Original Article

Propofol Inhibits Breast Cancer Cells Proliferation and Migration by Down-Regulating TGFβ1/Smad2/MMP-9 Signal

Lei Zhou[1], Xi He[1], Gong Chen[1], Jian-Bin Tong[1,2], Yu-Hui Wu[3], and Wen Ouyang[1,4]

ABSTRACT

Background: Breast cancer is the second leading cause of cancer-related death among women worldwide. Previous studies have suggested that propofol plays key roles in cancer progression by suppressing the growth and metastasis of tumor cells. However, the function and molecular mechanism of propofol on breast cancer cells remain unknown.

Methods: The effects of propofol on the proliferation and migration capacity of MCF-7 and MDA-MB-231 breast cancer cells were detected by cell counting kit-8 (CCK-8) and wound healing assays, respectively. The mRNA and protein levels of transforming growth factor-β1 (TGF-β1), Smad2 and MMP-9 in MCF-7 and MDA-MB-231 cells were analyzed by reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis. In addition, pcDNA3.1 + TGF-β1 vector was transfected into MCF-7 and MDA-MB-231 cells to assess the role of TGF-β1 in the effects of propofol on the biological behavior of the cells.

Results: Propofol inhibited the proliferation and migration capacity of MCF-7 and MDA-MB-231 cells in a dose- and time-dependent manner. Meanwhile, the mRNA and protein levels of TGF-β1, Smad2 and MMP-9 were down-regulated in MCF-7 and MDA-MB-231 cells after treatment with propofol. Moreover, exogenous over-expression of TGF-β1 in propofol-treated breast cancer cells indicated that propofol inhibited the cells growth and migration via TGFβ1/Smad2/MMP-9 signaling pathway.

Conclusions: These findings demonstrated that propofol inhibited breast cancer cells proliferation and migration by down-regulating TGFβ1/Smad2/MMP-9 signal.

From [1]Department of Anesthesiology, The Third Xiangya Hospital of Central South University, Changsha, China; [2]The Medical Central Laboratory, The Third Xiangya Hospital of Central South University, Changsha, China; [3]Department of Breast Surgery, Xiangya Hospital of Central South University, Changsha, China; [4]The Seniors Anesthesia and Perioperative Management Research Center; The State Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, China.

Correspondence to Dr. Wen Ouyang at ouyangwen15@sina.com or Dr. Yu-Hui Wu at wuyuhui56@sina.com.

Breast cancer is one of the most frequently diagnosed tumor types and the leading cause of death in women all over the world (1-3). At present, a large number of etiological factors and the clinical complexity of breast cancer present a major challenge for diagnose and treatment (4, 5). Breast cancer occurs though a multi-step process in which a normal cell undergoes complicated transformation as it becomes malignant. Many studies have found that numerous oncogenic and tumor-suppressive genes play key roles in breast cancer progression (6-8), however, the molecular mechanisms that maintain the malignant proliferation and metastasis remain unclear.

Propofol is one of the most universal and extensively used intravenous anesthetics reagents since the late 1980s (9, 10). In addition to its multiple anesthetic advantages, propofol also exerts a few non-anesthetic effects in the perioperative period. Many studies have indicated a close correlation between propofol and cancer development and progression (11-13). For example, suppression of cell in-

vasion and migration by propofol is involved in down-regulating p38-MAPK and MMP-2 signaling in A549 human lung adenocarcinoma epithelial cells (14). Propofol inhibits growth, invasion and angiogenesis by down-regulating ERK-VEGF/MMP-9 signal in Eca-109 esophageal squamous cell carcinoma cells (15). Propofol represses invasion and promotes apoptosis of osteosarcoma cells via down- regulating TGF- β1 expression (16). However, the potential anti-tumor effects and molecular mechanism of propofol in breast cancer cells remain unknown.

In the present study, we demonstrated that propofol significantly inhibited growth and migration of MCF-7 and MDA-MB-231 cells in a dose- and time- dependent manner. The MCF- 7 and MDA- MB- 231 cells treated with propofol also showed decreased mRNA and protein levels of transforming growth factor-β1 (TGF-β1), Smad2 and MMP- 9. Moreover, transfection of pcD-NA3.1 + TGF- β1 vector partly reversed the effects of propofol on the proliferation and migration behavior of MCF-7 and MDA-MB-231 cells. Thus, propofol may serve as a potential therapeutic strategy for breast cancer in the future.

MATERIALS AND METHODS

Cell Culture and Transfection
MCF- 7 and MDA- MB- 231 cell lines were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China). All cell lines were cultured in Invitrogen Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 100 U/ml penicillin (Sigma, St. Louis, MO, USA) and 100 mg/ml streptomycin (Sigma, St. Louis, MO, USA) at 37 ℃ in a 5% CO_2 atmosphere.

