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Original Research Article

Sevoflurane induces lung cancer cell apoptosis via inhibition of the expression of miRNA155 gene

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Abstract

Purpose: *To determine the apoptotic effect of sevoflurane on lung cancer cells, and the underlying mechanism of action.*
Methods: *Lung adenocarcinoma A549 cells were cultured for 24 h and divided into control group, 1% sevoflurane group and 3% sevoflurane group. The two levels of sevoflurane were provided through a gas monitor connected to each of the sevoflurane groups. The control group was not treated. Flow cytometry was used to analyze A549 cell apoptosis, while qRT-PCR was used for assay of the levels of miRNA155 in A549 cells. The protein expression of Bcl-2 was determined with immunoblotting. The percentage of apoptosis and levels of miRNA155 and Bcl-2 in the two cell lines were compared.*
Results: *Significant differences in miRNA146a level were seen between the 3 % sevoflurane and control groups at 3 h. There was higher apoptosis in the 3 % sevoflurane group, relative to control, but miRNA155 levels in the 3 % sevoflurane group were generally less than that of the control ($p < 0.05$). There was lower Bcl-2 content in the 3 % sevoflurane group than in control group ($p < 0.05$).*
Conclusion: *Sevoflurane exerts strong apoptotic and anti-proliferative effects on lung adenocarcinoma A549 cells via a mechanism which may be related to the downregulation of miRNA155, thereby inhibiting the expression of anti-apoptotic protein Bcl-2. This provides a new direction for research on anti-lung adenocarcinoma drugs.*

Keywords: *Sevoflurane, Lung cancer cells, Apoptosis, Inhibition, miRNA155, Expression, Induction*

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INTRODUCTION

Malignant tumors are diseases that seriously endanger human health. Tumor cells are characterized by high malignant growth, rapid metastasis and high degree of recurrence, all of which impede the effectiveness of current cancer treatment strategies [1]. Lung cancer is a malignancy with origin in bronchial epithelium. The mortality associated with lung cancer ranks first among cancer-related deaths. Thus, lung cancer is a serious threat to human health and a source of heavy economic burden on the global medical and health system [2].

Lung cancer is divided into two types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC accounting for 80 % of lung cancers. Although NSCLC is treated with surgery, the disease is associated with high postoperative metastasis, while its 5-year survival is low [3]. Studies have found that in addition to surgical factors, anesthesia techniques and drugs affect the invasion and migration of lung cancer cells to a certain extent [4]. Therefore, it is important to identify anesthesia methods and drugs that can significantly slow down tumor metastasis in patients with lung cancer so as to improve their life expectancy.

Sevoflurane is a frequently-used inhalation anesthetic drug in clinical practice. It induces rapid, highly efficient and stable general anesthesia, and the depth of anesthesia is easy to adjust. It is often used as a general anesthetic drug in thoracic surgery [5]. In recent years, studies have revealed that sevoflurane inhibits tumor proliferation and migration to some extent, although the mechanism involved in the process has not been elucidated [6]. The present study was carried out to determine the apoptotic effect of sevoflurane on lung cancer cells, and the mechanism involved.

EXPERIMENTAL

Experimental cells, drugs, equipment and reagents

Lung carcinoma A549 cell line was obtained from Wuxi Xinrun Biological Technology Co. Ltd. Sevoflurane was product of Xiamen Huijia Biological Technology Co. Ltd. The equipment and reagents used, and their manufacturers (in brackets) were: ultra-clean workbench (Guangzhou Haohan Instrument Co. Ltd), CO_2 constant temperature incubator (Shanghai Xinyu Biotechnology Co. Ltd.), biological safety cabinet (Esco Trading Co. Ltd), centrifuge (Texang Technology Co. Ltd), vertical ultra-low temperature refrigerator (Guangzhou Hohan Instrument Co. Ltd), high-speed desktop centrifuge (Shanghai Safe Biotechnology Co. Ltd), low-temperature desktop high-speed centrifuge (Sichuan Shuke Instrument Co. Ltd) and microscope (Puma Precision Medical Technology Co. Ltd). The others were rabbit anti-Bcl-2 polyclonal antibody (Xiamen Yanke Biotechnology Co. Ltd), DMEM (Wuhan Purity Biotechnology Co. Ltd), reverse transcription kit (Beijing Biolab Technology Co. Ltd.), Trizol (Beijing Kairuiji Biotechnology Co. Ltd), apoptosis detection Reagent Kit (Beijing Kairuiji Biotechnology Co. Ltd), and flow cytometer (Shanghai Ranzhe Instrument Equipment Co. Ltd).

