiments were done to test the overexpression efficacy of pcDNA3.1/RAB2A in U87 cells (Figure 5(a)). GBM cells were then co-transfected with sh-SOX2-OT#1 and pcDNA3.1/RAB2A for the following experiments. CCK-8 and EdU experiments illustrated that transfection with sh-SOX2-OT#1+ pcDNA3.1/RAB2A recovered the inhibited GBM cell proliferation caused by sh-SOX2-OT#1 (Figure 5(b) and Figure 5(c)). JC-1 assay later exhibited that the treatment of sh-SOX2-OT#1+ pcDNA3.1/RAB2A counteracted the stimulative impact of SOX2-OT reduction on GBM cell apoptosis (Figure 5(d)). Western blot results manifested that sh-SOX2-OT#1+ pcDNA3.1/RAB2A transfection reversed the promoting function of silencing SOX2-OT on cell apoptosis (Figure 5(e)). In summary, these results revealed that SOX2-OT stimulated proliferation and hampered apoptosis of GBM cells through the regulation of miR-192-5p/RAB2A axis in a ceRNA manner.

SOX2-OT regulates ERK signaling pathway in GBM cell lines

Reviewing published research work, we found that RAB2A pushes tumorigenesis of breast cancer stem cells by activation of ERK signaling pathway

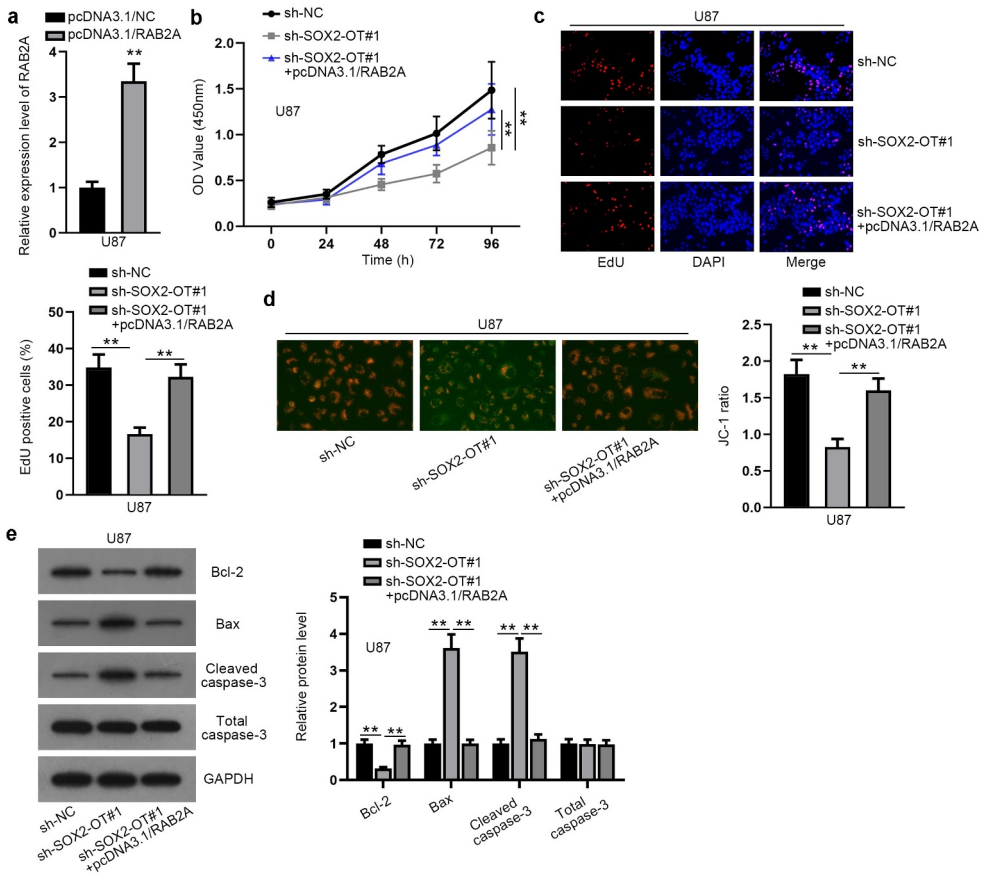

Figure 5. SOX2-OT promotes GBM cell growth via modulating RAB2A expression.

