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Propofol suppresses proliferation and invasion of gastric cancer cells via downregulation of microRNA-221 expression

Z.T. Wang¹, H.Y. Gong², F. Zheng³, D.J. Liu¹ and X.Q. Yue²

¹Department of Anesthesiology,
The Second Affiliated Hospital of Zhengzhou University, Jinzhui District,
Zhengzhou, China

²Department of Anesthesiology,
The First Affiliated Hospital of Xinxiang Medical University, Weihui,
Xinxiang, Henan, China

³Department of Medical Parasitology,
The First Affiliated Hospital of Xinxiang Medical University, Weihui,
Xinxiang, Henan, China

Corresponding author: X.Q. Yue
E-mail: xianyue1971@163.com

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ABSTRACT. Propofol is one of the extensively and commonly used intravenous anesthetic agents. The current study aimed to evaluate the effects of propofol on the behavior of human gastric cancer cells and the molecular mechanisms of this activity. The effects of propofol on SGC7901 and AGS cell proliferation, apoptosis, and invasion

were detected by MTT assay, flow cytometric analysis, and matrigel invasion assay. Real-time polymerase chain reaction (PCR) was used to assess microRNA (miR)-221 expression. miR-221 mimics were transfected into SGC7901 and AGS cells to assess the role of miR-221 in propofol-induced anti-tumor activity. Propofol significantly inhibited cell proliferation and invasion and promoted apoptosis of SGC7901 and AGS cells. Propofol also efficiently reduced miR-221 expression. Moreover, transfection of miR-221 mimics reversed the effects of propofol on the biological behavior of gastric cancer cells. Propofol can effectively inhibit proliferation and invasion and induce apoptosis of gastric cancer cells through, at least partly, downregulation of miR-221 expression.

Key words: Propofol; Gastric cancer; miR-221; Proliferation; Invasion

INTRODUCTION

Gastric cancer (GC) is the most common gastrointestinal malignancy and the second leading cause of cancer-related death in East Asia (Herszenyi and Dulassi, 2010). Despite advances in clinical and experimental oncology, the clinical outcome of patients with advanced GC is still disappointing, with a 5-year overall survival rate of 15% or less (Hartgrink et al., 2009). Recent studies have revealed many GC-associated deregulated genes and signaling pathways, but the in-depth molecular mechanisms underlying gastric carcinogenesis, progression, and aggressiveness have not been fully elucidated.

MicroRNAs (miRs) are a class of short (about 22 nucleotides in length), endogenous, single-stranded, non-protein-coding RNAs that directly bind to the 3'-untranslated regions (3'-UTRs) of target messenger RNA (mRNAs), leading to mRNA degradation or translational suppression (Bartel, 2009). Accumulating research suggests that miRs play essential roles in the biology of human cancers, which may provide a new and promising approach to treat cancer (Honegger et al., 2010). Aberrant expression of miRs has been well described in human GC (Pan et al., 2013). Specifically, miR-221 has been confirmed to be upregulated and may act as an oncogene in many types of human malignancies (Gimenes-Teixeira et al., 2013; Sarkar et al., 2013; Ergun et al., 2014; Sun et al., 2014; Ye et al., 2014a). Liu et al. (2012) reported that upregulation of miR-221 in GC correlated with aggressive clinicopathological features and shorter overall survival. Functional analyses showed that knockdown of miR-221 in GC cells should reduce cell proliferation and invasion and promote cell apoptosis (Chun-Zhi et al., 2010).

Propofol is one of the extensively and commonly used intravenous anesthetics. Apart from its multiple anesthetic advantages, propofol exerts a number of non-anesthetic effects (Vasileiou et al., 2009). Recently, increasing evidence indicates a possible correlation between propofol and cancers. Propofol has the ability to inhibit the adhesion, proliferation, and invasion, and induce apoptosis of cancer cells (Mammoto et al., 2002; Miao et al., 2010; Altenburg et al., 2011; Zhang et al., 2013b). Therefore, propofol might be a better agent than other anesthetics for cancer surgery (Inada et al., 2011). However, there is no information available on the anti-tumor action of propofol in GC. Our study aimed to investigate the

effects of propofol on the biological behavior of human GC cells and its related molecular mechanisms.

MATERIAL AND METHODS

Cell culture and reagents

Human GC cell lines SGC7901 and AGS were obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. These cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin, at 37°C in 5% CO₂. Propofol was acquired from Sigma Aldrich Chemical Co. (Sigma, St. Louis, MO, USA) and diluted in dimethyl sulfoxide (DMSO) (Sigma) for *in vitro* assays.