A549 cell culture and cryopreservation

The A549 cells were maintained in DMEM which contained 10 % FBS pre-warmed to 37 °C, pH 7.2. The cells were maintained at 37 °C in a 5 % CO_2 and saturated humidity incubator. Cell growth status was monitored under a microscope. The culture medium was changed in line with changes in pH of the medium or the cell status. After aspirating the medium, the cells were washed twice with sterile PBS solution, followed by addition of 0.5 – 1 mL of 0.25 % pancreatin and incubation. Under the microscope, the cell cytoplasm was shrunken. When the cells became rounded, the digestion was immediately stopped. Then, 10 mL of complete culture medium was added, with gentle pipetting so as to convert the cells into a single cell suspension. Care was taken to avoid introducing air bubbles into the culture flask. The cell suspension was transferred to a new culture flask and subjected to a 5-min centrifugation at 8000 rpm.

The supernatant was discarded, and the cells were re-suspended in 10 mL of complete culture medium. The culture medium was changed 24 h prior to cryopreservation, and cells at logarithmic growth phase were selected for use in subsequent experiments. The cells were centrifuged at 8000 rpm for 5 min. Then, the supernatant was replaced with complete culture medium, and sterile dimethyl sulfoxide (DMSO) was added dropwise as a protective agent. The cells were cryopreserved in a refrigerator at -80 ^{o}C overnight, after which they were transferred to liquid nitrogen for long-term storage.

A549 cell grouping and processing

The cell suspension was inoculated in A549 cell culture plate and cultured in an incubator at 37°C in an atmosphere of 5 % CO_2. The cells were divided into 3 groups: control group, 1 % sevoflurane group and 3 % sevoflurane group. The two levels of sevoflurane were provided through a gas monitor connected to each of the sevoflurane groups. The control group was not treated. After 1 and 3 h, samples were collected from each group, and the cells were photographed.

Measurement of apoptosis using flow cytometry

Adherent cells were digested with trypsin and centrifuged at 1000 rpm for 5 min. The cells were

washed twice with PBS solution, centrifuged at 1000 rpm for 5 min, and resuspended in 400 µL of binding buffer. Thereafter, the cells were treated with FITC, followed by treatment with PI, gentle mixing and incubation for 15 min at about 20 °C. The degree of apoptosis of the cancer cells was measured using computerized flow cytometry.

Determination of miRNAs levels in A549 cells

TRIzol was employed for RNA extraction. The cells were rinsed with PBS solution, followed by addition of 1ml of TRIzol to each well of the culture plate. The cell lysates were transferred to centrifuge tubes at room temperature for 5 min, followed by addition of 250 µL of chloroform to each centrifuge tube. The tubes were allowed to stand for 15 min at room temperature, prior to centrifugation at 12000 rpm for 15 min at 4 ^{o}C. Then, pre-cooled isopropanol (-20 °C) was added to each tube, with thorough mixing, followed by centrifugation in the cold at 4 °C. Then, 1 mL of 75 % ethanol was added, and the mixture was centrifuged at for 10 min at 4 °C, and dried at room temperature. Following addition of 20 µL of H_2O without RNase, the amount of the extracted RNA was determined via UV analysis.

Into an RNase-free PCR tube was put DEPC·H_2O mixed well and incubated on ice bath. Then, 4.1 µL of 5×reaction buffer, 0.6 µL of RiboLockTM ribonuclease inhibitor, 2.1 µL of dNTP mix, 1.1 µL of Reverse Tra Ace, and 1.1 µL Oligo (dT) primer were added to the tube. The reaction mixture was incubated at 42 °C for 620 min, and at 72 °C for 10 min. The resultant cDNA was freeze-dried at -20 °C or immediately used for PCR. The cDNA was put into a IQ5 fluorescent quantitative PCR instrument and subjected to SYBR Green method fluorescent quantitative PCR to determine the expression level of the tested gene. The relative quantification of gene expression was done with $2^{\triangle\triangle Ct}$ procedure.

