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Sevoflurane suppresses hepatocellular carcinoma cell progression via circ_0001649/miR-19a-3p/SGTB axis

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Summary. Background. Sevoflurane is a widely used anesthetic agent and is reported to play an anti-tumor action in many cancers. However, the underlying mechanisms are largely unclear.

Methods. Hepatocellular carcinoma (HCC) cells were treated with sevoflurane for 12 or 24 h. HCC cell proliferation, migration, invasion, and apoptosis were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, 5-ethynyl-2'-deoxyuridine (EdU) assay, transwell assay, and flow cytometry assay, respectively. The protein levels were determined by western blot. The expression of circular RNA (circ)_0001649, microRNA (miR)-19a-3p, and small glutamine rich tetratricopeptide repeat containing Beta (SGTB) were detected by quantitative real-time polymerase chain reaction (qRT-PCR). The relationship between miR-19a-3p and circ_0001649 or SGTB was predicted by Starbase and confirmed by dual-luciferase reporter and RNA immunoprecipitation (RIP) assays.

Results. Sevoflurane inhibited HCC cell proliferation, migration, and invasion, but promoted apoptosis. Sevoflurane could affect the expression of circ_0001649 and knockdown of circ_0001649 reversed the effects of sevoflurane on HCC cell progression. Subsequently, miR-19a-3p was identified as a target of circ_0001649 and directly targeted SGTB. In addition, circ_0001649 suppressed the development of sevoflurane-induced HCC cells through miR-19a-3p/SGTB axis.

Conclusion. Our study demonstrated that sevoflurane inhibited HCC cell development via circ_0001649/miR-19a-3p/SGTB axis.

Key words: Sevoflurane, Hepatocellular carcinoma, circ_0001649, miR-19a-3p, SGTB

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Introduction

Hepatocellular carcinoma (HCC) is the principal type of primary liver cancer with an increasing incidence, which is characterized by metastasis and high frequent recurrence (Salem and Lewandowski, 2013). Recent scientific advances suggested that hepatitis B virus (HBV), hepatitis C virus (HCV), and non-alcoholic fatty liver disease (NAFLD) are major risk factors for HCC development (Giannakoulis et al., 2021). Morbidity and mortality are very prominent in China, as approximately 50% of all HCC cases and deaths appeared in China (Zhu et al., 2016). Currently, the only strategies available for advanced-stage patients are surgical resection and liver transplantation (Dutta and Mahato, 2017; El Jabbour et al., 2019). Deeply understanding the underlying mechanism of HCC tumorigenesis is of great importance for seeking a prognostic marker for HCC.

Sevoflurane is frequently used in surgery for anesthesia, which could regulate the progression of various cancers. For instance, sevoflurane impeded glioma cell migration and invasion by up-regulating miR-637 as well as down-regulating AKT1 (Yi et al., 2016). Sevoflurane suppressed the cell cycle and proliferation in breast cancer development (Liu et al., 2018a). The abilities of migration and invasion in colorectal cancer were also repressed by sevoflurane treatment (Sun et al., 2019; Fan et al., 2019). As for HCC, a previous study revealed that sevoflurane inhibited the development of HCC cells (Song et al., 2019). Nonetheless, the mechanism of sevoflurane in HCC is worthy of further investigation.

Circular RNAs (circRNAs) were studied to engage in the progress of various diseases, including cancers (Patop and Kadener, 2018; Lux and Bullinger, 2018). CircRNA-Cdr1as was found to be aberrantly expressed in bladder cancer, and its overexpression suppressed bladder cancer progression (Liu et al., 2018b). Hsa_circ_100395 overexpression repressed cell cycle and proliferation in lung cancer via sponging miR-1228 to regulate TCF21 (Chen et al., 2018). In addition, due to

the stability of circRNAs relative to linear RNAs, they were considered promising diagnostic and therapeutic targets in the treatment of cancers (Zhang et al., 2017, 2018b). A previous study reported that hsa_circ_0 091579 functioned as a potential diagnostic and predictive target for HCC (Zhang et al. 2018a). And an existing study has shown that circ_0001649 was down-regulated in HCC, and the decreased circ_0001649 level indicates a poor prognosis (Qin et al., 2016; Zhang et al., 2018d). Nevertheless, the molecular mechanism of circ_0001649 in HCC still needs further exploration.

On the grounds of competing endogenous RNA (ceRNA) conjecture, circRNAs could function as ceRNA molecules for microRNAs (miRNAs) and subsequently regulate target gene expression to regulate the progression of cancers (Salmena et al., 2011; Tay et al., 2014). For instance, hsa_circ_104348 promoted HCC progression by regulating miR-187-3p/RTKN2 axis (Huang et al., 2020). Besides, circRNA-5692 worked as a miR-328-5p sponge to mediate the DAB2IP level, therefore inhibiting the development of HCC (Liu et al., 2019). Even though several studies have presented the circRNA/miRNA/mRNA axis in HCC, the regulatory networks underlying circRNAs in HCC still need further investigation.

Here, our study is dedicated to improving our comprehension of the mechanism underlying sevoflurane in HCC tumorigenesis. Besides, the effects and association between circ_0001649, miR-19a-3p, and small glutamine-rich tetratricopeptide repeat co-chaperone beta (SGTB) in sevoflurane treated HCC cells, would provide a novel implication for the study of investigating the regulatory mechanism of sevoflurane in HCC.

Materials and methods

Cell culture and sevoflurane treatment

The Chinese academy of sciences cell bank (Shanghai, China) supplied the human normal hepatic cell line THLE-2 and HCC cell lines (Huh7 and MHCC97), which were respectively cultured in Bronchial epithelial cell growth basal medium (BEBM, Lonza, Basel, Switzerland) and Dulbecco's modified Eagle medium (DMEM; Invitrogen) with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) at 37ºC with 5% CO_2.

For sevoflurane treatment, cells were incubated with 1%, 2%, or 4% sevoflurane for 12 or 24h. 4% sevoflurane treated for 24h was used for further functional experiments.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The cells were grown in 96-well plates (4×10^3 cells/well) and treated with sevoflurane. 20 μL MTT (5 mg/mL; Sigma, Louis, MO, USA) was supplemented into each well and allowed for a 4 h incubation. After that, dimethyl sulfoxide (150 μL) was applied to dissolving formazan. A microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) was employed to determine the absorbance at 490 nm.

Transwell assay

The chamber that was precoated with Matrigel (BD Bioscience, San Diego, CA, USA) was employed to detect invasion capability, while the uncoated chamber was utilized for cell migration. Transfected cells (5×10^4 cells for migration, and 1×10^5 cells for invasion) in serum-free medium were vaccinated in the superior chamber, and the beneath one was attached to the cell medium with 10% serum. 48h later, migratory or invasive cells were settled by methanol and spotted by crystal violet, then photographed with a microscope (Thermo Fisher Scientific).