A. Overexpression effect of pcDNA3.1/RAB2A on U87 cells was examined using qRT-PCR. B, C. CCK-8 and EdU experiments were conducted to measure proliferation abilities of GBM cells transfected with sh-NC, sh-SOX2-OT#1 or sh-SOX2-OT#1+ pcDNA3.1/RAB2A. D. JC-1 experiment was performed to exhibit apoptosis ratio of transfected GBM cells. E. Western blot was applied to quantify the levels of Cleaved caspase-3, Bax, and Total caspase-3, and Bcl2 in transfected cells. **P < 0.01.

[30]. ERK signaling pathway has been proved to be involved in human cancers by assorted repots [31,32], including GBM [33]. Thus, we attempted to figure out whether ERK signaling pathway could be activated by SOX2-OT in GBM or not. The collected data in qRT-PCR and western blot assays suggested that RAB2A expression was decreased after the treatment of sh-SOX2-OT#1 (Figure 6(a) and Figure 6(b)). To determine that SOX2-OT could activate ERK signaling pathway, the level of key proteins in ERK signaling pathway was measured in western blot experiment, including phosphorylated extracellular-signal-regulated kinase 1/1 (p-ERK1/1), phosphorylated mitogen-activated protein kinase (p-MEK), total MEK (t-MEK), and total ERK 1/1 (t-ERK1/1). It was discovered that SOX2-OT knockdown reduced p-ERK1/1 and p-MEK expression, whereas the protein levels of other proteins exhibited no significant difference compared with sh-NC group (Figure 6(c)), indicating ERK signaling pathway was activated by SOX2-OT. To conclude, SOX2-OT regulates ERK signaling pathway in GBM cell lines.

Discussion

Glioblastoma (GBM) is the most frequently occurring tumor in the central nervous system. Its five-year overall survival rate is less than 10% [1]. Regulation of lncRNAs has been found to play a significant part in oncogenesis [34], and SOX2-OT is a lncRNA that many researchers have illustrated to enhance cell viability in various cancers.

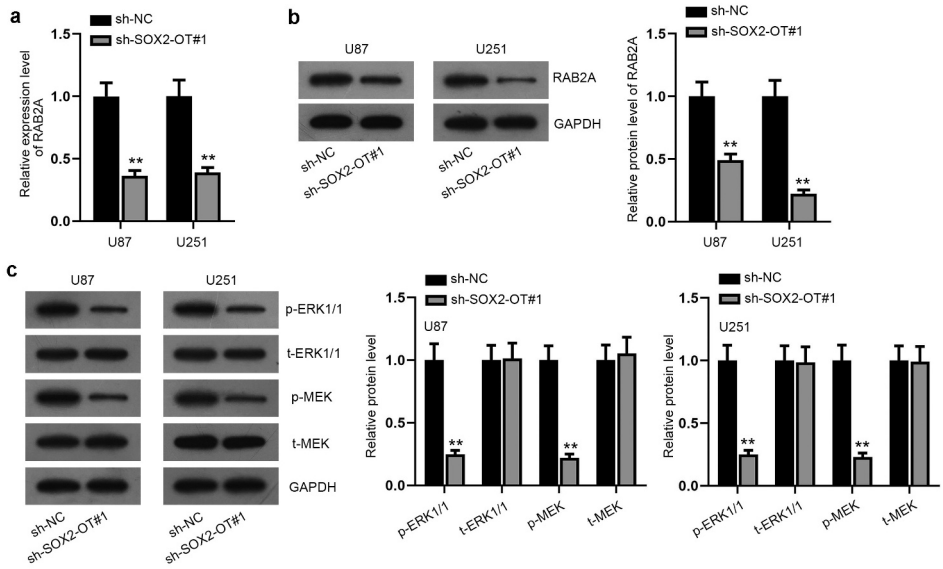

Figure 6. SOX2-OT regulates ERK signaling pathway in GBM cells.

