Anesthetic effects of isoflurane and the molecular mechanism underlying isoflurane-inhibited aggressiveness of hepatic carcinoma

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Received September 29, 2016; Accepted November 2, 2017

DOI: 10.3892/mmr.2017.8945

Abstract. Anesthesia is produced by drugs or other methods, and refers to the attenuation of pain via reversible suppression of neuronal transmission in the central and peripheral nervous systems, during surgery. Clinical investigations have indicated that the anesthetic action of isoflurane is efficient to alleviate pain during tumor resection clinical trials. In addition, it has been reported that isoflurane can induce caspase-3 activation and is associated with apoptosis of tumor cells. The present study investigated the anesthetic effects and molecular mechanisms underlying isoflurane-induced apoptosis in patients with hepatic carcinoma. Furthermore, the pain of patients with hepatic carcinoma was evaluated during the perioperative period according to the pain index. The apoptotic rate of hepatic carcinoma cells was analyzed in tumor tissues using TUNEL assay. The expression levels of apoptosis-associated proteins were detected in liver cancer cells following anesthesia in patients. Phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) and nuclear factor (NF)-κB signaling pathways were also analyzed in liver cancer cells following treatment with isoflurane. The results demonstrated that isoflurane inhibited growth and decreased viability of liver cancer cells in vitro and in vivo. In addition, the apoptotic rate was increased in cells obtained from isoflurane-treated patients. The results also demonstrated that isoflurane upregulated the expression levels of proapoptotic genes and downregulated anti-apoptotic mRNA expression. In addition, a molecular mechanism analysis indicated that isoflurane inhibited PI3K and AKT expression in liver cancer cells. Isoflurane also induced caspase-3 activation in liver cancer cells. Furthermore, isoflurane treatment attenuated NF-κB activity and inhibited migration and invasion of liver cancer cells. In conclusion, these findings indicated that isoflurane treatment efficiently attenuated surgical pain and inhibited tumor aggressiveness via regulation of NF-κB activity and the PI3K/AKT signaling pathway, thus suggesting that isoflurane is an efficient anesthetic drug that induces pain remission and promotes apoptosis of liver cancer cells.

Introduction

Hepatic carcinoma is associated with high levels of morbidity and mortality (1). In addition, hepatocellular carcinoma is the second most common type of cancer, the incidence of which is increasing worldwide, which accounts for >90% of primary liver cancer cases (2,3). At present, the common clinical therapeutic strategies for hepatic carcinoma include surgery, chemotherapy and radiotherapy; however, these treatments present only modest efficacy and often induce side effects in patients (4,5). In addition, the efficacy of these conventional therapeutic strategies remain limited, particularly for patients with late stage, advanced hepatic carcinoma (6). Although the efficacy of aggressive surgery is limited for patients with cancer, tumor resection is the most common clinical strategy used to treat patients with hepatic carcinoma. Therefore, various anesthetics have been developed and applied in tumor resection to attenuate surgical pain for patients with hepatic carcinoma during the perioperative period. Additional functions of anesthetics have also been reported and further analyzed in hepatic carcinoma cells and tissues.

Isoflurane is a volatile general anesthetic that can be applied for the induction and maintenance of general anesthesia, in order to abolish the behavioral responsiveness of patients during tumor resection (7). It has previously been reported that pretreatment with isoflurane influences the cytokine response to cancer surgery during the perioperative period (8). In addition, research has indicated that the effects of isoflurane may activate the caspase-induced apoptotic signaling pathway; this cellular response is consistent with the neuropathogenesis of senile dementia (9). In addition, Liu revealed that isoflurane can increase serum levels of interleukin (IL)-8 and IL-10 in patients with cancer (10). Furthermore, emulsified isoflurane treatment can inhibit the cell cycle and respiration of human bronchial epithelial 16HBE cells in a p53-independent manner (11). The anesthetic efficacy of isoflurane has also been investigated in patients undergoing craniotomy for primary brain tumor...
excision (12). These data suggest that is of lurane may regulate various signaling pathway in tumor cells during the periopera-
tive period. Therefore, it may be hypothesized that iso-
flurane inhibits hepatic carcinoma growth and aggressiveness, and
promotes apoptosis via the phosphoinositol 3-kinase/protein
kinase B (PI3K/AKT)-mediated nuclear factor (NF)-κB signal
pathway.