Western blot

Proteins were extracted and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After being blocked with 5% nonfat milk, the membranes were nurtured with primary antibodies against c-Myc (1:1000; ab32072; Abcam, Cambridge, UK), matrix metalloproteinase-9 (MMP-9; 1:1000; ab38898; Abcam), Cleaved Caspase-3 (Cleaved-cas-3; 1:1000; ab2302; Abcam), Cleaved-cas-9 (1:1000; ab2324; Abcam), SGTB (1:2000; ab202419; Abcam) or GAPDH (1:5000; ab181602; Abcam) at 4ºC for 12h,. Then, the membranes were allowed for a 2h incubation with a secondary antibody (1:5000; ab6721; Abcam). The blots were imagined through an enhanced chemiluminescence reagent (Millipore).

5-ethynyl-2'-deoxyuridine (EdU) staining assay

The Click-iT® EdU Imaging Kit (Thermo Fisher Scientific) was employed to detect cell proliferation. Briefly, after the transfected cells were maintained in a 96-well plate for 24h, the cells were then hatched with EdU solution (50 μM) for 2h. Subsequently, the cells were settled with 4% paraformaldehyde for 30 min and spotted with DAPI for an additional 30 min. Cell proliferation was then evaluated by a fluorescence microscope (Thermo Fisher Scientific).

Flow cytometry assay

Annexin V-FITC/ propidium iodide (PI) apoptosis kit (BD Bioscience) was applied for apoptosis detection. After treatment or transfection, annexin V-FITC and PI solution was mixed into cells and hatched for 20 min,

and examined by flow cytometry (BD Bioscience).

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

Total RNA was separated in line with the instruction of TRIZol (Invitrogen). Complementary DNA (cDNA) was acquired by inverse transcription and PrimeScript RT Reagent Kit (Takara, Dalian, China). qPCR was fulfilled by SYBR Green Master Mix (Takara) with DAPDH and U6 as internal controls. The expression of genes was estimated by the $2^{-\Delta\Delta Ct}$ approach. The primers were presented in Table 1.

Cell transfection

To construct the circ_0001649 overexpression vector (circ_0001649), the sequence of circ_0001649 was inserted into pCD-ciR vector (Geneseed, Guangzhou, China). The sequence of SGTB was inserted into pcDNA vector (Geneseed, Guangzhou, China), with an empty vector as a control. Small interfering RNA (siRNA) versus circ_0001649 (si-circ_0001649), miR-19a-3p mimic (miR-19a-3p), miR-19a-3p inhibitor (anti-miR-19a-3p) and the controls (si-NC, miR-NC or anti-NC) were bought from GenePharma (Shanghai, China). Lipofectamine 3000 (Invitrogen) was exploited for cell transfection at a density of 6×10^4 cells/well.

Dual-luciferase reporter assay

The sequences of circ_0001649 or SGTB 3'UTR harbored the wild type (WT) and mutant type (MUT) miR-19a-3p binding fragments were cloned into pGL3 luciferase reporter vectors (Promega, Madison, WI, USA) to construct circ_0001649 MUT, circ_0001649 WT, SGTB 3'UTR MUT or SGTB 3'UTR WT. These plasmids and miR-19a-3p or miR-NC were co-introduced into Huh7 and MHCC97 cells and allowed to culture for 48h. A Dual-Luciferase reporter system (Promega) was employed to determine the activities of luciferases.

Table 1. The primers used in qRT-PCR.

Name		Sequences (5'-3')
circ_0001649	Forward	5'-AGGCAATGCTGAAAACTGCT-3'
	Reverse	5'-TTGAGAAAACGAGTGCTTTGG-3'
miR-19a-3p	Forward	5'-GCGTGTGCAAATCTATGCAA-3'
	Reverse	5'-AGTGCAGGGTCCGAGGTATT-3'
SGTB	Forward	5'-CTGGTGGGTTCTGGGGTCTA-3'
	Reverse	5'-TTTTCCAAGCAGGAGGACGC-3'
GAPDH	Forward	5'-ACATCGCTCAGACACCATG-3'
	Reverse	5'-TGTAGTTGAGGTCAATGAAGGG-3'
U6	Forward	5'-GCTTCGGCAGCACATATACTAAAAT-3'
	Reverse	5'-CGCTTCACGAATTTGCGTGTCAT-3'

RNA immunoprecipitation (RIP) assay

An RNA-Binding Protein Immunoprecipitation Kit was applied for RIP assay (Millipore) following the instructions. Human anti-Ago2 and negative control anti-IgG were bought from Abcam. The co-precipitated RNAs were tested by qRT-PCR.

Statistical analysis

Data of three repetitions were examined by SPSS 22.0 and indicated as mean ± standard deviation. The differences between groups were analyzed by Student's t-test or analysis of variance (ANOVA) with Tukey's HSD test. $P<0.05$ was deemed as statistically significant.

Results

Sevoflurane suppressed proliferation, migration, and invasion and enhanced apoptosis in HCC cells

To begin with, Huh7 and MHCC97 cells were treated with sevoflurane (1%, 2%, or 4%) for 12h or 24h. Compared with the control group (without sevoflurane treatment), the cell vitality of Huh7 and MHCC97 cells was significantly reduced after being treated with sevoflurane (2%, 4% for 12h and 1%, 2%, 4% for 24h). When treated with 4% sevoflurane for 24h, the cell vitality of Huh7 and MHCC97 cells was decreased by about 50% (Fig. 1A,B). Thus, Huh7 and MHCC97 cells were treated with 4% sevoflurane for 24h for further experiments. EdU staining assay disclosed that sevoflurane treatment inhibited the proliferation of Huh7 and MHCC97 cells (Fig. 1C). Besides, sevoflurane treatment triggered remarkable repression in cell migration and invasion (Fig. 1D,E). Moreover, the western blot result showed that the protein levels of migration-related proteins (c-Myc and MMP-9) were downregulated in Huh7 and MHCC97 cells treated with sevoflurane compared to the control cells (Fig. 1F-H). In addition, apoptosis of Huh7 and MHCC97 cells was induced by sevoflurane (Fig. 1I). Sevoflurane treatment also resulted in elevation expression of Cleaved-cas-3 and Cleaved-cas-9 levels in Huh7 and MHCC97 cells (Fig. 1J). Therefore, our results suggested that sevoflurane suppressed HCC cell development.