Western-blot assessment of Bcl-2 protein content

After drug treatment, the cells were subjected to digestion with trypsin. The cells were recovered via centrifugation, and total protein was extracted using RIPA buffer containing protease inhibitors. The protein contents of the extracts were determined using BCA method. Equal amounts of total protein from each group were subjected to SDS-PAGE and transferred to PVDF membranes. The membranes were blocked by incubation with 5 % non-fat milk solution, prior to incubation with appropriate primary antibodies overnight at 4^{o}C. Thereafter, the membrane was washed and incubated with HRP-conjugated 2^{o} antibody at laboratory temperature for 1 h, followed by color development and quantitative analysis of protein expression levels. B-Actin was used as internal standard.

Statistical analysis

Measurement data are expressed as mean ± SD. Two-group comparison was carried put using Student's *t*-test. Count data are presented as numbers (n, %), and X^2 test was used for data analysis. The statistical analyses were done using SPSS 20.0 software. Values of $p < 0.05$ indicated significant differences.

RESULTS

Levels of miRNAs expression

As shown in Table 1, the miRNA146a level in 3 % sevoflurane group at 3 h differed statistically from the control value. In contrast, miRNA221, miRNA223 and miRNA34a levels were comparable between the two cell groups. Moreover, miRNA221, miRNA223, miRNA146a and miRNA34a levels did not differ within the 1 % sevoflurane group at 1 h and 3 h. These results are shown in Table 1.

Table 1: Expression levels of miRNAs

Group	miRNA34a		miRNA221		miRNA223		miRNA146a	
	1h	*3h*	*1h*	*3h*	*1h*	*3h*	*1h*	*3h*
Control	1.02 ± 0.09	0.97 ± 0.03	0.96 ± 0.04	0.95 ± 0.05	0.96 ± 0.04	0.94 ± 0.06	0.95 ± 0.05	0.91 ± 0.07
1% sevoflurane	0.94 ± 0.08	0.81 ± 0.07	0.90 ± 0.05	0.85 ± 0.06	0.92 ± 0.04	0.90 ± 0.05	0.92 ± 0.05	0.87 ± 0.05
3% sevoflurane	0.86 ± 0.15	0.75 ± 0.07	1.08 ± 0.04	1.05 ± 0.05	0.88 ± 0.07	0.89 ± 0.04	1.05 ± 0.07	4.15 ± 0.16[a]

[a]$P < 0.05$, compared with control group. Data are shown as mean ± SD

Apoptosis

As shown in Figure 1, apoptosis of cells in the 3 % sevoflurane group was significantly higher than that in the control group (4.1 % *vs* 16.2 % $p < 0.05$).

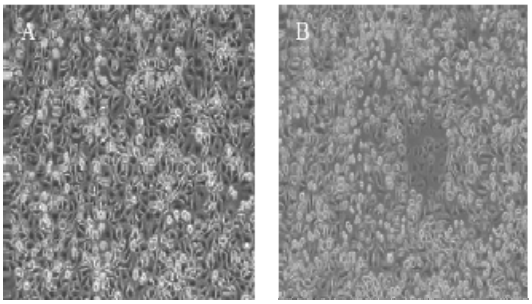

Figure 1: Apoptosis of cells in the two groups of cell lines. A: Apoptosis in control group; B: apoptosis in the 3% sevoflurane group

Relative expressions of miRNA155

Within the same group, miRNA155 at each time point did not differ statistically ($p > 0.05$; Figure 3). However, the relative expression of miRNA155 in the 3% sevoflurane group at each time point was significantly reduced in the control group.

