Sevoflurane Inhibits Metastasis in Hepatocellular Carcinoma by Inhibiting MiR-665-Induced Activation of the ERK/MMP Pathway

Xiaoyan Zhu¹,², Chuchu Peng¹, Zhiyong Peng³, Ruimin Chang²,⁴, and Qulian Guo¹,²

Abstract
Recent evidence has indicated that inhalational anesthetics may affect the growth and malignant potential of tumor cells and ultimately influence tumor recurrence after surgery. Sevoflurane, a volatile anesthetic, is used extensively in hepatectomy. However, the effect of sevoflurane on the growth of hepatocellular carcinoma (HCC) cells remains unknown. The aim of this study was to explore the effects of sevoflurane on HCC metastasis and its potential mechanisms in the human HCC cell lines, HepG2 and SMMC7721. HepG2 and SMMC7721 cells were treated with 1.7%, 3.4%, and 5.1% sevoflurane for 6 h. Cell migration was analyzed using invasion, migration, and scratch assays. Based on previous literature, several microRNAs (miRNAs) were screened to determine regulatory miRNA targets of sevoflurane in HepG2 and SMMC7721 cells; miR-665 was detected as a potential target and overexpressed or inhibited in HepG2 and SMMC7721 cells by a lentiviral system. The p-ERK/MMP pathway was also measured by western blotting. Sevoflurane inhibited the migration and invasion of HCC cells in a dose-dependent manner. It also inhibited miR-665 expression in HCC cells. We further observed that sevoflurane inhibited HCC metastasis via miR-665. Sevoflurane-induced downregulation of miRNA-665 led to phosphorylation of ERK and matrix metalloproteinase (MMP-9) via suppression of SPRED1. These results demonstrated that sevoflurane may inhibit invasion and migration via the p-ERK/MMP-9 signaling pathway in HCC cells.

Keywords
sevoflurane, anesthetics, hepatocellular carcinoma, miR-665, metastasis

Introduction
Hepatocellular carcinoma (HCC) is the sixth leading cause of cancer and the third leading cause of cancer-related deaths worldwide¹. Surgery remains the most effective therapy for HCC. However, recurrence or metastasis remains the most common cause of mortality after the intended curative therapy¹. Importantly, tumor dissemination may occur during surgery in patients. Anesthesia is a necessary procedure for hepatectomy. It has been reported that anesthesia techniques have an impact on the invasive and migratory abilities of cancer cells, which may affect the prognosis of patients.

The anesthetic agent used in surgery may be an important factor affecting the features of cancer cells. For example, midazolam enhances free radical production and suppresses proliferation and migration of human lung carcinoma cells². In contrast, morphine promotes tumorigenesis and cetuximab resistance in colorectal cancer via activation of epidermal growth factor receptor (EGFR) signaling³. Therefore, it is necessary to investigate the role of anesthetics in cancer. Moreover, relative inhibitors may be used with anesthetic agents to reduce the dissemination of tumor cells during surgery.

¹ Department of Anesthesiology, Xiangya Hospital, Central South University, Changsha, China
² National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, China
³ Department of Anesthesiology, Shenzhen Hospital, Southern Medical University, Guangdong, China
⁴ Department of Thoracic Surgery, Xiangya Hospital, Central South University, Changsha, China

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Corresponding Author:
Ruimin Chang, Department of Thoracic Surgery, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha 410008, Hunan, China.
Email: changruimin@163.com
Sevoflurane is a commercially available anesthetic that is widely used in liver surgery. In recent years, several studies have focused on the effect of sevoflurane on the progression of tumor cells. Kvolik et al.\textsuperscript{4} showed that sevoflurane induced late apoptosis in colonic and laryngeal cancer cells. Sevoflurane suppresses lung cancer metastasis by modulating hypoxia-inducible factor-1α (HIF-1α) or cancer-associated platelets\textsuperscript{5,6}. It has also been reported that sevoflurane may affect the metastasis of renal cell carcinoma\textsuperscript{7} and glioma\textsuperscript{8,9}. It is also reported to be associated with the function of breast\textsuperscript{10–12}, cervical\textsuperscript{13}, and colorectal cancer cells\textsuperscript{14}. All these studies proved that sevoflurane exhibits a specific impact on the metastasis of cancer cells. However, there have been few reports on the role of sevoflurane in HCC metastasis and its regulatory mechanisms.