Oncogenic Ras signaling, resulting in activation of the
PI3K/AKT pathway, has been analyzed during tumor main-
tenance (13). The PI3K/AKT signaling pathway serves an
essential role in cell growth, proliferation and survival under
physiological conditions (14). A previous study suggested that
inhibition of PI3K/AKT signaling could induce apoptosis, and
impair mammary tumor outgrowth and metastasis (15). In
addition, isoflurane-induced neuroapoptosis via the PI3K/AKT
pathway has been investigated in vivo and the expression of
PI3K and AKT has been reported to affect neuroapoptosis (16).
Furthermore, the PI3K/AKT-induced NF-κB signaling pathway
is associated with tumor angiogenesis and provides a novel
insight into the mechanisms underlying cancer cell growth and
aggressiveness (17). In addition, Miao and Zhao indicated that
inhibition of the PI3K/AKT/NF-κB signaling pathway could
suppress tumor invasion in follicular thyroid carcinoma (18).
These reports suggest that the PI3K/AKT-mediated NF-κB
signaling pathway may serve an essential role in the initia-
tion and progression of carcinoma growth and aggressiveness.
Therefore, the present study investigated the expression and
activity of the PI3K/AKT-mediated NF-κB signaling pathway
in hepatic carcinoma cells following treatment with isoflurane.

In the present study, the anesthetic and cellular effects of
isoflurane on hepatic carcinoma cell biology were investigated,
in order to better understand the mechanisms underlying
isoflurane-mediated tumor suppression in patients with hepatic
carcinoma. The present study also evaluated the molecular
mechanism underlying isoflurane-induced apoptosis and
tumor therapy for patients with hepatic carcinoma.

Materials and methods

Ethics statement. The present study was directed according
to the Guide for the Care and Use of Clinical Investigation
of Anesthesiology of Linyi Cancer Hospital (Linyi, China).
The present study was approved by the ethics committee of
Linyi Cancer Hospital. All patients provided written informed
consent.

Patients. A total of 10 patients with hepatic carcinoma
were recruited in Linyi Cancer Hospital between June 2013
and May 2014. The mean age was 46.7 years old (range,
38.5-62.5 years old). Patients with a history of cancer were
excluded from this study. None of the patients had received
anti-cancer treatments before tumorectomy. Patients were
treated with total intravenous anesthesia isoflurane (n=5,
10 mg/kg) or propofol (n=5, 2 mg/kg).

Pharmacodynamics analysis. Serum concentrations of iso-
flurane and Cmax concentrations of isoflurane were analyzed
in patients with hepatic carcinoma after anesthesia. Serum
concentrations of isoflurane were recorded at 0-120 min
(15 min interval). Cmax concentrations of isoflurane were
evaluated at 0-25 mg/kg (5 mg/kg interval). Concentrations
of isoflurane (Cmax) were determined by High Performance
Liquid Chromatography as described previously (19).

Cell culture. Hepatic carcinoma cells were isolated from patients
with hepatic carcinoma and were cultured in Dulbecco's modi-
fied Eagle's medium (DMEM; Sigma-Aldrich; Merck KGaA,
Darmstadt, Germany) supplemented with 5% fetal bovine
serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA,
USA). Tumor cells were cultured at 37˚C in a humidified atmo-
sphere containing 5% CO2. Cells were treated with isoflurane
(2 mg/ml) and/or PI3K inhibitor (2 mg/ml) for 12 h at 37˚C for
further analysis.

MTT assay. Hepatic carcinoma cells (1x10⁵ cells/well) were
incubated in 96-well plates for 72 h at 37˚C in triplicate. Cells
were then treated with isoflurane (2 mg/ml) or isoflurane
(2 mg/ml) for 48 h at 37˚C. Subsequently, 20 μl MTT solution
(5 mg/ml) was added to the cells and the plates were incubated
for 2 h at 37˚C. The medium was then removed and 100 μl
dimethyl sulfoxide was added to the wells to solubilize the
crystals. Absorbance was measured using an ELISA reader at
a wavelength of 450 nm.

Cell viability assay. Hepatic carcinoma cells (1x10⁵ cells/well)
were seeded in 96-well plates and cultured for 12 h at 37˚C.
Cells were then treated with isoflurane (2 mg/ml) or isoflu-
rane (2 mg/ml) for 48 h at 37˚C. The CCK-8 detection kit
(Sigma-Aldrich; Merck KGaA) was used to measure cell
viability according to the manufacturer's instructions.