Circ_0001649 was elevated by sevoflurane and inhibited HCC cell development

The molecular mechanism of sevoflurane in regulating HCC cell development was further explored. The qRT-PCR result showed that the expression of circ_0001649 was significantly decreased in Huh7 and MHCC97 cells than that in THLE-2 cells (Fig. 2A). Circ_0001649 level was induced by sevoflurane in a dose-dependent manner in Huh7 and MHCC97 cells (Fig. 2B,C). In comparison to the negative control, the transfection of circ_0001649 overexpressing plasmid

significantly elevated the expression of circ_0001649 in Huh7 and MHCC97 cells (Fig. 2D). MTT and EdU staining assays displayed that cell proliferation was inhibited by transfection with circ_0001649, as confirmed by the reduced cell viability (Fig. 2E,F), as well as the decrease of Edu- and DAPI-positive cell rate (Fig. 2G, H). Besides, overexpression of circ_0001649 repressed migration and invasion of Huh7 and MHCC97 cells (Fig. 2I,J). The protein levels of c-Myc and MMP-9 were remarkably downregulated by circ_0001649 overexpression in both Huh7 and MHCC97 cells (Fig. 2K,L). Additionally, overexpression of circ_0001649 enhanced cell apoptosis (Fig. 2M). Circ_0001649 overexpression resulted in increased Cleaved-cas-3 and Cleaved-cas-9 expressions in Huh7 and MHCC97 cells (Fig. 2N,O). Our data indicated that circ_0001649 expression was elevated by sevoflurane and played an inhibitory effect in HCC cell development.

Knockdown of circ_0001649 reversed the effects of sevoflurane on proliferation, migration, invasion, and apoptosis of HCC cells

To investigate the function of circ_0001649 in sevoflurane-treated HCC cells, Huh7 and MHCC97 cells were exposed to sevoflurane and transfected with si-NC or si-circ_0001649. The expression of circ_0001649 was greatly reduced in Huh7 and MHCC97 cells transfected with si-circ_0001649 (Fig. 3A). As shown in Fig. 3B and 3C, cell proliferation was restrained by sevoflurane, which was restored by circ_0001649 knockdown in Huh7 and MHCC97 cells. Besides, the inhibitory effects of sevoflurane on migration and invasion were abolished by si-circ_0001649 (Fig. 3D,E). Western blot results showed that the downregulated c-Myc and MMP-9 levels induced by sevoflurane were elevated by circ_0001649 knockdown in Huh7 and MHCC97 cells (Fig. 3F). Flow cytometry assay disclosed that cell apoptosis was promoted by sevoflurane, whereas it was reverted by circ_0001649 knockdown in Huh7 and MHCC97 cells treated with sevoflurane (Fig. 3G). Additionally, sevoflurane-induced the increase of Cleaved-cas-3 and Cleaved-cas-9 protein levels were partly reversed by circ_0001649 knockdown in Huh7 and MHCC97 cells (Fig. 3H). These findings indicated that the knockdown of circ_0001649 reversed the effects of sevoflurane on HCC cells.

Circ_0001649 targeted miR-19a-3p to regulate its expression

To further investigate the molecular mechanism of

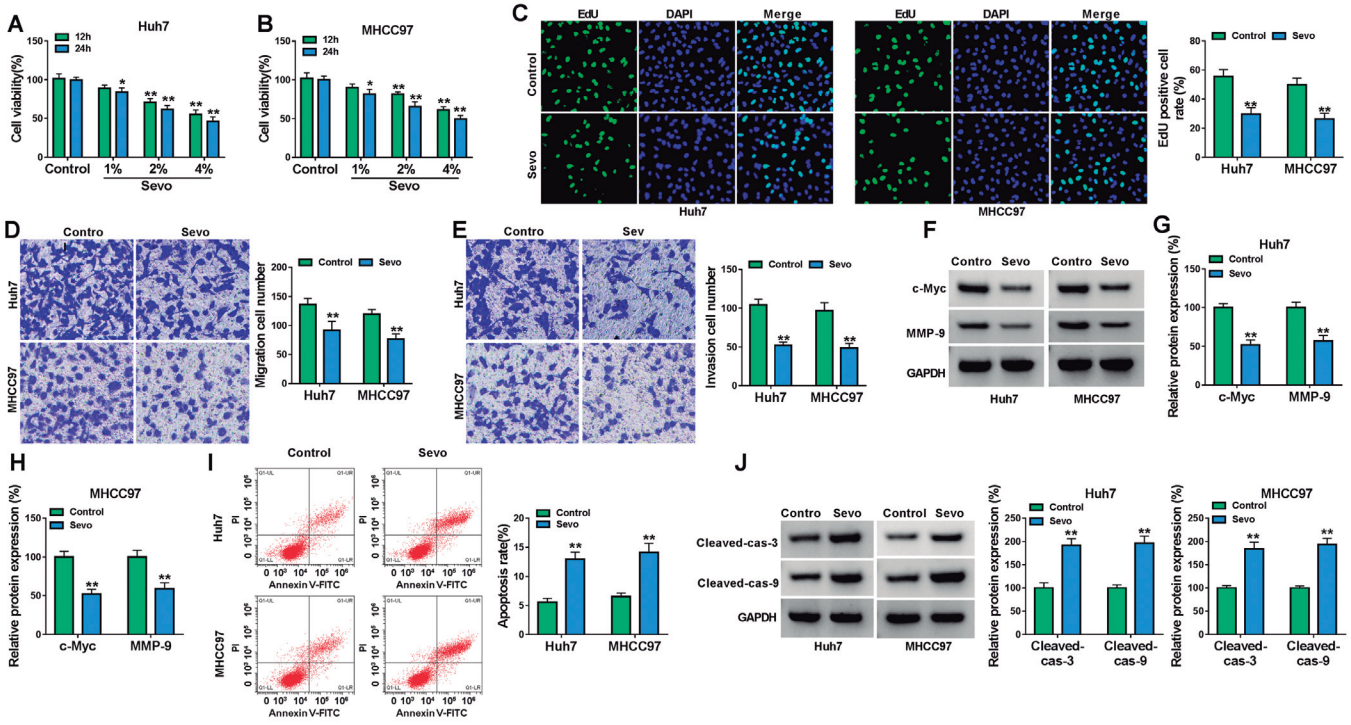

Fig. 1. Sevoflurane suppressed the progression of HCC cells. **A and B.** Cell viability of Huh7 and MHCC97 cells was assessed by MTT assay after treatment with 1%, 2%, or 4% sevoflurane (Sevo) for 12h or 24h. **C-J.** Huh7 and MHCC97 cells were treated with 4% Sevo for 24h. **C**. The proliferation of Huh7 and MHCC97 cells was detected by EdU staining assay. **D and E.** Cell migration and invasion were evaluated by transwell assay. **F-H.** The protein levels of migration-related proteins c-Myc and MMP9 were detected by western blot. **I.** Apoptosis was determined by flow cytometry. **J.** The protein levels of apoptosis-related proteins Cleaved-cas-3 and Cleaved-cas-9 were detected by western blot. *P<0.05, **P<0.01.