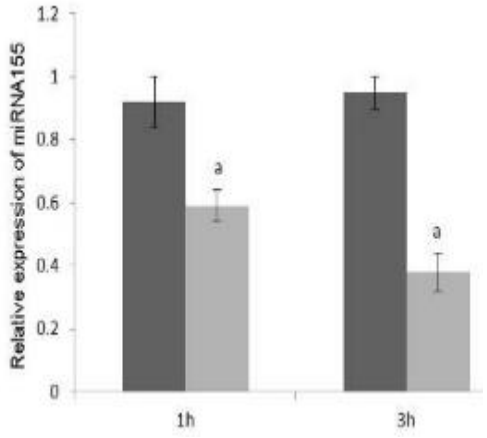

Figure 2: Relative expressions of miRNA155 within and between the 3 % sevoflurane group (grey bars) and control (dark bars). $^{a}P < 0.05$, compared with the control group

Bcl-2 protein contents

The Bcl-2 content of the 3% sevoflurane-treated cells group was significantly decreased, relative to control cells (Figure 3).

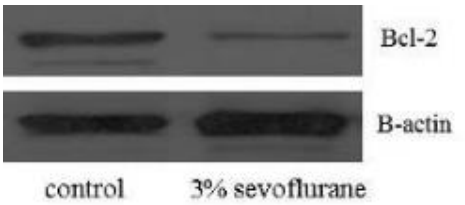

Figure 3: Bcl-2 contents of the 3 % sevoflurane group and control

DISCUSSION

The incidence of lung cancer in China has shown an increasing trend recently, with total prevalence ranking first among male malignant tumors [7]. At present, the pathogenesis of lung cancer is not well understood. An understanding of the pathogenesis of lung cancer, and targeted treatment for the disease are of great significance for prolonging the survival of patients and improving their quality of life [8]. The current methods used for the treatment of lung cancer involve surgery, chemotherapy and radiotherapy, among which surgical treatment is first-choice and the most effective method [9]. With increasing incidence of lung cancer and continuous improvements in thoracic surgery, the high degrees of recurrence and metastasis of lung cancer in patients after lung tissue resection have increasingly attracted the attention of clinicians [10].

Sevoflurane is one of the frequently used clinical anesthetics which mitigate inflammatory response during one-lung ventilation and reduce the aggressiveness of tumors [11]. Studies have shown that sevoflurane anesthesia maintains stable intraoperative hemodynamics and suppresses the proliferative potential and metastasis of pulmonary carcinoma tissue cells [12]. Excessive cell proliferation and obstruction of apoptosis of cancer cells play an important role in tumorigenesis. Previous studies have mainly focused on cell proliferation. In recent years, it has been found that reduction of cell death is involved in the survival of cancer cells [13]. Inhibition of apoptosis promotes tumor transformation and affects the response of tumor cells to anti-cancer treatments. The influence of sevoflurane on apoptosis and expressions of miRNA155 and Bcl-2 in A549 human lung adenocarcinoma cells was determined in this investigation. Apoptosis of cell lines in the 3 % sevoflurane group was significantly higher than that in the control group, suggesting that sevoflurane effectively enhanced apoptosis of the lung cancers, and inhibited lung cancer proliferation and metastasis.

It is known that miRNAs are involved in the regulation of cancer [14]. The miRNAs are implicated in prevention, treatment and prognosis of various respiratory diseases such as pulmonary fibrosis, chronic obstructive pulmonary disease, asthma, viral infectious respiratory diseases, tuberculosis, and lung malignancies. The expression of miRNA155 is related to NSCLC [15]. In this study, miRNA155 within the same group at different time points did not differ statistically. However, the relative expression of miRNA155 in the 3 % sevoflurane group at each time point was significantly lower than that in the control group. Moreover, Bcl-2 content of the 3 % sevoflurane-treated cells was markedly reduced, relative to the control value. These findings suggest that sevoflurane significantly increases apoptosis of lung adenocarcinoma A549 cells most likely through downregulation of miRNA155 and inhibition of Bcl-2 production.

CONCLUSION

The results obtained in this study indicate that sevoflurane exerts strong apoptotic and anti-proliferative effects on lung adenocarcinoma A549 cells through a mechanism which may be related to downregulation of miRNA155, thereby inhibiting production of Bcl-2. This provides a new direction for research on anti-lung adenocarcinoma drugs.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents. The study was conceived and designed by Na Li. Huaizhao Wang, Bin Wang, Jingyan Jing, Na Li collected and analyzed the data. Huaizhao Wang wrote the manuscript. All authors read and approved the manuscript for publication.