MicroRNAs (miRNAs) are short non-coding RNAs (19–22 nucleotides) that function as important regulators of tumor metastasis\textsuperscript{15,16}. Currently, 2,693 mature miRNAs have been identified in humans (http://www.mirbase.org/). A fraction of them have been shown to regulate the metastasis of liver cancer\textsuperscript{15,16}. Sevoflurane may regulate HCC metastasis by targeting miRNAs. In the present study, we aimed to explore whether sevoflurane has some impact on the metastasis of liver cancer. We also aimed to investigate whether sevoflurane regulates miRNA expression and influences the potential downstream pathways.

**Materials and Methods**

**Cell Lines and Cell Culture**

The human HCC cell lines HepG2 and SMMC7721 and the human hepatic cell line L02 were purchased from the Type Culture Collection (ATCC, Manassas, VA) in the United States. SMMC7721 and L02 were purchased from the Cell Bank of Typical Culture Preservation Committee of Chinese Academy of Science, Shanghai, China. Cells were stored in liquid nitrogen and cultured in an atmosphere containing 5% CO\textsubscript{2} at 37°C in high-glucose Dulbecco’s modified Eagle medium (Gibco, Grand Island, NY, USA) supplemented with penicillin (100 IU/ml), streptomycin (100 mg/ml), and 10% fetal bovine serum (FBS; Gibco).

**Exposure to Sevoflurane**

HepG2, SMMC7721, and L02 cells were treated under the following conditions: 95% air with 5% CO\textsubscript{2}, 1.7% sevoflurane (Abbott, Abbott Park, IL, USA) mixed with 95% air and 5% CO\textsubscript{2}, 3.4% sevoflurane mixed with 95% air and 5% CO\textsubscript{2}, or 5.1% sevoflurane mixed with 95% air and 5% CO\textsubscript{2}. Cells in the exponential growth phase were seeded in plates and cultured in a CO\textsubscript{2} incubator (Thermo Fisher Scientific, Waltham, MA, USA). According to an experimental protocol described previously\textsuperscript{18,19}, cell culture plates were placed in an airtight glass chamber with inlet and outlet connectors. The inlet port of the chamber was connected to an anesthesia machine (Cicero EM 8060; Dräger, Lübeck, Germany), and sevoflurane was delivered into the chamber using a sevoflurane vaporizer (Sevorane; Abbott) attached to the anesthesia machine. A gas monitor (PM 8060; Dräger) installed with an anesthetic machine at the chamber exit port was used to detect the concentrations of sevoflurane in the chamber. After being exposed to various concentrations of sevoflurane for 6 h, the cells were grown at 37°C in a CO\textsubscript{2} incubator for an additional 24 h and then assayed for cell migration and invasion or subjected to molecular analyses.

**RNA Isolation and Quantitative Real-Time PCR**

RNA was extracted from tissues and cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The PrimeScript RT reagent Kit (Takara, Kusatsu, Shiga, Japan) was used for reverse transcription. Quantitative polymerase chain reaction (PCR) was performed using SYBR Green Master. U6 was used as a control for miRNA, whereas GAPDH was used as a control for messenger RNA (mRNA). The primers for miR-665, miR-214, miR-206, miR-27a-3p, miR-135b-5p, miR-665, miR-200c, miR-7a, miR-15b, miR-634, and U6 were purchased from RiboBio (Guangzhou, China).

**Western Blotting**

The RIPA (Radio-Immunoprecipitation Assay) lysis buffer was used to extract total proteins. Proteins were separated by dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride membrane (PVDF) (Roche Life Sciences, Basel, Switzerland). Subsequently, the PVDF membrane was blocked with 5% skim milk. The membrane was incubated with the appropriate primary antibody overnight, followed by incubation with the secondary antibody. The antigen–antibody complex on the membrane was detected using enhanced chemiluminescence reagents (Thermo Scientific, Waltham, MA, USA). The antibodies against Sprouty-related EVH1 domain–containing protein 1 (SPRED1) (ab77079) and β-actin (ab8226) were purchased from Abcam (Cambridge, UK). Antibodies against extracellular signal–regulated kinases (ERKs) (sc-514302), matrix metalloproteinase (MMP-9) (sc-21733), p-ERK (sc-81492), and the corresponding secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

**Lentiviral Vector Construction**

miR-665-overexpressing lentivirus and miR-665-inhibiting lentivirus, and their corresponding negative control (NC) lentiviral vectors were purchased from GeneChem (Shanghai, China). The SPRED1 wild-type vector and mutant vector were purchased from GeneChem.
**Dual-Luciferase Reporter Assay**

The wild-type or mutant 3′-untranslated region (UTR) sequences of SPRED1 were inserted into the restriction sites located at the 3′ end of the luciferase gene of the pGL3 vector (GeneChem). The wild-type or mutant sequence is shown in Fig. 4B. The pGL3 vector with the wild-type or mutant 3′-UTR sequences of SPRED1 and pRL-TK vectors were co-transfected into the corresponding cells using Lipofectamine LTX (Invitrogen). Luciferase activity was assayed 48 h after transfection according to the manufacturer’s protocol (Promega, Madison, WI, USA). Firefly and Renilla luciferase activities were detected using a Dual-luciferase Reporter Assay System Kit (Promega) with a Victor X machine (Perkin-Elmer, Boston, MA, USA).