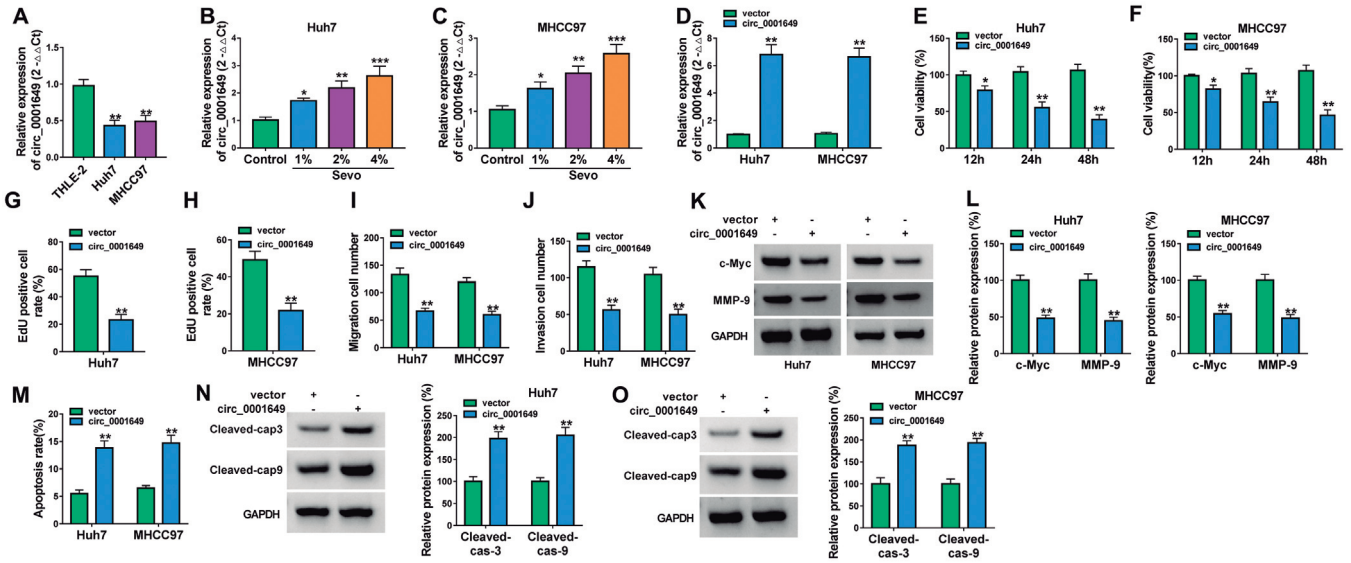

Fig. 2. Overexpression of circ_0001649 repressed the progression of HCC cells. **A.** The expression of circ_0001649 in THLE-2, Huh7, and MHCC97 cells was detected by qRT-PCR. **B and C.** The expression of circ_0001649 in Huh7 and MHCC97 cells treated with 1%, 2%, or 4% Sevo for 24h was detected by qRT-PCR. **D-O.** Huh7 and MHCC97 cells were transfected with vector or circ_0001649. **D.** The expression of circ_0001649 was detected by qRT-PCR. **E and F.** Cell viability was assessed by MTT assay. **G and H.** EdU assay was conducted to evaluate the proliferation of Huh7 and MHCC97 cells. **I and J.** Cell migration and invasion were evaluated by transwell assay. **K and L.** The protein levels of c-Myc and MMP9 were detected by western blot. **M.** HCC cell apoptosis was determined by flow cytometry. **N and O.** The protein levels of apoptosis-related proteins Cleaved-cas-3 and Cleaved-cas-9 were detected by western blot. *P<0.05, **P<0.01, ***P<0.001.

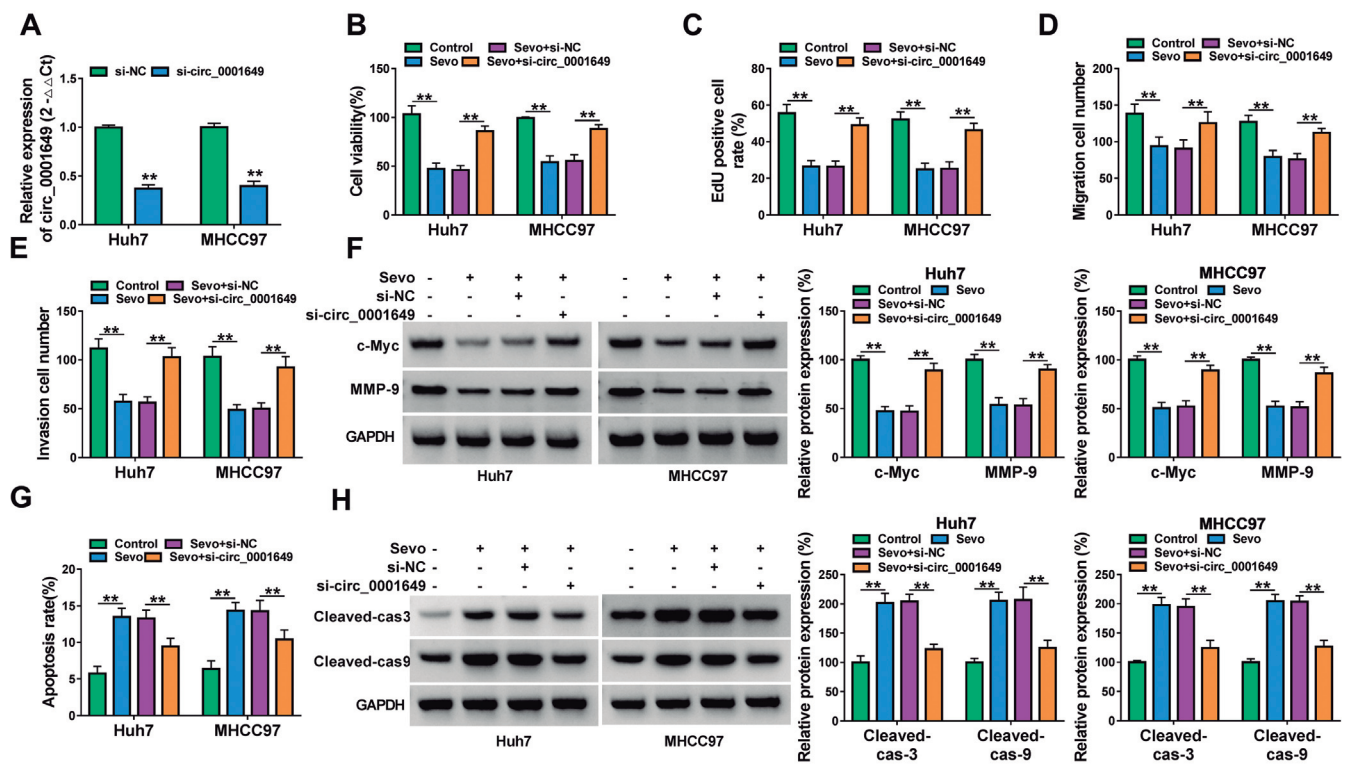

Fig. 3. Knockdown of circ_0001649 reversed the effects of sevoflurane on HCC cells. **A.** The expression of circ_0001649 was detected by qRT-PCR in Huh7 and MHCC97 cells transfected with si-NC or si-circ_0001649. **B-H.** Huh7 and MHCC97 cells were treated with Sevo, Sevo+si-NC, or Sevo+si-circ_0001649; untreated cells were served as Control. **B and C.** Cell proliferation was assessed by MTT and EdU staining assays. **D and E.** Cell migration and invasion were evaluated by transwell assay. **F.** The protein levels of c-Myc and MMP9 were detected by western blot. **G.** Apoptosis was determined by flow cytometry. **H.** The protein levels of apoptosis-related proteins Cleaved-cas-3 and Cleaved-cas-9 were detected by western blot. **P<0.01.