Cell viability assay

Cell viability was determined with the thiazolyl blue tetrazolium bromide (MTT) (Sigma) assay. Briefly, the cells were seeded at a density of 5×10^3 cells/well on 96-well plates containing 180 μ L of culture medium and incubated at 37°C with 5% CO₂ overnight. At the indicated time after treatment, 20 μ L 5 mg/mL MTT was added into each corresponding test well and incubated for 4 h. The reaction was then solubilized by adding 200 μ L DMSO to each well. Optical density (OD) was evaluated on a multi-detection microplate reader (BMG LABTECH, Durham, NC, USA) by measuring the absorbance at a wavelength of 570 nm. The experiments were repeated three times independently, and the results are reported as means \pm SD.

Detection of apoptosis by flow cytometry

Apoptosis was detected by flow cytometric analysis. Briefly, the cells were washed and resuspended at a concentration of 1×10^6 cells/mL. Then, the cells were stained with annexin V and propidium iodide (PI), using the Annexin V Apoptosis Detection Kit (Merck, Frankfurt, Darmstadt, Germany). After incubation at room temperature in the dark for 15 min, cell apoptosis was analyzed on a FACSCalibur (Becton, Dickinson and Company, San Jose, CA, USA).

Matrigel invasion assay

Invasion assays were performed in triplicate using a 24-well invasion chamber system coated with Matrigel (BD Biosciences, Bedford, MA, USA). Cells were seeded in the upper chamber at 1×10^5 cells/well in serum-free DMEM. DMEM containing 10% FBS was added to the lower chambers as a chemoattractant. After incubation for 24 h, nonmigratory cells in the upper chamber were removed with a cotton-tip applicator. Migrated cells on the lower

surface were fixed with 95% ethanol, stained with 0.1% crystal violet, and counted under a microscope (Olympus Corp., Tokyo, Japan).

Detection of miRNA expression by quantitative real-time PCR

After treatment with or without (control group) propofol for 24 h, approximately 5×10^6 cells were collected, and miRNAs were extracted using Trizol reagent (Invitrogen). The miR-221- and RNA U6-specific complementary DNA were synthesized from total RNA using gene-specific primers and the TaqMan[®] miRNA assay system. Real-time quantitative PCR was performed using an ABI7500 real-time PCR detection system (Applied Biosystems, Curitiba, PR, Brasil). PCR amplification was initiated at 95°C for 10 min, followed by 40 cycles of 94°C for 15 s, 60°C for 60 s, and 72°C for 50 s. The relative amount of miR-221 to U6 was calculated using the equation $2^{-\Delta Ct}$, where $\Delta Ct = (Ct^{miR-221} - Ct^{U6})$.

Cell transfection

To selectively upregulate miR-221, SGC7901 and AGS cells were seeded into each well of a 24-well plate and incubated overnight, then transfected with miR-221 mimics (GenePharma, Shanghai, China) at a concentration of 10 nM using Lipofectamine 2000 (Invitrogen). At 24 h after transfection, the cells were harvested for further analysis.

Statistics

Data are reported as mean \pm SD. Statistical analysis was performed with SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Differences between groups were assessed by the unpaired Student's *t*-test. All tests were two-tailed, and the significance level was set at $P < 0.05$.

RESULTS

Effects of propofol on cell proliferation, apoptosis, invasion, and miR-221 expression in SGC7901 and AGS cells

As displayed in Figure 1A, MTT analysis showed that the proliferation of SGC7901 and AGS cells was inhibited by propofol in a dose- and time-dependent manner. Propofol at concentrations of 5 and 10 $\mu\text{g}/\text{mL}$ remarkably inhibited the proliferation at 48 and 72 h. To further detect cell apoptosis, flow cytometric analysis was carried out in our study. After exposure to propofol for 48 h, SGC7901 and AGS cells exhibited increased apoptosis, as indicated in Figure 1B. The matrigel invasion assay also revealed that propofol significantly reduced cell invasion when administered at concentrations of 5 $\mu\text{g}/\text{mL}$ and 10 $\mu\text{g}/\text{mL}$ (Figure 1C). Figure 1D shows that propofol treatment reduced miR-221 expression in SGC7901 and AGS cells in a dose-dependent fashion.