**Cell Viability Assay**

Cell viability was determined using the cell counting kit-8 (CCK-8; Dojindo, Kumamoto, Japan). In short, CCK-8 solution was added into each well, and the cells were incubated for 1 h at 37°C in a humidified atmosphere containing 95% air and 5% CO2. The absorbance was detected at 490 nm using a Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**Lactate Dehydrogenase (LDH) Release Assay**

Cells were tested with LDH released using a colorimetric assay kit (Jiancheng Bioengineering Institute). Briefly, the supernatant of cells was removed. After 150 μl of LDH release reagent was added and mixed, the compounds were incubated at 37°C in 5% CO2 for 1 h. Cell cytotoxicity was measured by the absorbance at 490 nm by a Microplate Reader (Bio-Rad Laboratories, Inc.).

**Wound Healing Assays**

HepG2 and SMMC7721 cells were seeded into 35-mm dishes and cultured under the above-mentioned conditions for 1 day. When the cells reached 100% confluence, a scraped line was created with a 200-μL pipette tip by gliding the tip across the cell surface once. The dishes were washed twice with phosphate-buffered saline (PBS). The medium was replaced with medium without fetal bovine serum (FBS). The dishes were then cultured for 24 h. The speed of wound closure was captured using an inverted microscope (TE-2000S; Nikon, Tokyo, Japan), and the rate of closure was assessed.

**Transwell Invasion and Migration Assays**

Cells (1 × 106) cultured in medium containing 0.1% bovine serum albumin were seeded into the upper chamber with (invasion assay) or without (migration assay) the Matrigel membrane (BD Biosciences, Franklin Lakes, NJ, USA). Medium containing 10% bovine serum albumin was placed in the chamber. After 24 h of incubation at 37°C, the gel and cells in the upper chamber were carefully removed. Cells adhering to the underside of the membrane were fixed and stained with ethanol and 0.1% crystal violet (Beyotime Institute of Biotechnology, Changsha, China). The number of cells was counted and analyzed.

**Statistical Analysis**

Data are expressed as mean ± standard deviation (SD) from four independent experiments. The differences between groups were analyzed by Student’s t-test when only two groups were compared or by one-way analysis of variance (ANOVA) for multiple comparisons. A two-tailed P value of less than 0.05 was considered statistically significant.