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REFERENCES

1. *Zheng R, Zeng H, Zuo T, Zhang S, Qiao Y, Zhou Q, Chen W. Lung cancer incidence and mortality in China, 2011. Thorac Cancer 2016; 7(1): 94-99.*
2. *Masters GA, Temin S, Azzoli CG, Giaccone G, Baker S Jr, Brahmer JR, Ellis PM, Gajra A, Rackear N, Schiller JH, et al. Systemic Therapy for Stage IV Non-Small-Cell Lung Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. J Oncol Pract 2017; 33(30): 832-837.*
3. *Shi Q, Zhou Z, Ye N, Chen Q, Zheng X, Fang M. MiR-181a inhibits non-small cell lung cancer cell proliferation by targeting CDK1. Cancer Biomark 2017; 20(4): 539-546.*
4. *Ou SH, Ahn JS, De Petris L, Govindan R, Yang JC, Hughes B, Lena H, Moro-Sibilot D, Bearz A, Ramirez SV, et al. Alectinib in crizotinib-refractory alk-rearranged non-small-cell lung cancer: a phase II global study. J Clin Oncol 2016; 34(7): 661-668.*
5. *Flatman K, Raj D. Obstructive sleep apnoea and anaesthesia. Anaesth Intensive Care Med 2017; 18(4): 185-189.*
6. *Wang A, Li Y, Wang T, Wang S. Copper ion-Selective Fluorescent Sensor Using Salicyl Fluorone Immobilized on Glutaraldehyde-Coated Eggshell Membrane. Anal Methods 2016; 8(9): 2112-2117.*
7. *Song MH, Kim BH, Ahn SJ, Yoo SH, Kang SW, Kim YJ, Kim DH. Peri-articular injections of local anaesthesia can replace patient-controlled analgesia after total knee arthroplasty: a randomised controlled study. Int Orthop 2016; 40(2): 295-299.*
8. *Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, Felip E, van den Heuvel MM, Ciuleanu TE, Badin F, et al. First-line nivolumab in stage IV or recurrent non–small-cell lung cancer. N Engl J Med 2017; 376(25): 2415-2426.*
9. *Hida T, Nokihara H, Kondo M, Kim YH, Azuma K, Seto T, Takiguchi Y, Nishio M, Yoshioka H, Imamura F, et al. Alectinib versus crizotinib in patients with ALK-positive non-small-cell lung cancer (J-ALEX): An open-label, randomised phase 3 trial. Lancet 2017; 390(10089): 29-39.*
10. *Zhao Z, Lv B, Zhang L, Zhao N, Lv Y. miR-202 functions as a tumor suppressor in non-small cell lung cancer by targeting STAT3. Mol Med Rep 2017; 16(2): 2281-2289.*
11. *Botman J, Dugdale A, Gabriel F, Vandeweerd JM. Cardiorespiratory parameters in the awake pigeon and during anaesthesia with isoflurane. Vet Anaesth Analg 2016; 43(1): 63-71.*
12. *Euasobhon P, Dej-Arkom S, Siriussawakul A, Muangman S, Sriraj W, Pattanittum P, Lumbiganon P. Lidocaine for*

reducing propofol-induced pain on induction of anaesthesia in adults. Cochrane Database Syst Rev 2016; 82(3): 3670-3676.

13. *Low AE, McEwan JC, Karanam S, North J, Kong KL. Anaesthesia-associated hypersensitivity reactions: seven years\" data from a British bi-specialty clinic. Anaesthesia 2016; 71(1): 76-84.*
14. *De Palma A, Cheleschi S, Pascarelli NA, Giannotti S, Galeazzi M, Fioravanti A. Hydrostatic pressure as epigenetic modulator in chondrocyte cultures: A study on miRNA-155, miRNA-181a and miRNA-223 expression levels. J Biomech 2018; 66: 165-169.*
15. *Gu J, Lu Z, Ji C, Chen Y, Liu Y, Lei Z, Wang L, Zhang HT, Li X. Melatonin inhibits proliferation and invasion via repression of miRNA-155 in glioma cells. Biomed Pharmacother 2017; 93: 969-975.*