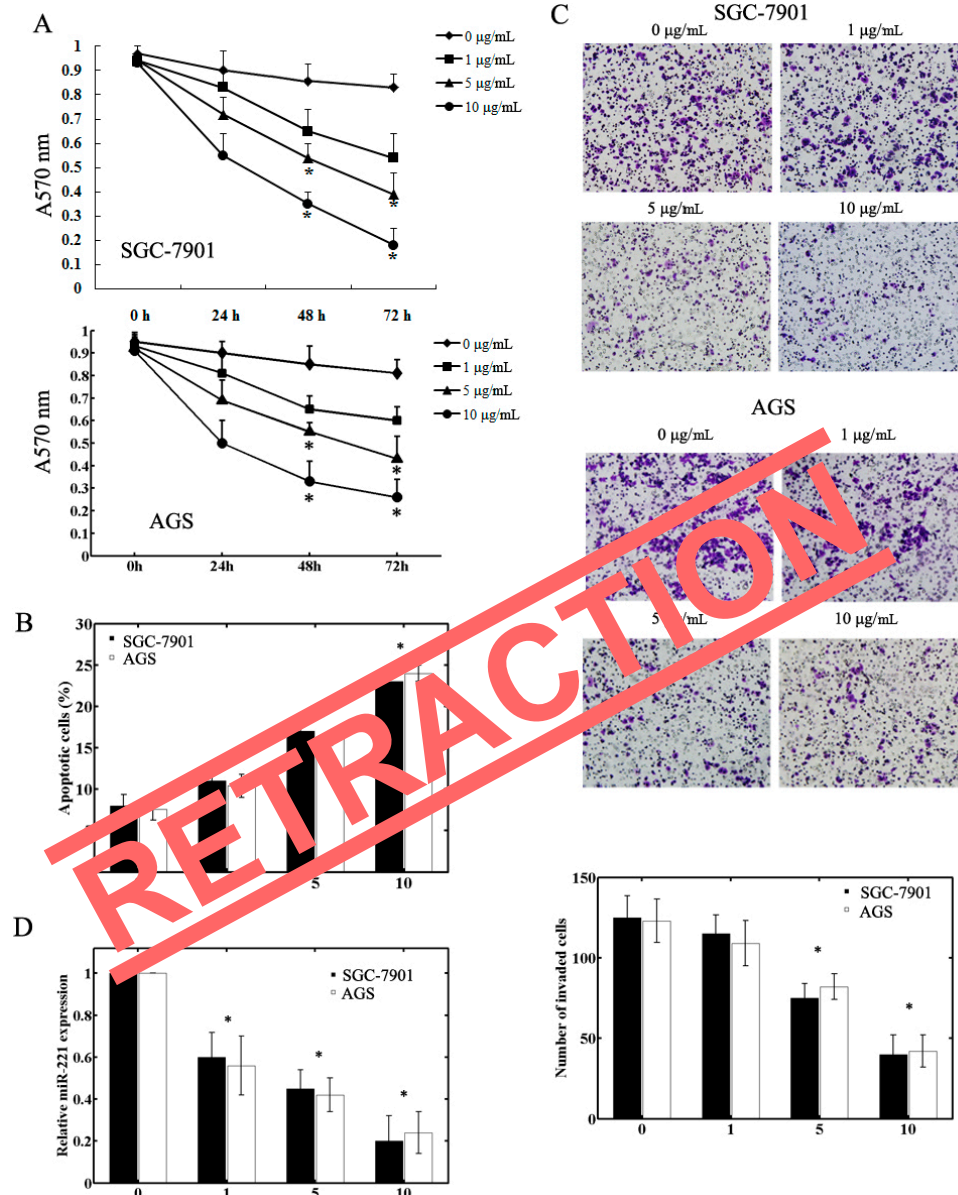


Figure 1. Effects of propofol on cell proliferation, apoptosis, invasion, and miR-221 expression in SGC7901 and AGS cells. Propofol treatment inhibited cell proliferation (A), promoted apoptosis (B), suppressed invasion (C), and reduced miR-221 expression (D) in SGC7901 and AGS cells.

MiR-221 over-expression reverses the effect of propofol

To further explore the role of miR-221 in the effect of propofol on gastric cancer cells, miR-221 mimics were transfected in SGC7901 and AGS cells. First, miR-221 mimics

remarkably increased the expression of miR-221, suggesting that miR-221 mimics successfully penetrated into SGC7901 and AGS cells (Figure 2A). Secondly, the inhibitory effects of propofol (10 $\mu\text{g/mL}$) on cell proliferation and invasion and its promoting effect on apoptosis significantly reversed after transfection with miR-221 mimics (Figures 2B-2D).