**Results**

**Sevoflurane Inhibits Migration and Invasion of HCC Cells**

To investigate the role of sevoflurane in metastasis of HCC cells, HepG2 and SMMC7721 cells were cultured with different concentrations of sevoflurane (1.7%, 3.4%, and 5.1%). Invasion, migration, and scratch assays were performed to assess the metastatic ability of HCC cells. Invasion assay showed that the number of HepG2 cells under the Transwell membrane of the 1.7% sevoflurane group (106.5 ± 7.4), 3.4% sevoflurane group (79.1 ± 8.8), and 5.1% sevoflurane group (45.6 ± 9.7) were all significantly lower than that of the control group (149.0 ± 5.2, P < 0.01) (Fig. 1A). The number of SMMC7721 cells of the 1.7% sevoflurane group (110.2 ± 11.1), 3.4% sevoflurane group (80.8 ± 7.6), and 5.1% sevoflurane group 53.2 ± 3.7) were significantly lower than that of the control group (150.2 ± 5.0, P < 0.01) (Fig. 1B). The migration assay also showed that the migratory ability of HepG2 and SMMC7721 cells in the control group (256.0 ± 16.37, 181.6 ± 10.45) was higher than that of the 1.7% sevoflurane group (207.6 ± 13.7, P<0.01, 139.6 ± 12.0, P<0.01), 3.4% sevoflurane group (157.0 ± 9.1, 112.2 ± 7.4), and 5.1% sevoflurane group (97.6 ± 8.3, 79.4 ± 6.2) (Fig. 1C, D). Furthermore, the scratch assay showed that the healing rate of HepG2 and SMMC7721 cells in the control group (53.1%, ± 4.3%, 68.5% ± 2.8%) was higher than that of the 1.7% sevoflurane group (53.0% ± 4.3%, 40.61% ± 5.0%), 3.4% sevoflurane group (46.7% ± 2.5%, 35.2% ± 2.3%), and 5.1% sevoflurane group (32.5% ± 2.2%, 20.1% ± 1.7%) (Fig. 1E, F). These data indicated that sevoflurane may suppress the migration and invasion of HCC cells. The inhibitory effect of sevoflurane increased gradually in a dose-dependent manner. Then, we detected the cytotoxicity of sevoflurane on HCC cell lines and normal liver cells with CCK-8 assay and LDH assay. Interestingly, the data...
Figure 1. Sevoflurane inhibits the metastasis of hepatocellular carcinoma cells. HepG2 cells and SMMC7721 cells of different groups were incubated with different concentrations of sevoflurane. (A, B) Transwell assays with a Matrigel were used to detect the metastasis of HepG2 cells and SMMC7721 cells of each group. The cell number in the visual field of each group was counted and compared. (C, D) Transwell assay without the Matrigel was employed to detect migration of HepG2 cells and SMMC7721 cells. The cell number in the visual field was counted and compared. (E, F) The migration capacity of HepG2 cells and SMMC7721 cells in each group was detected with a wound healing assay. The wound healing speed was compared after incubation with different concentrations of sevoflurane for 24 h. The width of the scratch was measured at 0 and 24 h time points. The wound healing speed is represented as a histogram. *P < 0.05, **P < 0.01.
showed that sevoflurane can cause HCC cell damage in a dose-dependent manner (Supplementary Fig. 1A–D), while only high concentrations of sevoflurane lead to significant damage to normal liver cells (Supplementary Fig. 1E, F), which is in accordance with previous studies\textsuperscript{20,21}. These results suggest that sevoflurane may be more toxic to tumor cells with high proliferative capacity due to some mechanism, which is not fully understood at present.

**Sevoflurane Inhibits MiR-665 Expression in HCC Cells**

To determine whether miRNAs are regulated by sevoflurane, real-time PCR was employed to determine the expression of miRNAs associated with sevoflurane exposure under other pathological states\textsuperscript{22,23}. We observed that miR-665 was significantly suppressed more than two folds in the 5.1% sevoflurane group than NC in HepG2 cells (Fig. 2A). And similarly results also can be seen in SMMC7721 cells (Fig. 2B). Thus, we speculated that sevoflurane may inhibit HCC metastasis by suppressing miR-665.

**Sevoflurane Inhibits Metastasis of HCC via MiR-665**

As sevoflurane (5.1%) inhibited metastasis and miR-665 expression more than two folds, we used miR-665 overexpression or miR-665 inhibition lentiviral vectors in these two groups. After 5.1% sevoflurane treatment, the expression of miR-665 decreased. This decline was reversed by miR-665 overexpression. Meanwhile, the expression of miR-665 in the sevoflurane 5.1% + NC group was mimicked in the control + anti-miR-665 group (Fig. 2C, D). Invasion, migration, and scratch assays were performed to evaluate the metastasis of HCC cells in these groups. The invasion assay showed that the invasive ability of the HepG2 and SMMC7721 cells in 5.1% sevoflurane + miR-665 group (162.0 ± 8.4, 122.6 ± 2.1) was similar to that of the control + NC group (154.2 ± 7.8, 128.0 ± 6.3) and was significantly higher than that of the 5.1% sevoflurane + NC group (47.0 ± 4.3, 79.0 ± 10.3). The invasive ability of the control + anti-miR-665 group (52.0 ± 5.8, 71.2 ± 13.3) was lower than that of the control + NC group (154.2 ± 7.8, 128.0 ± 6.3) and was similar to that of the 5.1% sevoflurane + NC group (47.0 ± 4.3, 79.0 ± 10.3) (Fig. 3A, B). The migration assay also showed that after normalizing the expression of miR-665, the migration of HepG2 and SMMC7721 cells was recovered. After inhibiting miR-665 expression, the migration of HepG2 and SMMC7721 cells was also suppressed (Fig. 3C, D). The wound healing assay showed that the closure speed of the 5.1% sevoflurane + miR-665 group (67.5% ± 5.9%, 74.7% ± 2.1%) was similar to that of the control + NC group (67.6% ± 5.9%, 70.3% ± 4.6%) and was higher than that of the 5.1% sevoflurane + NC group (40.0% ± 4.2%, 35.1% ± 3.3%). The closure speed of the control + anti-miR-665 group (38.0% ± 2.5%, 37.5% ± 1.6%) was lower than that of the control + NC group (67.6% ± 5.9%, 70.3% ± 4.6%) (Fig. 3E, F). These results showed that overexpression or inhibition of miR-665 expression may reverse or mimic the effect of sevoflurane on HCC metastasis, indicating that sevoflurane can inhibit metastasis of HCC cells via miR-665.