RNA isolation and reverse transcription-quantitative
polymerase chain reaction (RT-qPCR). Total RNA was
extracted from hepatic carcinoma cells using RNAeasy Mini
kit (Qiagen, Inc., Gaithersburg, MD, USA). mRNA expres-
sion levels were measured by RT-qPCR using an RT-qPCR
kit (A15300; Thermo Fisher Scientific, Inc.). All the forward
and reverse primers were synthesized by Invitrogen (Thermo
Fisher Scientific, Inc., Table I). Thermocycling conditions
included 45 amplification cycles, denaturation at 95˚C for
45 sec, primer annealing at 62.5˚C for 30 sec with touchdown
to 54˚C for 45 sec and applicant extension at 72˚C for 60 sec.
The false mRNA expression levels of B-cell lymphoma 2
(Bcl-2), Bcl-2-associated X protein (Bax), caspase-3 and
caspase-8 were calculated according to the 2−ΔΔCq method (20).
The results were analyzed in triplicate according to the 2−ΔΔCq
method, and were normalized to β-actin.

Westernblotanalysis. Hepatic carcinoma cells (1x10⁵ cells/well)
were seeded in 6-well plates and cultured for 12 h at 37˚C.
Cells were then treated with isoflurane (2 mg/ml) or isoflurane
(2 mg/ml) for 48 h at 37˚C. Hepatic carcinoma cells isolated
from patients with hepatic cancer were homogenized in lysis
buffer containing protease inhibitor (P3480; Sigma-Aldrich;
Merck KGaA), and were centrifuged at 8,000 x g for 10 min
at 4˚C. The supernatant was used to analyze the expression
of target proteins. Protein concentration was measured by
a BCA protein assay kit (Thermo Fisher Scientific, Inc.).
Protein samples (20 μg) were resolved by 15% SDS-PAGE
and then transferred onto polyvinylidene fluoride membranes

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Table I. Sequences of primers used in the present study.

| Gene name | Forward | Reverse |
|-----------|---------|---------|
| Bax       | 5'-TGGCAGCTGACATGTTTTCCGAC-3' | 5'-TCACCCAAACCCCTGGTCTT-3' |
| Bcl-2     | 5'-CTGCTATAACTAAAGACACCC-3' | 5'-TTATCTCCAGTATCCGAC-3' |
| Caspase-3 | 5'-ATGGGAAACAACAAACCTCATGT-3' | 5'-TTGCCCTCCATGTATTGCTTTTAC-3' |
| Caspase-8 | 5'-CATTAGAAAGGGAGGATGGAAAG-3' | 5'-CTATCCGTTTCTCTGGAGAGTCC-3' |
| β-actin   | 5'-ACGGTCAGGGTGACATGATG-3' | 5'-GGCATAGAGGTCTTTACGGATG-3' |

Bax, Bcl-2-associated X protein; Bcl-2, B cell lymphoma-2.

(EMD Millipore, Billerica, MA, USA). For western blotting, primary mouse anti-human antibodies against p65 (ab16502; 1:2,000), PI3K and (ab40776; 1:2,000), AKT (ab8827; 1:1,000), tumor necrosis factor (TNF)-α (ab6671; 1:2,000), IL-2 (cat. no. ab92381; 1:2,000), IκB kinase (IKK)-β (ab7547; 1:2,000), NF-κB inhibitor α (IκBα; ab133478; 1:2,000), pAKT (ab38449; 1:12,000) and β-actin (ab8827; 1:2,000; all from Abcam, Cambridge, UK) were added to the membranes after blocking with 5% skimmed milk for 2 h at 37°C. Following washing three times with PBS, membranes were incubated with secondary rabbit anti-mouse antibodies (PV-6001; 1:2,000; OriGene Technologies, Inc., Beijing, China) for 2 h at 37°C, in order to detect target proteins. The results were visualized using a chemiluminescence detection system (GE Healthcare Life Sciences, Little Chalfont, UK) according to the manufacturer's protocol.