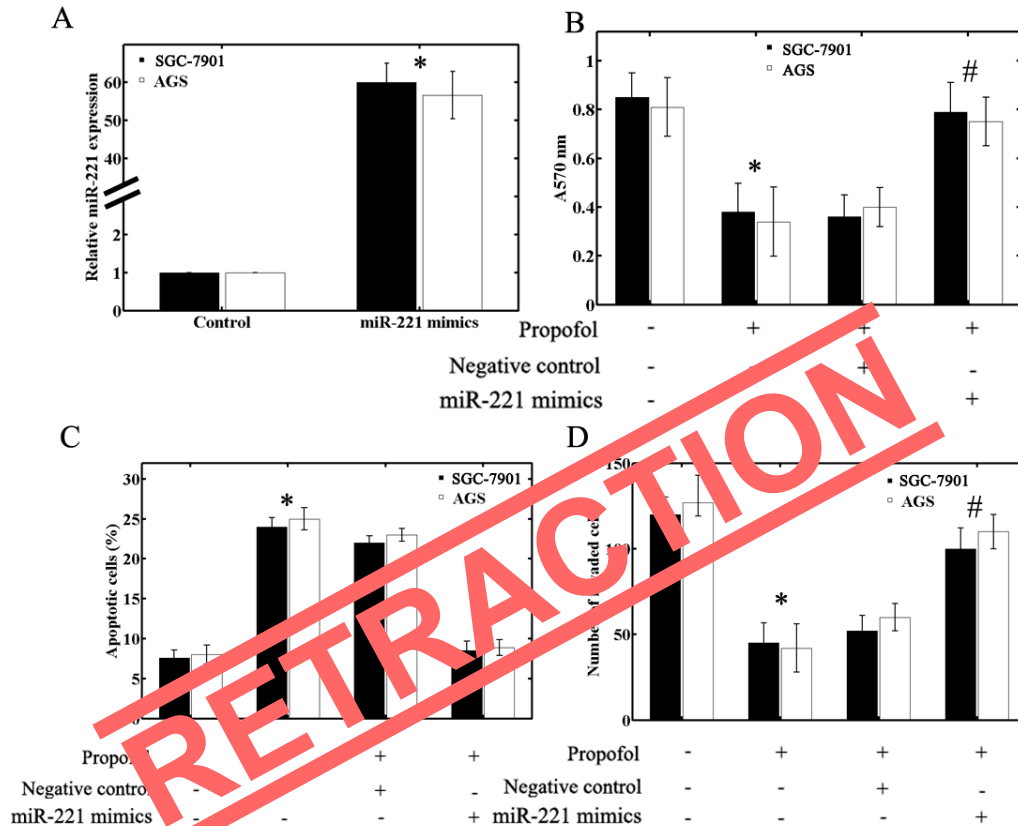


Figure 2. Over-expression of miR-221 can reverse the effect of propofol. Transfection of miR-221 mimics significantly increased the expression of miR-221 in SGC7901 and AGS cells (A). miR-221 over-expression evidently promoted cell proliferation (B), inhibited cell apoptosis (C), and enhanced invasion (D) of SGC7901 and AGS cells after propofol treatment. * $P < 0.01$ compared with the control group without propofol treatment and # $P < 0.01$ compared with the propofol-treated group transfected with negative control.

DISCUSSION

Although there have been advances in the treatment of GC, the survival rate of patients with GC is disappointing. This is mainly owing to the high incidence rate of local recurrence or metastatic spread and multifactorial resistance to treatments. In this study, we evaluated the effects of propofol on the behavior of human GC cells and found that propofol inhibited cell proliferation and invasion and promoted apoptosis of SGC7901 and AGS cells. Our results were consistent with those of other studies. For example, Mammoto et al. (2002) demonstrated that propofol decreased the invasion ability of human cancer cells (HeLa, HT1080, HOS, and

RPMI-7951). Miao et al. (2010) reported that propofol inhibited invasion of LOVO colon cancer cells. Zhang et al. (2013a,b) showed that propofol could effectively induce apoptosis and inhibit invasiveness of hepatocellular carcinoma cells. Taken together, propofol may be a particularly suitable anesthetic for the peri-operative phase in cancer surgery (Inada et al., 2011).

To clarify the mechanism involved in the suppression of SGC7901 and AGS cells, the effect of propofol on miR-221 expression was examined. We observed that propofol significantly reduced miR-221 expression. More importantly, over-expression of miR-221 with transfection of miR-221 mimics reversed the effects of propofol in gastric cancer cells. These results suggested that the anti-tumor function of propofol might be owing partly to the down-regulation of miR-221. miR-221 over-expression has been confirmed in bladder cancer (Gottardo et al., 2007), colorectal cancer (Schetter and Harris, 2009), breast cancer (Iorio, et al., 2005), pancreatic cancer (Lee et al., 2007), lymphoma (Metzler et al., 2004), and gastric cancer (Liu et al., 2012). In these cancers, increased miR-221 expression results in the induction of cell proliferation, inhibition of apoptosis, or promotion of cell invasion, indicating that miR-221 acts as a potential oncogene. Previous studies have shown that propofol may affect the biological behaviors of cancer cells via regulation of miRNA expression. A recent study reported that propofol inhibited proliferation and invasion of osteosarcoma cells through miR-143 upregulation (Ye et al., 2014a,b). However, the detailed mechanisms of how propofol influences miRNA expression are still unclear, and further clarification is needed in the future.

In conclusion, the results of our study suggest that propofol could inhibit proliferation and invasion and induce apoptosis of gastric cancer cells and that modulation of miR-221 may contribute to these anti-tumor actions. Further studies are needed to validate the clinical relevance of propofol.

Conflicts of interest

The authors declare no conflict of interest.

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RETRACTION