**Sevoflurane-Induced Downregulation of MiRNA-665 Leads to Phosphorylation of ERK and MMP-9 Expression via the Suppression of SPRED1**

To identify the target of miR-665, we screened the predicted target mRNAs of miR-665 from three miRNA databases.
Figure 3. Reintroduction of miR-665 abrogates the tumor suppressive ability of sevoflurane in HepG2 cell and SMMC7721 cells. Transwell assay with Matrigel (A), Transwell assay without Matrigel (B) and wound healing assays (C) were used to detect metastasis and migration capacities of HepG2 cells and SMMC7721 cells. Histogram represents the statistical results of corresponding assays. NC: negative control. $^\#P \geq 0.05$; $^*P < 0.05$; $^{**}P < 0.01$. 
These predicted targets contained intersection elements and are represented as a Venn diagram in Fig. 4A. Relevant literature about these 94 common predictive targets was reviewed to identify mRNAs that may function as tumor suppressors. SPRED1 was a predictive mRNA target with three predicted binding sites (Fig. 4B). Mutant or wild-type 3′-UTR of SPRED1 was cloned on the respective PGL3 vectors containing the firefly luciferin gene, as shown in Fig. 4B. The wild-type or mutant PGL3 vector was transfected with the pRL-TK vector into HepG2 and SMMC7721 cells. The dual-luciferase reporter assay showed that the intensity of firefly luciferin was significantly suppressed in the wild-type group than the vector group and mutant group, but the intensity of firefly luciferin was similar to the vector group and mutant group because of the inhibition of miR-665. The intensity of firefly luciferin in the three groups was equal after inhibiting miR-665 expression (Fig. 4C, D). These results indicate that miR-665 may directly bind to the 3′-UTR of SPRED1. SPRED1 is an important inhibitor of the Ras MAPK pathway in cancer. Thus, we determined the expression of important metastasis-related proteins by western blotting in cells treated with different concentrations of sevoflurane. Concordant with the increase in sevofluran concentrations, p-ERK and MMP-9 (an endopeptidase that digests basement-membrane type IV collagen) expression decreased (Fig. 5A–C). This result indicated that sevoflurane activates the MAPK pathway. We reversed miR-655 expression to evaluate whether miR-655 expression may reverse the effect of sevoflurane. MiR-655 overexpression attenuated the function of sevoflurane, whereas miR-655 inhibition
mimicked it (Fig. 5D–H). Taken together, the present findings suggest that sevoflurane can regulate metastasis of HCC via the miR-665/SPRED1/p-ERK/MMP-9 pathway (Fig. 6).

**Discussion**

General anesthesia is routinely employed for hepatectomy. Recently, an increasing number of studies have shown that anesthetics used in surgery exhibit positive or negative effects on tumor recurrence. It is important for anesthesiologists to choose anesthetics that have anticancer effects during cancer surgery to reduce the risk of recurrence and metastasis in patients. Sevoflurane is a widely used analgesic in clinical surgery. It is suitable for anesthesia induction and maintenance because of its rapid recovery time and relatively low risk.

The aim of this study was to explore the effect of sevoflurane on the metastasis of HCC cells, as the mechanism of this drug is still unknown. To simulate the clinical anesthesia setting, HepG2, SMMC7721, and L02 cells were treated for 6 h with three commonly used concentrations of sevoflurane. Translational findings based on a clinical trial revealed that sevoflurane protects hepatocytes from ischemic injury by reducing reactive oxygen species–associated signaling in hematopoietic stem cells. Also, 2% sevoflurane does not trigger hepatocyte apoptosis in rat; it has no significant hepatotoxicity. Even after 3 days of 3% sevoflurane exposure, there was no evidence of hepatic or renal toxicity both from histologic studies and from serum biomarkers. Sevoflurane can increase proliferation and migration in an in vitro model of breast cancer. A recent study showed that the biological processes of HCC cells were suppressed by sevoflurane, and 4% sevoflurane exerts antiapoptotic activity and inhibits cell viability and invasive behavior in vitro. In this study, we demonstrated that inhalation of sevoflurane inhibits the metastasis of HCC cells by inhibiting miR-665/SPRED1/p-ERK/MMP-9 pathway (Fig. 6).