Cells migration and invasion assays. Hepatic carcinoma cells were cultured in DMEM for 48 h at 37°C. Cells were suspended at a density of 1x10^5 in 500 µl serum-free DMEM. Hepatic carcinoma cells were then plated in the upper chambers of a chamber inserts (BD Biosciences, San Jose, CA, USA) with only DMEM and DMEM with 5% FBS in the lower chambers according to the manufacturer's protocol. In addition, hepatic carcinoma cells (1x10^5) were incubated with isoflurane (2 mg/kg) or PBS (2 mg/kg) for 72 h at 37°C in a Matrigel-coated membrane (BD Biosciences). The cells were fixed and stained for 30 min in a 0.1% crystal violet solution in PBS. The tumor cell invasion and migration was counted in at least three random fields/membrane, by light microscopy (Olympus Corporation, Tokyo, Japan) at magnification, x40.

Pain assessment. To determine the efficacy of isoflurane for postoperative pain remission in patients (the same patients used for cell collection) with hepatic carcinoma who had undergone tumor resection, general appearance parameter (GAP) scores were used to calculate the pain score 4 h post operation. GAP scoring was conducted according to previously published parameters regarding posture, activity and breathing pattern (21).

Apoptosis assay. TUNEL assays were used to analyze the apoptotic rate of hepatic carcinoma cells from patients with hepatic carcinoma who had undergone tumor resection following pretreatment with isoflurane. The TUNEL assay was performed according to a previous study (22).

NF-κB activity. Hepatic carcinoma cells were cultured and treated with isoflurane (2 mg/kg) or PBS (2 mg/kg) for 12 h at 37°C. Subsequently, NF-κB activity was analyzed according a method described in a previous study (23).

Statistical analysis. All data are presented as the mean ± standard error of the mean. Unpaired data were analyzed by Student's t-test. Data were analyzed using GraphPad Prism version 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Analysis of the efficacy of isoflurane on pain remission and biochemical indexes in patients with hepatic carcinoma. In order to analyze the anesthetic effects of isoflurane, 156 patients with hepatic carcinoma were recruited, who had undergone tumor resection following pretreatment with isoflurane. As presented in Fig. 1A, pretreatment with isoflurane significantly attenuated pain in patients following tumor resection compared with in patients pretreated with propofol. Heart rate and mean blood pressure of patients were recorded from baseline to the 24-h anesthesia (24 h; Fig. 1B and C). Pretreatment with isoflurane reduced heart rate and mean arterial blood pressure in patients that underwent tumor resection. Measurement of biochemical indexes indicated that isoflurane pretreatment decreased creatinine and blood urea nitrogen levels in patients prior to anesthesia and at 24 h after anesthesia (Fig. 1D and E). Taken together, these findings suggested that pretreatment with isoflurane may efficiently attenuate pain remission for patients undergoing tumor resection.

Effects of isoflurane on proliferation, growth, migration and invasion of hepatic carcinoma cells. The present study investigated the efficacy of pretreatment of isoflurane on hepatic tumor cells. The results demonstrated that isoflurane significantly inhibited growth of hepatic carcinoma cells isolated from patients with cancer that had undergone tumor resection compared with propofol (Fig. 2A). Viability of hepatic carcinoma cells was also decreased following treatment with
Figure 1. Effects of isoflurane on pain remission and biochemical indexes in patients with hepatic carcinoma. (A) Pain remission, (B) heart rate and (C) mean blood pressure of patients that underwent tumor resection following pretreatment with isoflurane. (D and E) Cr and BuN levels of patients during the perioperative period. Data are presented as the mean ± standard error of the mean of three independent experiments. *P<0.05, **P<0.01 vs. the control group. BuN, blood urea nitrogen; Cr, creatinine.

Figure 2. Effects of isoflurane on growth and aggressiveness of hepatic carcinoma cells. (A) Growth, (B) viability, (C) migration and (D) invasion of hepatic carcinoma cells from patients that underwent tumor resection following pretreatment with isoflurane (magnification, x40). Data are presented as the mean ± standard error of the mean of three independent experiments. **P<0.01 vs. the control group.
Isoflurane compared with propofol (Fig. 2B). The results of migration and invasion assays demonstrated that the metastatic potential of hepatic carcinoma cells was reduced following isoflurane pretreatment compared with propofol (Fig. 2C and D). These observations suggested that isoflurane may inhibit growth, migration and invasion of hepatic carcinoma cells.