circ_0001649 in regulating HCC cell development, the downstream target of circ_0001649 was predicted. Starbase predicted that miR-19a-3p was a direct target of circ_0001649, and the putative binding sites were shown in Fig. 4A. Dual-luciferase reporter assay was performed to confirm the relationship between circ_0001649 and miR-19a-3p. The luciferase activity was significantly reduced by miR-19a-3p in Huh7 and MHCC97 cells co-transfected with circ_0001649 WT rather than circ_0001649 MUT (Fig. 4B,C). Subsequently, RIP assay showed that the enrichment of both circ_0001649 and miR-19a-3p was strikingly enhanced in anti-Ago2 group relative to anti-IgG group (Fig. 4D,E). Besides, the level of miR-19a-3p was elevated in Huh7 and MHCC97 cells compared with THLE-2 cells (Fig. 4F). The expression of miR-19a-3p was decreased in Huh7 and MHCC97 cells by sevoflurane treatment in a dose-dependent method (Fig. 4G,H). Moreover, miR-19a-3p was downregulated by circ_0001649 overexpression and upregulated by circ_0001649 knockdown (Fig. 4I,J). These results revealed that miR-19a-3p was a direct target of circ_0001649 and was negatively regulated by circ_0001649.

Sevoflurane affected HCC cell development via circ_0001649/miR-19a-3p axis

To explore the interaction between circ_0001649 and miR-19a-3p in sevoflurane- HCC, Huh7 and MHCC97 cells were treated with sevoflurane and then transfected with si-NC, si-circ_0001649, si-circ_0001649+anti-NC or si-circ_0001649+anti-miR-19a-3p. The expression of miR-19a-3p was effectively downregulated in Huh7 and MHCC97 cells transfected

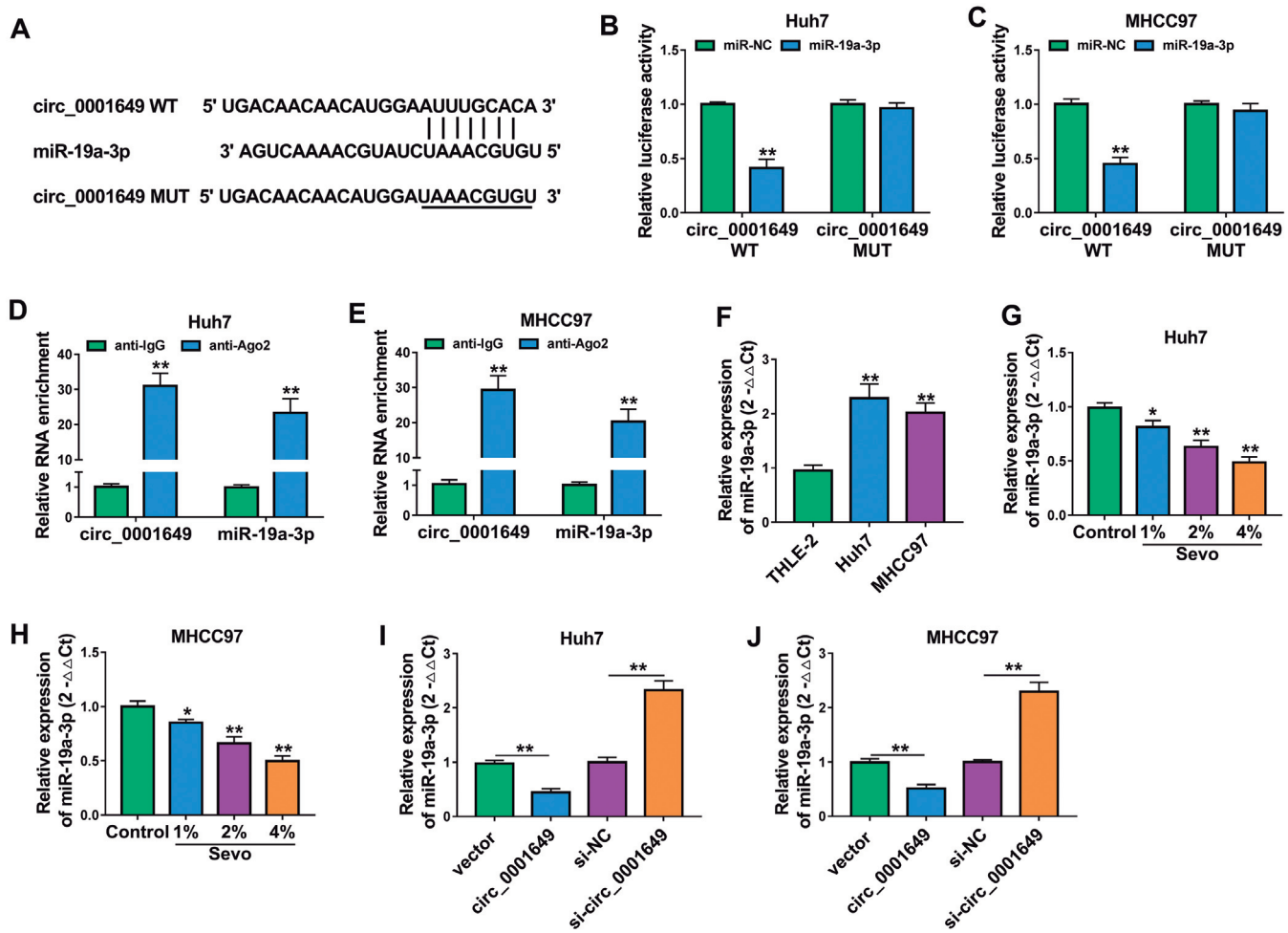

Fig. 4. Circ_0001649 acted as a sponge for miR-19a-3p. **A.** The putative binding sites between circ_0001649 and miR-19a-3p were exhibited. **B and C.** The luciferase activities of circ_0001649 WT and circ_0001649 MUT were detected in Huh7 and MHCC97 cells transfected with miR-NC or miR-19a-3p. **D and E.** The enrichment of circ_0001649 and miR-19a-3p was detected by qRT-PCR when incubated with anti-IgG or anti-Ago2 in RIP assay. **F.** The expression of miR-19a-3p in THLE-2, Huh7, and MHCC97 cells was detected by qRT-PCR. **G and H.** The expression of miR-19a-3p in Huh7 and MHCC97 cells treated with 1%, 2%, or 4% Sevo for 24h was detected by qRT-PCR. **I and J.** The expression of miR-19a-3p was detected by qRT-PCR in Huh7 and MHCC97 cells transfected with vector, circ_0001649, si-NC or si-circ_0001649. *P<0.05, **P<0.01.