### Figure 5

MiR-665 targets SPRED1 and activates ERK and MMP-9. HepG2 cells were incubated with different concentrations of sevoflurane. Western blot bands indicate p-ERK and MMP-9 expression in each group (A). The gray value of p-ERK/β-actin and MMP-9/β-actin was measured and is shown in the figure (B, C). (D) Western blot shows SPRED1, ERK, ERK phosphorylation status, and MMP expression in HepG2 cells treated with sevoflurane or upon reinfecation with miRNA expression vectors. The gray value of SPRED1/β-actin, ERK/β-actin, p-ERK/β-actin, and MMP-9/β-actin is shown in the figure (E, H). ERK: extracellular signal–regulated kinase; MMP-9: matrix metalloproteinase-9; NC: negative control. *P < 0.05; **P < 0.01.
ERK/MMP-9 axis. Sevoflurane is more toxic to HCC cells than to normal liver cells. These results indicated that sevoflurane has certain antitumor activity. However, the mechanism of sevoflurane inhibits the expression of miR-665, the cause of inducing tumor cytotoxicity, and the EC50 of sevoflurane is still not fully understood. Further research is still needed.

MiRNAs are involved in regulating various cell processes, such as cell proliferation, migration, invasion, and apoptosis. Several miRNAs have been reported to participate in tumor progression, and thus, these functional miRNAs are considered potential therapeutic targets for cancer treatment. MiR-665 has been reported to be closely associated with pivotal signaling pathways in the pathogenesis of lung cancer and hepatocellular carcinoma. Upregulated miR-665 expression independently predicts poor prognosis of lung cancer and facilitates tumor cell proliferation, migration, and invasion. Another study revealed that it promotes HCC cell migration, invasion, and proliferation by decreasing Hippo signaling by targeting PTPRB (Protein Tyrosine Phosphatase Receptor Type B) both in vivo and in vitro. Widely known as a tumor suppressor, SPRED1 inhibits the Ras/Raf-1/ERK pathway and reduces the expression levels of MMP-9 and MMP-2. The SPRED1 cascade is also involved in tumor growth and metastasis in breast cancer.

**Conclusion**

In conclusion, we identified that miR-665 targets the SPRED1/p-ERK/MMP9 pathway and participates in the metastasis of HCC, and sevoflurane was able to inhibit the invasion and migration of HCC cells by inhibiting miR-665-induced activation of the p-ERK/MMP pathway in HCC. Our study supported that sevoflurane inhalation used in surgical anesthesia may reduce the postoperative metastasis. However, more clinical evidences are needed to confirm this hypothesis. This study will help elucidate the pharmacological effects of inhaled general anesthetics such as sevoflurane and emphasize that the selection of volatile anesthetics for patients with HCC during surgery is clinically significant.

**Data Availability Statement**

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding authors.
CRediT Author Statement

Xiaoyan Zhu: Conceptualization, Methodology, Writing – Original draft preparation.
Chuchu Peng: Methodology, Software.
Zhiyong Peng: Methodology, Software.
Ruimin Chang: Supervision, Conceptualization, Writing – Original draft preparation.
Qulian Guo: Supervision, Validation.

Ethical Approval

This project was approved by the Independent Ethics Committee of the Xiangya Hospital, Central South University, Hunan Province, China.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