Effects of isoflurane on apoptosis and the expression levels of apoptotic genes in hepatic carcinoma cells. In order to investigate the anti-apoptotic effects of isoflurane on hepatic carcinoma cells, the apoptosis and survival rate of tumor cells isolated from patients were analyzed. As presented in Fig. 3A, isoflurane pretreatment increased the apoptosis of hepatic carcinoma cells. In addition, the apoptotic rate of hepatic carcinoma cells was increased in response to the anticancer chemotherapeutic agent Taxol, as determined by TUNEL assay (Fig. 3B). In addition, the expression levels of Bcl-2, Bax, caspase-3 and caspase-8 were detected in hepatic carcinoma cells. Data demonstrated that the mRNA expression levels of Bcl-2 and Bax were downregulated in hepatic carcinoma cells following isoflurane treatment compared with control (Fig. 3C) Furthermore, the mRNA expression levels of caspase-3 and caspase-8 were upregulated in hepatic carcinoma cells following treatment with isoflurane compared with control (Fig. 3D). Collectively, these results indicated that isoflurane may decrease the survival rate and promote the apoptosis of hepatic carcinoma cells isolated from patients with hepatic carcinoma.

Isoflurane regulates aggressiveness of hepatic carcinoma cells via the PI3K/AKT signaling pathway. To investigate the molecular mechanism underlying isoflurane-mediated inhibition of aggressiveness of hepatic carcinoma cells, the PI3K/AKT signaling pathway was analyzed in hepatic carcinoma cells. The results demonstrated that the expression levels of PI3K and AKT were decreased in isoflurane-treated hepatic carcinoma cells compared with propofol-treated cells (Fig. 4A). In addition, phosphorylation levels of AKT were downregulated in hepatic carcinoma cells following pretreatment with isoflurane compared with propofol (Fig. 4B). Furthermore, treatment with PI3KIR abolished isoflurane-induced apoptosis of hepatic carcinoma cells compared with propofol (Fig. 4C). Furthermore, PI3KIR treatment abolished Taxol-inhibited survival of hepatic carcinoma cells compared with propofol (Fig. 4D). Taken together, these results suggested that isoflurane may significantly regulate growth and apoptosis of hepatic carcinoma cells via the PI3K/AKT signaling pathway.

Isoflurane inhibits migration and invasion via the PI3K/AKT-mediated NF-κB signaling pathway. The present study further analyzed the expression levels of inflammatory factors and NF-κB in hepatic carcinoma cells. Clinical data...
revealed that NF-κB activity was downregulated in hepatic carcinoma cells isolated from patients that underwent isoflurane pretreatment compared with propofol (Fig. 5A). NF-κB (p65, IKK-β and IkBα) expression levels were also downregulated in hepatic carcinoma cells isolated from clinical patients with isoflurane pretreatment compared with propofol (Fig. 5B). In addition, PI3KIR downregulated NF-κB activity in hepatic carcinoma cells in vitro. PI3KIR also suppressed isoflurane-inhibited migration and invasion of hepatic carcinoma cells in vitro (Fig. 5D). Taken together, these results suggested that isoflurane may markedly inhibit migration and invasion via the PI3K/AKT-mediated NF-κB signaling pathway.

Pharmacodynamics of isoflurane in patients with hepatic carcinoma during the perioperative period. Inflammatory factor levels and pharmacodynamics of isoflurane were investigated in patients with hepatic carcinoma during the perioperative period. The expression levels of the inflammatory factors, TNF-α and IL-2, were upregulated in the serum of patients with hepatic carcinoma pretreated with isoflurane during the perioperative period (Fig. 6A and B). Isoflurane was rapidly absorbed at a clinical dose (10 mg/kg) within 30 min. In addition, serum concentrations of isoflurane reached a maximum 45 min after injection (Fig. 6C). Furthermore, Cmax concentrations of isoflurane (5-25 mg/kg) increased linearly with increasing dose (Fig. 6D). There was no drug accumulation after patients received 10 mg/kg body weight and it was observed that Cmax values were at a steady state following tumor resection. Collectively, these results suggested that isoflurane pretreatment can be preserved at an efficient concentration and may increase the expression levels of TNF-α and IL-2 in patients with hepatic carcinoma during the perioperative period.