with anit-miR-19a-3p (Fig. 5A). MTT and EdU staining assays manifested that knockdown of circ_0001649 facilitated cell proliferation, and this effect was reverted by miR-19a-3p knockdown in sevoflurane-induced Huh7 and MHCC97 cells (Fig. 5B, C). Besides, cell migration and invasion were promoted by circ_0001649 knockdown, whereas these effects were restored by downregulation of miR-19a-3p (Fig. 5D,E). Furthermore, the elevated c-Myc and MMP-9 levels induced by circ_0001649 knockdown were overturned by anti-miR-19a-3p after Huh7 and MHCC97 cells were treated with sevoflurane (Fig. 5F). Additionally, the silence of miR-19a-3p abolished the suppression effect of circ_0001649 knockdown on cell apoptosis (Fig. 5G). Western blot results uncovered that miR-19a-3p knockdown overturned the suppression effect of si-circ_0001649 on Cleaved-cas-3 and Cleaved-cas-9 protein levels (Fig. 5H). Therefore, sevoflurane inhibited HCC cell proliferation, migration, and invasion, but promoted apoptosis via circ_0001649/miR-19a-3p axis.

SGTB was targeted by miR-19a-3p

Considering that miRNA could exert its function by binding to the 3'UTR of target mRNA, we further predicted the target mRNAs of miR-19a-3p by Starbase. And the results indicated that SGTB contained the complementary binding sites of miR-19a-3p (Fig. 6A). Dual-luciferase reporter assay disclosed that transfection of miR-19a-3p significantly decreased the luciferase activity of the SGTB 3'UTR WT group, but had little effect on the SGTB 3'UTR MUT group (Fig. 6B,C). In addition, SGTB protein level was significantly decreased in Huh7 and MHCC97 cells (Fig. 6D). Moreover, we found that sevoflurane elevated the protein level of SGTB, and circ_0001649 knockdown partly overturned the promotion effect of sevoflurane on SGTB protein level. And miR-19a-3p partly reversed the effect of circ_0001649 knockdown on SGTB protein level in sevoflurane-treated Huh7 and MHCC97 cells (Fig. 6E). These results indicated that SGTB is the target of miR-19a-3p.

Sevoflurane affected HCC cell development via miR-19a-3p/SGTB axis

Subsequently, gain-of-function assays were performed to evaluate the function of SGTB in miR-19a-

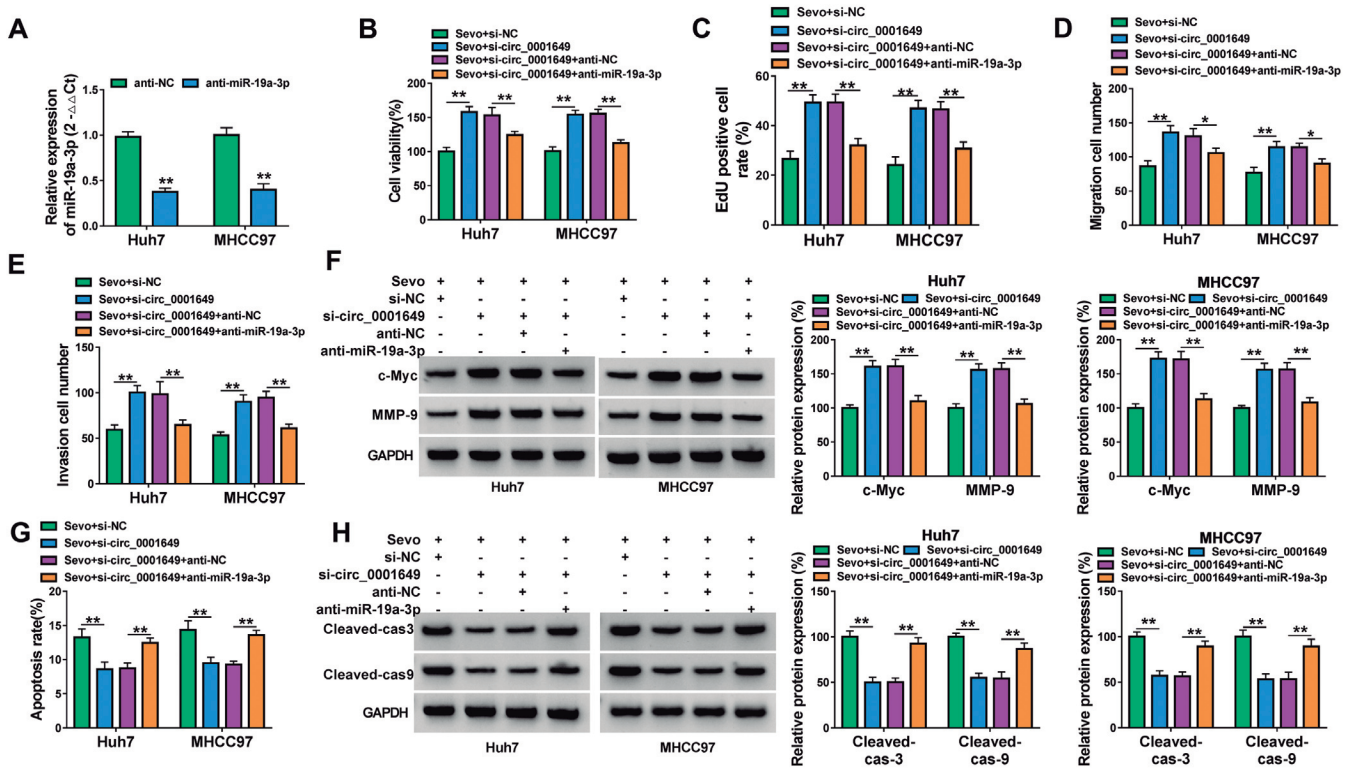

Fig. 5. Knockdown of miR-19a-3p restored the effect of circ_0001649 silencing in sevoflurane-treated HCC cells. **A.** The expression of miR-19a-3p was detected by qRT-PCR in Huh7 and MHCC97 cells transfected with anti-NC or anti-miR-19a-3p. **B-I.** Huh7 and MHCC97 cells were treated with Sevo+si-NC, Sevo+si-circ_0001649, Sevo+si-circ_0001649+anti-NC or Sevo+si-circ_0001649+anti-miR-19a-3p. **B and C.** Cell proliferation was assessed by MTT and EdU assays. **D and E.** Cell migration and invasion were evaluated by transwell assay. **F.** The protein levels of c-Myc and MMP9 were detected by western blot. **G.** Apoptosis was determined by flow cytometry. **H.** The protein levels of apoptosis-related proteins Cleaved-cas-3 and Cleaved-cas-9 were detected by western blot. *P<0.05, **P<0.01.