A. RAB2A expression in GBM cell lines was quantified by means of qRT-PCR. B. Protein expression level of RAB2A was shown in western blot when SOX2-OT was inhibited. C. Data of western blot and qRT-PCR showed the levels of proteins related to ERK signaling pathway. **P < 0.01.

For instance, Jiang et al. discover that SOX2-OT promotes cholangiocarcinoma progression through modulating SOX2 and PI3K/AKT signaling [31]. We et al. report that SOX2-OT-miR-369-3p-CFL2 axis contributes to prostate cancer progression [32]. Li et al. manifest that the SOX2-OT facilitates nasopharyngeal carcinoma carcinogenesis via miR-146b-5p/HNRNPA2B1 pathway [33]. Wan et al. elucidate that SOX2-OT functions as a laryngeal cancer promoter by sponging miR-654 [34]. Song et al. reveal that SOX2-OT encourages prostate cancer cell proliferation via targeting miR-452-5p/HMGB3 and activating Wnt/β-catenin pathway [35]. Even so, the molecular mechanisms of SOX2-OT in GBM have not been studied yet. In this study, it was indicated that SOX2-OT was overexpressed in GBM cells. Functional assays showed that reduced SOX2-OT accelerated GBM cell proliferation but inhibited GBM cell apoptosis. Western blots further uncovered that SOX2-OT could repress the cell apoptosis in GBM. Briefly, SOX2-OT serves as an oncogene in GBM cells.

LncRNAs can function as a ceRNA to promote or inhibit tumor development by sponging miRNAs to regulate mRNA expression [11,35]. Yu et al. have analyzed and compared different ceRNA gene interaction network in GBM subtypes [36]. Our study testified that SOX2-OT bound to miR-192-5p as a ceRNA in GBM cells via bioinformatics tools and a series of mechanism assays. In previous research, miR-192-5p has been reported to repress bladder cancer cell proliferation by targeting YY1 [23]. Also, miR-192-5p could inhibit lung cancer progression by targeting TRIM44 [24]. Moreover, lncRNA FTX is verified to facilitate the progression of colorectal cancer through the regulation of miR-192-5p/EIF5A2 axis [37]. In line with the findings of former literature, our study also found miR-192-5p played a inhibitory part in GBM cells, as knockdown of miR-192-5p reversed the repressive function of inhibiting SOX2-OT on GBM cell growth. These results implied that SOX2-OT sponged miR-192-5p to promote GBM cell growth. Unlike the conclusion of previous research that miR-451 restricts cell proliferative ability in human hepatocellular carcinoma through direct inhibition of IKK-β[38], our study first proved miR-192-5p impeded GBM cell growth in SOX2-OT-involved ceRNA mechanism.

Moreover, published research has uncovered that RAB2A could promote carcinogenesis through activating ERK signaling pathway [30], and enhance metastasis by controlling both canonical polarized Golgi-to-Plasma membrane

trafficking of the junctional protein E-cadherin, and post-endocytic trafficking of the membrane-bound metalloprotease, MT1-MMP [39]. Our experiments also showed that RAB2A bound with miR-192-5p in GBM cells. RAB2A had a positive association with SOX2-OT and negative correlation with miR-192-5p. Moreover, functional assays demonstrated RAB2A overexpression restored the repressed malignant behaviors of GBM cells induced by SOX2-OT reduction. Mechanism assays confirmed that SOX2-OT could activate ERK signaling pathway in GBM cells. To conclude, SOX2-OT promotes GBM cell malignant behavior via regulating miR-192-5p/RAB2A axis and ERK signaling pathway.

Taken together, our report first illustrated roles of SOX2-OT in GBM. Our study proved that SOX2-OT contributed to GBM tumorigenesis by sponging miR-192-5p to upregulate RAB2A and targeting ERK signaling pathway in GBM cell lines. In fact, ERK/MAPK pathway could participate in complicated modulation of biological activities in GBM cells. In our study, we merely focused on the potential relationship between SOX2-OT and ERK/MAPK pathway. In the future, we may explore more about ERK/MAPK pathway. Aside from that, other potential targets influenced by miR-192-5p sequestration will also be further discussed in the future. Hopefully, the SOX2-OT/miR-192-5p/RAB2A axis may blaze a new trail to GBM treatment.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Not applicable.

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