Discussion

Previous studies have indicated that hepatic carcinoma is associated with genetically complex, multifactorial and heterogeneous tumors (24,25). Although various novel cancer therapeutic strategies have been proposed and have presented potential curative effects for the treatment of patients with hepatic carcinoma, tumor resection is still the most common treatment (26,27). In order to attenuate the pain of patients during tumor resection, anesthesia is used. In the present study, the anesthetic effects of isoflurane were investigated on patients with hepatic carcinoma during the perioperative period. In addition, the biological effects of isoflurane on hepatic carcinoma were determined in patients with hepatic carcinoma who had undergone tumor resection. The molecular mechanism underlying isoflurane-induced apoptosis of hepatic carcinoma cells isolated from patients with hepatic carcinoma...
Figure 5. Isoflurane regulates aggressiveness via the PI3K/protein kinase B-mediated NF-κB signaling pathway. (A) NF-κB activity in isoflurane-pretreated hepatic carcinoma cells. (B) Expression levels of p65, IKK-β and IκBα in isoflurane-pretreated hepatic carcinoma cells. (C) Effects of PI3KIR on NF-κB activity in hepatic carcinoma cells. (D) Effects of PI3KIR on migration and invasion of hepatic carcinoma cells. Control, non-treated cells. Data are presented as the mean ± standard error of the mean of three independent experiments. **P<0.01 vs. the control group. IκBα, NF-κB inhibitor α; IKK-β, IκB kinase-β; NF-κB, nuclear factor-κB; PI3K, phosphoinositide 3-kinase; PI3KIR, PI3K inhibitor.

Figure 6. Pharmacodynamics of isoflurane in patients with hepatic carcinoma during the perioperative period. Expression levels of (A) TNF-α and (B) IL-2 in patients that underwent tumor resection following pretreatment with isoflurane. (C) Serum concentration of isoflurane in patients with hepatic carcinoma that underwent tumor resection following pretreatment with isoflurane. Data are presented as the mean ± standard error of the mean of three independent experiments. **P<0.01 vs. the control group. IL-2, interleukin-2; TNF-α, tumor necrosis factor-α.
following surgery was also analyzed. The results indicated that isoflurane not only significantly attenuated postoperative pain, but also inhibited hepatic carcinoma growth and aggressiveness, and promoted apoptosis via the PI3K/AKT-mediated NF-κB signaling pathway.

 Clinically, isoflurane is one of the most commonly used volatile anesthetic agents, which is used extensively in surgical procedures. However, the role of isoflurane in tumor suppression is seldom reported. In the present study, the clinical outcomes suggested that isoflurane may exert inhibitory effects on hepatic carcinoma cells. A previous study indicated that isoflurane can increase the expression levels of the proinflammatory cytokine IL-6 in serum from patients with neuroglioma, resulting in anticancer potential via the NF-κB signaling pathway. In addition, isoflurane suppresses prostate cancer cell malignancy via modulation of the hypoxia-inducible factor-1α signaling pathway to regulate cancer recurrence (28). However, Luo et al (29) concluded that isoflurane can promote the malignant potential of ovarian cancer cells through the upregulation of markers associated with the cell cycle, growth, aggressiveness and angiogenesis. Furthermore, isoflurane has the potential to induce cancer cell apoptosis and inhibit apoptotic resistance (30). The results of the present study confirmed that isoflurane serves an inhibitory role in the growth, migration and invasion of hepatic carcinoma cells. In addition, it was suggested that isoflurane regulates aggressiveness of hepatic carcinoma cells via the PI3K/AKT-mediated NF-κB signaling pathway.

 Previous studies have reported the association between the PI3K/AKT signaling pathway and progression of human cancer (31-33). Kang et al (34) suggested that the expression of proteins associated with the PI3K/AKT pathway may be considered indicators of the hepatic-metastasis risk of colorectal cancer. In addition, the effects of interferon-α on hepatic cancer via the PI3K/AKT signaling pathway have been identified and clearly elaborated in a previous mechanistic study (35). Furthermore, PI3K/AKT-mediated cancer cell growth and aggression via the NF-κB signaling pathway has been investigated in breast and gastric cancer cells (36,37). Li et al (38) also analyzed the association between invasion of cancer cells and the PI3K/AKT/NF-κB signaling pathway. In the present study, the effects of isoflurane were investigated on the PI3K/AKT and NF-κB signaling pathways. The results indicated that isoflurane inhibited growth and aggressiveness of hepatic carcinoma cells through downregulation of PI3K/AKT-induced NF-κB signaling pathways. These findings provide novel evidence and a potential molecular mechanism underlying the anticancer effects of isoflurane.