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ORCID iD

Ruimin Chang https://orcid.org/0000-0003-3237-6478

Supplemental Material

Supplemental material for this article is available online.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
2. Wang C, Datoo T, Zhao H, Wu L, Date A, Jiang C, Sanders RD, Wang G, Bevan C, Ma D. Midazolam and dexamethasone affect neuroglioma and lung carcinoma cell biology in vitro and in vivo. Anesthesiology. 2018;129(5):1000–14.
3. Lu H, Zhang H, Weng ML, Zhang J, Jiang N, Cata JP, Ma D, Chen WK, Miao CH. Morphine promotes tumorigenesis and cetuximab resistance via EGFR signaling activation in human colorectal cancer. J Cell Physiol. 2021;236:4445–54.
4. Kvolik S, Dobrosevic B, Marzci S, Prlic L, Glavas-Obrovac L. Different apoptosis ratios and gene expressions in two human cell lines after sevoflurane anaesthesia. Acta Anaesthesiol Scand. 2009;53(9):1192–99.
5. Liang H, Yang CX, Zhang B, Wang HB, Liu HZ, Lai XH, Liao MJ, Zhang T. Sevoflurane suppresses hypoxia-induced growth and metastasis of lung cancer cells via inhibiting hypoxia-inducible factor-1alpha. J Anesth. 2015;29(6):821–30.
6. Liang H, Yang CX, Zhang B, Zhao ZL, Zhong YJ, Wen XJ. Sevoflurane attenuates platelets activation of patients undergoing lung cancer surgery and suppresses platelets-induced invasion of lung cancer cells. J Clin Anesth. 2016;35:304–12.
7. Ciechanowicz Z, Zhao H, Chen Q, Cui J, Mi E, Mi E, Lian Q, Ma D. Differential effects of sevoflurane on the metastatic potential and chemosensitivity of non-small-cell lung adenocarcinoma and renal cell carcinoma in vitro. Br J Anaesth. 2018;120(2):368–75.
8. Hurmath FK, Mittal M, Ramaswamy P, Umapaheswara Rao GS, Dalavakodihalli Nanjaiah N. Sevoflurane and thioental preconditioning attenuates the migration and activity of MMP-2 in U87MG glioma cells. Neurochem Int. 2016;94:32–38.
9. Yi W, Li D, Guo Y, Zhang Y, Huang B, Li X. Sevoflurane inhibits the migration and invasion of glioma cells by upregulating microRNA-637. Int J Mol Med. 2016;38(6):1857–63.
10. Li R, Huang Y, Lin J. Distinct effects of general anesthetics on lung metastasis mediated by IL-6/JAK/STAT3 pathway in mouse models. Nat Commun. 2020;11(1):642.
11. Wall TP, Crowley PD, Sherwin A, Foley AG, Buggy DJ. Effects of lidocaine and Src inhibition on metastasis in a murine model of breast cancer surgery. Cancers (Basel). 2019;11(10):1414.
12. Freeman J, Crowley PD, Foley AG, Gallagher HC, Iwasaki M, Ma D, Buggy DJ. Effect of perioperative lidocaine, propofol and steroids on pulmonary metastasis in a murine model of breast cancer surgery. Cancers (Basel). 2019;11(5):613.
13. Zhang W, Sheng B, Chen S, Zhao H, Wu L, Sun Y, Cui J, Zhu X, Ma D. Sevoflurane enhances proliferation, metastatic potential of cervical cancer cells via the histone deacetylase 6 modulation in vitro. Anesthesiology. 2020;132(6):1469–81.
14. Fan L, Wu W, Yang J, He J, Han X. Sevoflurane inhibits the migration and invasion of colorectal cancer cells through regulating ERK/MMP-9 pathway by up-regulating miR-203. Eur J Pharmacol. 2019;850:43–52.
15. Chang RM, Xiao S, Lei X, Yang H, Fang F, Yang LY. miRNA-487a promotes proliferation and metastasis in hepatocellular carcinoma. Clin Cancer Res. 2017;23(10):2593–604.
16. Fang T, Lv H, Lv G, Li T, Wang C, Han Q, Yu L, Su B, Guo L, Huang S, Cao D, et al. Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. Nat Commun. 2018;9(1):191.
17. Guo W, Qiu Z, Wang Z, Wang Q, Tan N, Chen T, Chen Z, Huang S, Gu J, Li J, Yao M, et al. MiR-199a-5p is negatively associated with malignancies and regulates glycolysis and lactate production by targeting hexokinase 2 in liver cancer. Hepatology. 2015;62(4):1132–44.

18. Liang H, Gu M, Yang C, Wang H, Wen X, Zhou Q. Sevoflurane inhibits invasion and migration of lung cancer cells by inactivating the p38 MAPK signaling pathway. J Anesth. 2012;26(3):381–92.

19. Roesslein M, Frick M, Auwaerter V, Humar M, Goebel U, Schwer C, Geiger KK, Pahl HL, Pannen BH, Loop T. Sevoflurane-mediated activation of p38-mitogen-activated stresskinase is independent of apoptosis in Jurkat T-cells. Anesth Analg. 2008;106(4):1150–60.

20. Ruxanda F, Gal AF, Ratiu C, Miclaus V, Rus V, Oana LI. Comparative immunohistochemical assessment of the effect of repetitive anesthesia with isoflurane and sevoflurane on rat liver. Braz J Anesthesiol. 2016;66(5):465–69.

21. Puig NR, Ferrero P, Bay ML, Hidalgo G, Valenti J, Amerio N, Elena G. Effects of sevoflurane general anesthesia: immunological studies in mice. Int Immunopharmacol. 2002;2(1):95–104.

22. Gao C, Shen J, Meng ZX, He XF. Sevoflurane inhibits glioma cells proliferation and metastasis through miRNA-124-3p/ROCK1 axis. Pathol Oncol Res. 2020;26:947–54.