3p-mediated the progression of HCC. qRT-PCR and western blot assays confirmed the transfection efficiencies of miR-19a-3p and SGTB overexpression vector (Fig. 7A,B). Cell proliferation was elevated by miR-19a-3p, while this effect was overturned by SGTB overexpression (Fig. 7C,D). Besides, transwell assay uncovered that SGTB overexpression revoked the promotion effects of miR-19a-3p on cell migration and invasion in sevoflurane-treated Huh7 and MHCC97 cells (Fig. 7E,F). Western blot also confirmed that SGTB overexpression partly overturned the promotion effects of miR-19a-3p on c-Myc and MMP-9 protein levels in sevoflurane-treated Huh7 and MHCC97 cells (Fig. 7G,H). Furthermore, SGTB overexpression relieved miR-19a-3p-mediated the inhibitory effect on cell apoptosis (Fig. 7I) and the protein levels of Cleaved-casp3 and Cleaved-casp9 (Fig. 7J,K) in sevoflurane-treated Huh7 and MHCC97 cells. Altogether, miR-19a-3p mediated HCC cell progression by regulating SGTB in sevoflurane-treated HCC cells.

Discussion

Sevoflurane has been found to suppress tumor progression in many cancers, comprising glioma, head and neck squamous cell cancer, breast cancer, colorectal cancer, and HCC (Yi et al., 2016; Liu et al., 2018a; Yang et al., 2018; Fan et al., 2019; Song et al., 2019; Sun et al., 2019). In our study, we also proved that sevoflurane curbed the development of HCC cells. Moreover, circ_0001649 was upregulated when HCC cells were treated with sevoflurane. Subsequent experiments revealed that sevoflurane affected HCC cell development via circ_0001649. Likewise, miR-19a-3p was the target of circ_0001649 and directly targeted SGTB. Our data demonstrated that sevoflurane suppressed the development of HCC cells via the circ_0001649/miR-19a-3p/SGTB axis.

Sevoflurane was revealed to exert a suppression function in HCC and has been used for clinical therapy (Zhang et al., 2018c; Song et al., 2019). Song et al. suggested sevoflurane inhibited Huh7 and HepG2 cells progression via downregulating DNA methyltransferase 3 alpha (Dnmt3a) through miR-29a (Song et al., 2019). Consistent with this study, we uncovered that sevoflurane repressed cell vitality in a dose-dependent manner in HCC cells. Besides, sevoflurane hindered metastasis and enhanced apoptosis of HCC cells. Subsequently, we uncovered that the circ_0001649 level was elevated in sevoflurane-treated HCC cells, implying that circ_0001649 might mediate the sevoflurane-induced inhibitory effect on HCC cells. Circ_0001649 was reported to be closely related to tumor progression. Down-regulation expression was found in HCC, colorectal cancer, cholangiocarcinoma, non-small cell lung cancer, and so on, and a lower expression of circ_0001649 is associated with poor prognosis, thereby circ_0001649 might be a probable prognostic target in these cancers (Qin et al., 2016; Ji et al., 2018; Liu et al., 2018c; Xu et al., 2018). In the present study,

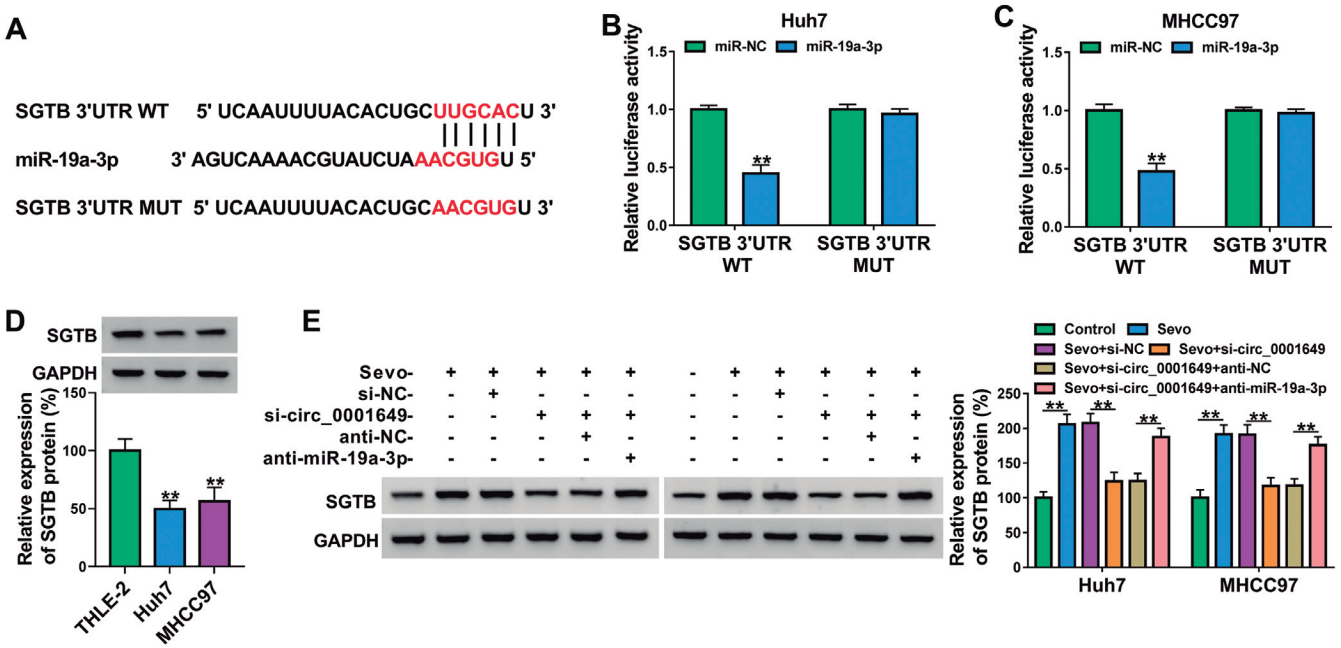

Fig. 6. SGTB was the target of miR-19a-3p. **A.** The complementary binding sites of SGTB and miR-19a-3p were shown. **B and C.** The luciferase activities of Huh7 and MHCC97 cells transfected with SGTB 3'UTR WT or SGTB 3'UTR MUT and miR-NC or miR-19a-3p. **D.** The protein level of SGTB in THLE-2, Huh7 and MHCC97 cells was detected by qRT-PCR. **E.** SGTB protein level in Huh7 and MHCC97 cells treated with Sevo, Sevo+si-NC, Sevo+si-circ_0001649, Sevo+si-circ_0001649+anti-NC, or Sevo+si-circ_0001649+anti-miR-19a-3p. **P<0.01.

overexpression of circ_0001649 retarded HCC cell progression. Besides, circ_0001649 knockdown annulled the inhibitory influence of sevoflurane on HCC cells, confirming that sevoflurane suppressed HCC cell development via inducing circ_0001649 expression.