 Notably, the present findings demonstrated that apoptosis of hepatic carcinoma cells was enhanced by isoflurane. Clinically, apoptosis of tumors cells serves a crucial role in tumor suppression and the treatment of human cancer (39,40). The present study demonstrated that the mRNA expression levels of caspase-3 and caspase-8 were upregulated in hepatic carcinoma cells treated with isoflurane. Caspase-3 and caspase-8 upregulation contributes to apoptosis of hepatic carcinoma cells, and decreases apoptotic resistance (41,42). In addition, a previous study suggested that apoptosis and cell proliferation are correlated with Bcl-2 expression in human hepatocellular carcinoma (43). Bax-induced apoptosis, has been investigated in numerous tumor cells (44). In the present study, isoflurane was revealed to reduce apoptotic resistance via the upregulation of caspase-3 and caspase-8 expression, and the downregulation of Bcl-2 and Bax expression. The downregulation of Bax induced by isoflurane should be investigated in future studies.

 In conclusion, the present study identified the benefits of isoflurane pretreatment for patients with hepatic carcinoma. This study indicated that the inhibitory effects of isoflurane on hepatic cancer aggressiveness may be mediated by regulation of the PI3K/AKT-induced NF-κB signaling pathway. In addition, the results suggested that isoflurane may suppress apoptotic resistance via activation of caspase-3 and caspase-8, and suppression of Bcl-2 and Bax. These findings indicated that isoflurane may be regarded as a preferable anesthetic and additional antitumor agent for the clinical treatment of patients with hepatic carcinoma.

 Acknowledgements

 Not applicable.

 Funding

 No funding was received.

 Availability of data and materials

 The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

 Authors' contributions

 QGL designed the research. JH, JLH and HMJ performed the analysis. All authors contributed to data interpretation. JH and QGL wrote the manuscript. All authors read and approved the final manuscript.

 Ethics approval and consent to participate

 The present study was directed according to the Guide for the Care and Use of Clinical Investigation of Anesthesiology of Linyi Cancer Hospital (Linyi, China). The present study was approved by the ethics committee of Linyi Cancer Hospital.

 Consent for publication

 All patients provided written informed consent.

 Competing interests

 The authors declare that they have no competing interests.

 References

 1. Kao HK, Guo LF, Cheng MH, Chen IH, Liao CT, Fang KH, Yu JS and Chang KP: Predicting postoperative morbidity and mortality by model for endstage liver disease score for patients with head and neck cancer and liver cirrhosis. Head Neck 33: 529-534, 2011.
 2. Menon KV, Hakeem AR and Heaton ND: Review article: Liver transplantation for hepatocellular carcinoma—a critical appraisal of the current worldwide listing criteria. Aliment Pharmacol Ther 40: 893-902, 2014.
3. Sharifi MI, Cox JJ, Gomaa AI, Khan SA, Gedroc W and Taylor-Robinson SD: Hepatocellular carcinoma: Current trends in worldwide epidemiology, risk factors, diagnosis and therapeutic approaches. Gastrointest Cancer Rep 2012: 902178, 2012.

4. Philip-Ephraim EE, Eyong KI, Williams UE and Ephraim RP: The role of radiotherapy and chemotherapy in the treatment of primary adult high grade gliomas: Assessment of patients for these treatment approaches and the common immediate side effects. J Neurooncol 102: 363-367, 2009.

5. Bai P, Zhang R, Li XG, Ma SK, Wu LY and Zhang WH: Efficiency and side effects of concurrent radiotherapy and chemotherapy for advanced cervical cancers. Zhonghua Zhong Liu Za Zhi 29: 467-469, 2007 (In Chinese).

6. Huang YH, Wu JC, Chen SC, Chen CH, Chiang JH, Hsu TI, Lee PC, Chang FY and Lee SD: Survival benefit of transcatheter arteriovenous embolization in patients with hepatocellular carcinoma larger than 10 cm in diameter. Alim Pharmacol Ther 23: 129-135, 2006.