23. Wang L, Wang T, Gu JQ, Su HB. Volatile anesthetic sevoflurane suppresses lung cancer cells and miRNA interference in lung cancer cells. Onco Targets Ther. 2018;11:5689–93.

24. Pasmant E, Gilbert-Dussardier B, Petit A, de Laval B, Luscan A, Gruber A, Lapillonne H, Deswarte C, Goussard P, Laurendeau I, Uzan B, et al. SPRED1, a RAS MAPK pathway inhibitor that causes Legius syndrome, is a tumour suppressor downregulated in paediatric acute myeloblastic leukaemia. Oncogene. 2015;34(5):631–38.

25. Yan T, Zhang GH, Wang BN, Sun L, Zheng H. Effects of propofol/remifentanil-based total intravenous anesthesia versus sevoflurane-based inhalational anesthesia on the release of VEGF-C and TGF-beta and prognosis after breast cancer surgery: a prospective, randomized and controlled study. BMC Anesthesiol. 2018;18(1):131.

26. Huang X, Teng Y, Yang H, Ma J. Propofol inhibits invasion and growth of ovarian cancer cells via regulating miR-9-NF-kappaB signal. Braz J Med Biol Res. 2016;49(12):e5717.

27. Beck-Schimmer B, Roth Z'graggen B, Booy C, Koppel S, Spahn DR, Schlapfer M, Schadde E. Sevoflurane protects hepatocytes from ischemic injury by reducing reactive oxygen species signaling of hepatic stellate cells: translational findings based on a clinical trial. Anesth Analg. 2018;127(4):1058–65.

28. Erimovic P, McHugh B, Murray D, Doran P, Buggy DJ. Effects of sevoflurane on breast cancer cell function in vitro. Anticancer Res. 2013;33(10):4255–60.

29. Song G, Tian L, Cheng Y, Liu J, Wang K, Li S, Li T. Antitumor activity of sevoflurane in HCC cell line is mediated by miR-29a-induced suppression of Dnmt3a. J Cell Biochem. 2019;120(10):18152–61.

30. Dong M, Xie Y, Xu Y. miR-7-5p regulates the proliferation and migration of colorectal cancer cells by negatively regulating the expression of Kruppel-like factor 4. Oncol Lett. 2019;17(3):3241–46.

31. Takahashi T, Nakano Y, Onomoto K, Yoneyama M, Ui-Tei K. LGBP2 virus sensor enhances apoptosis by upregulating apoptosis regulatory genes through TRBP-bound miRNAs during viral infection. Nucleic Acids Res. 2020;48(3):1494–507.

32. Yoshida K, Yokoi A, Kato T, Ochiya T, Yamamoto Y. The clinical impact of intra- and extracellular miRNAs in ovarian cancer. Cancer Sci. 2020;111(10):3435–44.

33. Qiao G, Li J, Wang J, Wang Z, Bian W. miR381 functions as a tumor suppressor by targeting ETS1 in pancreatic cancer. Int J Mol Med. 2019;44(2):593–607.

34. Hu Y, Yang C, Yang S, Cheng F, Rao J, Wang X. miR-665 promotes hepatocellular carcinoma cell migration, invasion, and proliferation by decreasing Hippo signaling through targeting PTPRB. Cell Death Dis. 2018;9(10):954.

35. Jin X, Guan Y, Sheng H, Liu Y. Crosstalk in competing endogenous RNA network reveals the complex molecular mechanism underlying lung cancer. Oncotarget. 2017;8(53):91270–80.

36. Xia J, Li D, Zhu X, Xia W, Qi Z, Li G, Xu Q. Upregulated miR-665 expression independently predicts poor prognosis of lung cancer and facilitates tumor cell proliferation, migration and invasion. Oncol Lett. 2020;19(5):6056–66.

37. Yoshida T, Hisamoto T, Akiba J, Koga H, Nakamura K, Tokunaga Y, Hanada S, Kunemura H, Maeyama M, Harada M, Ogata H, et al. Spreds, inhibitors of the Ras/ERK signal transduction, are dysregulated in human hepatocellular carcinoma and linked to the malignant phenotype of tumors. Oncogene. 2006;25(45):6056–66.

38. Jiang CF, Shi ZM, Li DM, Qian YC, Ren Y, Bai XM, Xie YX, Wang L, Ge X, Liu WT, Zhen LL, et al. Estrogen-induced miR-196a elevation promotes tumor growth and metastasis via targeting SPRED1 in breast cancer. Mol Cancer. 2018;17(1):83.