Propofol was obtained from Sigma Aldrich Chemical Co. and diluted in dimethyl sulfoxide (Sigma, St. Louis, MO, USA). Propofol at the concentration of 0, 5 or 10 μg/ml was added to MCF- 7 and MDA- MB- 231 cells. The over- expression plasmid vector of TGF-β1, pcDNA3.1+TGF-β1 vector, was synthesized from GenePharma (Shanghai, China). The pcDNA3.1 + empty vector was used as a negative control. A final concentration of 2 μg of pcDNA3.1+TGF-β1 or pcDNA3.1 + empty vector was transfected into MCF-7 and MDA-MB-231 cells by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. After 24 or 48 hours of transfection, the cells were harvested for the further experiments.

Reverse Transcription Polymerase Chain Reaction
Total RNA of MCF- 7 and MDA- MB- 231 cells were extracted by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. Then, 1 μg of RNA was reverse transcribed to cDNA via SuperScript First Strand cDNA System (Invitrogen, Carlsbad, CA, USA). Reverse transcription polymerase chain reaction (RT- PCR) assay was performed on the ABI PRISM 7000 Fluorescent Quantitative PCR System (Applied Biosystems, Foster City, CA, USA) by SYBR® Premix EX TaqTMII PCR Kit (Takara, Japan) according to the manufacturer's instructions. The primer information was shown as followings: 5'-TGATGTCACCGGAGTTGTG-3' and 5'- CATGAGAAGCAGGAAAGG- 3' for TGF- β1; 5'-AACCTTTCCAAGCCTCTT-3' and 5'- TGGTGCCAGCAGTATCAA- 3' for Smad2; 5'- CGCTGGGCTTAGATCATTC- 3' and 5'-AGGGCGAGGACCATAGAGG- 3' for MMP- 9; 5'- CCACATCGCTCAGACACC- 3' and 5'- ATG-GCAACAATATCCACTTTA- 3' for GAPDH. The level of each gene was normalized internally by the CT value of the GAPDH gene. The relative quantitative value was calculated by the $2^{-\Delta\Delta Ct}$ method (17). Each experiment was performed in triplicates and repeated at least three times.

Western Blot Analysis
Total protein of the cells was isolated by using cold RIPA reagent (Beyotime Biotech, Shanghai, China), according to the manufacturer's protocols. The cell lysates were washed with PBS for three times and incubated on cold RIPA buffer for 30 minutes. Then, the cell lysates were centrifuged at 13,000 g for 15 minutes at 4 ℃. After that, 10% of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Beyotime Biotech, Shanghai, China) was used for separating total proteins into equal amount and then transferred to polyvinylidene fluoride (PVDF) (Millipore, MA, USA) membranes. After blocking with 5% non-fat milk for 1 hour, the

membranes were incubated with the mouse monoclonal anti- TGFβ1 (1: 1000; ab64715), rabbit monoclonal anti- Smad2 (1: 500; ab33875) and rabbit monoclonal anti- MMP- 9 (1: 2000; ab137867) antibody over- night, followed by horseradish peroxidase (HRP)- linked corresponding secondary antibodies (Santa Cruz Biotechnology, USA).
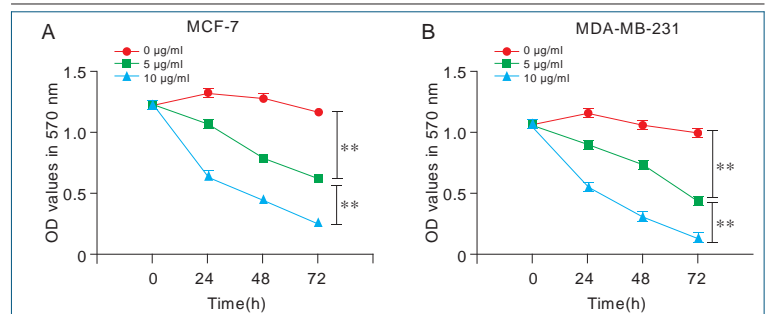
Cell Proliferation Assay

The proliferation ability of MCF- 7 and MDA- MB- 231 cells was detected by using cell counting kit- 8 (CCK- 8) (Dojindo, shanghai, Japan), according to instructions. MCF- 7 and MDA- MB- 231 cells were seeded in a 96- well plate and cultured in normal medium for 24 hours, then added 0, 5 or 10 μg/ml propofol and transfected with pcDNA3.1 + TGF- β1 or pcDNA3.1 + empty vector. At 0, 24, 48, and 72 hours after transfection, the absorbance of each well at wavelength of 570 nm was measured by amicroplate reader (Bio- Rad, Hercules, CA, USA). All experiments were performed three times and repeated at least three times.