Emerging research manifested that circRNAs targeted miRNAs to regulate gene expression (Xiong et al., 2018; Verduci et al., 2019). Online software forecasted that circ_0001649 had complementary binding sites of miR-19a-3p. Furthermore, circ_0001649 negatively regulated miR-19a-3p level in HCC cells. MiR-19a-3p contributed to cell growth via modulating PI3K/AKT axis in gastric cancer (Li et al., 2019). Moreover, miR-19a-3p interacted with nuclear factor-kappaB (NF-κB) and promote ovarian cancer development (Bai et al., 2019). Additionally, miR-19a-3p enhanced the resistance to sorafenib in HCC (Jiang et al., 2018). In accordance with these studies, we detected that miR-19a-3p level was elevated in HCC cells, and knockdown of miR-19a-3p restored circ_0001649 silencing-mediated promoting influences on proliferation, migration, and invasion, and the inhibitory influence on apoptosis in sevoflurane-treated HCC cells, intimating that the tumor suppressor role of miR-19a-3p in HCC. Also, the regulatory network of the circ_0001649/miR-19a-3p axis was identified in sevoflurane-induced HCC cells.

In addition, we predicted that the SGTB sequence harbored the complementary binding fragments of miR-19a-3p. SGTB belongs to the SGTB family, which has been suggested to exert essential functions in cancers and diseases (Cao et al., 2013; Bao et al., 2016; Sun et al., 2020; Zhou et al., 2021). In addition, SGTB was suggested to be regulated by miR-365b and constrained HCC cell migration and invasion (Tian et al., 2019). These results indicate the pivotal role of SGTB in HCC cancer development. Consistent with previous research, SGTB was found to be lower expressed in HCC tissues. Besides, sevoflurane elevated the expression of SGTB by regulating the circ_0001649/miR-19a-3p axis. In addition, overexpression of SGTB partly overturned the elevation impact of miR-19a-3p in sevoflurane-treated HCC cells. Altogether, sevoflurane repressed the malignant progress of HCC cells by regulating the circ_0001649/miR-19a-3p/SGTB axis.

There were also some deficiencies in the present study. Firstly, we didn't collect clinical samples to verify the role of sevoflurane in HCC tissue due to time and condition limitations. Moreover, an in vivo experiment was preferable to support our conclusion.

In conclusion, we found that sevoflurane suppressed HCC cell progression, and the subsequent experiments proved that sevoflurane regulated the behaviors of HCC cells via the modulating circ_001649/miR-19a-3p/SGTB

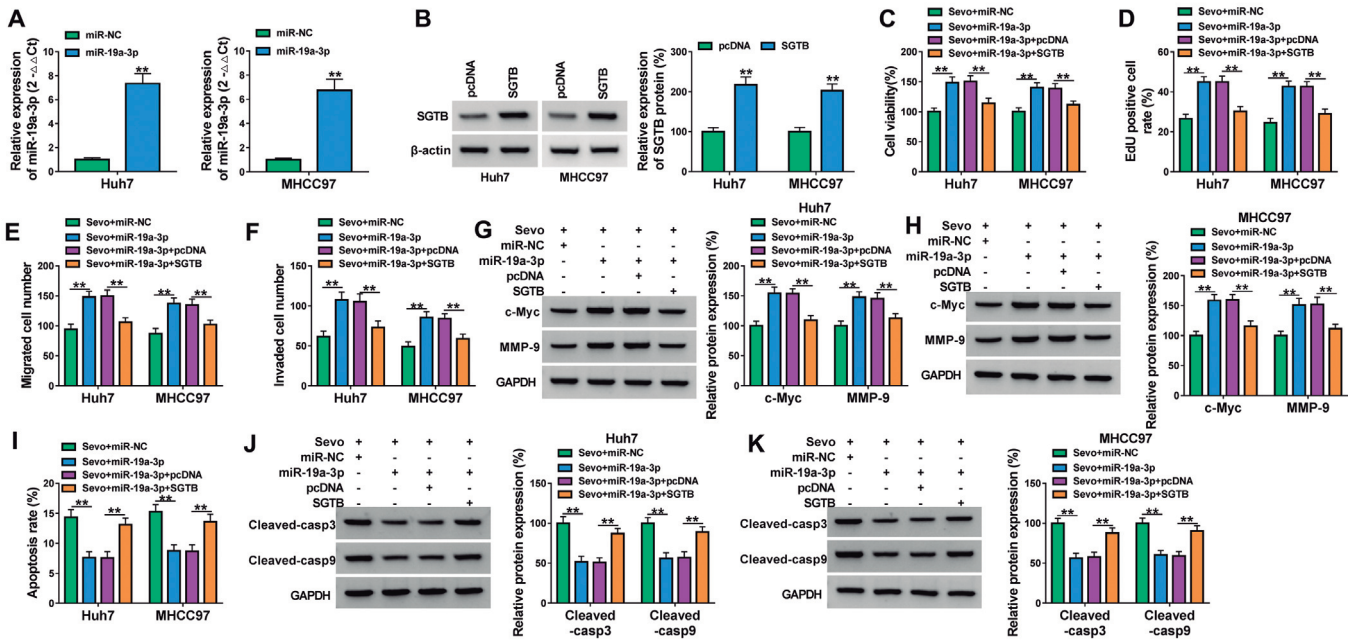

Fig. 7. Overexpression of SGTB partly overturned the effect of miR-19a-3p in sevoflurane-treated HCC cells. **A.** The expression of miR-19a-3p was detected by qRT-PCR in Huh7 and MHCC97 cells transfected with miR-NC or miR-19a-3p. **B.** The protein level of SGTB in Huh7 and MHCC97 cells transfected with SGTB or pcDNA. **C-K.** Huh7 and MHCC97 cells were treated with Sevo+miR-NC, Sevo+miR-19a-3p, Sevo+miR-19a-3p+pcDNA or Sevo+miR-19a-3p+SGTB. **C and D.** Cell proliferation was assessed by MTT and EdU assays. **E and F.** Transwell assay was conducted to evaluate cell migration and invasion. **G and H.** The protein levels of c-Myc and MMP9 were detected by western blot. **I.** Cell apoptosis was determined by flow cytometry. **J and K.** The protein levels of apoptosis-related proteins Cleaved-cas-3. **P<0.01.

axis, which might contribute to the improvement of therapeutic strategy for HCC.

Authors' contributions. Nai Sun conceived and designed the experiments, performed the experiments and analyzed and interpreted the data, wrote the paper. Jianzhuang Gong performed the experiments, analyzed and interpreted the data and participated in the methodology. Wei Zhang and Xiaochen Yang analyzed interpreted the data, performed the experiments. Jiaying Liu analyzed and interpreted the data.

Availability of Data and Material. All data generated or analyzed during this study are included in this article.

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