7. Joseph JD, Peng Y, Mak DO, Cheung KH, Vais H, Foskett JK and Wei H: General anesthetic anesthetic isoform modulates inositol 1,4,5-trisphosphate receptor calcium channel opening. Anesthesiology 121: 528-537, 2014.

8. Yu C, Luo YL, Xiao SS, Zhang Q and Chen SL: Influence of propofol and isoflurane on cytokines response to cancer surgery during perioperative period. Hu Min Xiu Kou Qiu Qian Yi Xue Za Zhi 25: 554-556, 2007 (In Chinese).

9. Jiang J and Jiang H: Effect of the inhaled anesthetics isoflurane, sevoflurane and desflurane on the neurophysiopathology of Alzheimer's disease (review). Mol Med Rep 12: 3-12, 2015.

10. Liu TC: Inhaled anesthetics and end-tidal levels on serum interleukin-8 and interleukin-10 in cancer patients. Asian Pac J Cancer Prev 15: 6703-6707, 2014.

11. Yang H, Deng J, Jiang Y, Chen J, Zeng X, He Z, Jiang X, Li Z and Jiang C: Emulsified isoflurane treatment inhibits the cell cycle and respiration of human bronchial epithelial 16HBE cells in a p53-independent manner. Mol Med Rep 14: 349-354, 2016.

12. Miura Y, Kamiya K, Kanazawa K, Okada M, Nakane M, Kamasaka A and Kawamoe K: Superior recovery profiles of propofol-based regimen as compared to isoflurane-based regimen in patients receiving chemotherapy for lung cancer primary brain tumor excision: A retrospective study. J Anesth 26: 721-727, 2012.

13. Lim KH and Counter CM: Reduction in the requirement of non-genetic Ras signaling to activation of PI3K/AKT pathway during tumor maintenance, Cancer cell 8: 381-392, 2005.

14. Shi LX, Wang JH and Shi XD: PI3K/AKT/mTOR Pathway and Pediatric T acute lymphoblastic leukemia -review. Zhongguo Shi Yan Xue Ye Xue Za Zhi 24: 1269-1274, 2016 (In Chinese).

15. Dey JH, Bianchi F, Voshol J, Bonenfant D, Oakeley EJ and Hymes NE: Targeting fibroblast growth factor receptor blockers PFS-236, an antiangiogenic molecule, induces apoptosis, tumor growth and metastasis. Cancer Res 70: 4151-4162, 2010.

16. Wang CM, Cai XL and Wen QP: Astaxanthin reduces isoflurane-induced neuroapoptosis via the PI3K/AKT pathway. Mol Med Rep 6: 251-254, 2012.

17. Zhao K, Song X, Huang Y, Yao J, Zhou M, Li Z, You Q, Gao Q and Lu N: Wogonin inhibits LPS-induced tumor angiogenesis via suppressing PI3K/AKT/NF-κB signaling. Eur J Pharmacol 737: 57-69, 2014.

18. Miao X and Zhao Y: ST6GalNAcII mediates tumor invasion through PI3K/AKT/NF-κB signaling pathway in follicular thyroid carcinoma. Oncol Rep 35: 2311-2140, 2014.

19. Al-Sobayil EA and Omer OH: Serum biochemical values of adult ostriches (Struthio camelus) anesthetized with xylazine, ketamine, and propofol. J Avian Med Surg 25: 97-101, 2011.

20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-DeltaDelta C(T)) method. Methods 25: 402-408, 2001.

21. Nilsson U and Idvall E: Pain assessments in day surgery patients. J Clin Nurs 19: 2942-2943, 2010.

22. Zheng N, Wei X, Zhang D, Chui W, Che M, Wang J and Du B: Hepatic resection or transarterial chemoembolization for hepatocellular carcinoma with portal vein tumor thrombus. Medicine (Baltimore) 95: e5959, 2016.

23. Jia YS, Hu XQ, Gabriella H, Qin LJ and Meggyshezi N: Antitumor activity of tenacissoside H on esophageal cancer through arresting cell cycle and regulating PI3K/AKT-NF-κB transduction cascade. Evid Based Complement Altern Med 2015: 464937, 2015.

24. Simonetti RG, Cammà C, Fiorello F, Politi F, D’Amico G and Pagliaro L: Hepatocellular carcinoma: A worldwide problem and the major risk factors.Dig Dis Sci 36: 962-972, 1991.