Cell Migration Assay

A wound scratch assay was used to assess the migratory ability of the MCF-7 and MDA-MB-231 cells in vitro. For the assay, 6×10^6 cells were seeded per 6- well dish. After culture for 24 hours, 0, 5 or 10 μg/ml propofol was added to MCF-7 and MDA-MB-231 cells, then transfected with pcDNA3.1 + TGF- β1 or pcDNA3.1 + empty vector. At 6 hours post-transfection, vertical horizontal wounds were made in the cell layer by using a sterile 10 μl pipette tip, and markers were included to allow observation of cells at the same point. Then, the cells were then rinsed with PBS and cultured in an incubator at 37 ℃. Images of the wounds were collected with a digital camera system (Olympus Corporation, Tokyo, Japan) at 0 and 12 hours following creation of the wounds at the same points. The wound widths (lm) were measured by using a standard caliper (Caliper; PerkinElmer, Inc., Waltham, MA, USA). The experiments were performed in triplicate and repeated at least three times.

Statistical Analysis

SPSS v16.0 software (SPSS Inc., Chicago, IL,

Figure 1. Propofol Suppresses MCF-7 and MDA-MB-231 Cells Proliferation in Vitro.
A. The CCK-8 assay showed that the proliferation of MCF-7 cells was markedly suppressed by propofol in a dose- and time-dependent manner; B. Propofol treatment inhibited proliferation of the breast cancer cell line MDA-MB-231 in a dose- and time-dependent manner.
Data were expressed as means±SD (n=3). Each experiment was performed in triplicate for three independent times. **P<0.01.

USA) was used for statistical analysis. All of the results were represented as the means ± standard deviation (SD). The CCK- 8 assay was analyzed using analysis of variance (ANOVA). The independent- samples t- test was used to analyze others data. Values of P<0.05 were considered to be statistically significant.

RESULTS

Propofol Suppresses MCF-7 and MDA-MB-231 Cells Proliferation in Vitro

The present study aimed to determine the effects of propofol on proliferation and migration of the MCF-7 and MDA-MB-231 cells. Following treatment with various concentrations of propofol (0, 5 or 10 μg/ml), the proliferation ability of MCF- 7 and MDA- MB- 231 cells was markedly decreased by propofol in a dose- and time- dependent manner (Figure 1, P<0.01). The concentrations of propofol at 5 and 10 μg/ml significantly inhibited the proliferation of MCF- 7 and MDA- MB- 231 cells at 24, 48 and 72 hours (Figure 1, P<0.01).

Propofol Inhibits MCF- 7 and MDA- MB- 231 Cells Migration in Vitro

In vitro studies were done to determine the effects of propofol (0, 5 or 10 μg/ml) on migration capability of MCF-7 and MDA-MB-231 cells, which were markedly decreased in a dose- and time- dependent manner, as compared with the control cells (Figure 2, P<0.05). These results suggested that propofol inhibited breast cancer cell migration.
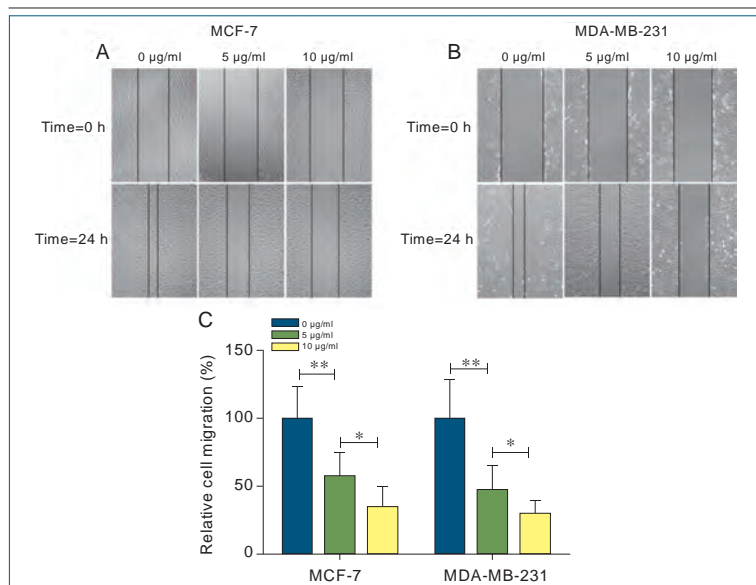
Figure 2. Propofol Inhibits MCF-7 and MDA-MB-231 Cells Migration in Vitro.
A. Propofol inhibited MCF-7 cell migration in vitro by wound healing assay; B. The migration ability of MDA-MB-231 cell was significantly decreased after treatment with propofol; C. The lines indicated the migration front. Comparison of cell migration in MCF-7 and MDA-MB-231 cells treated with propofol.
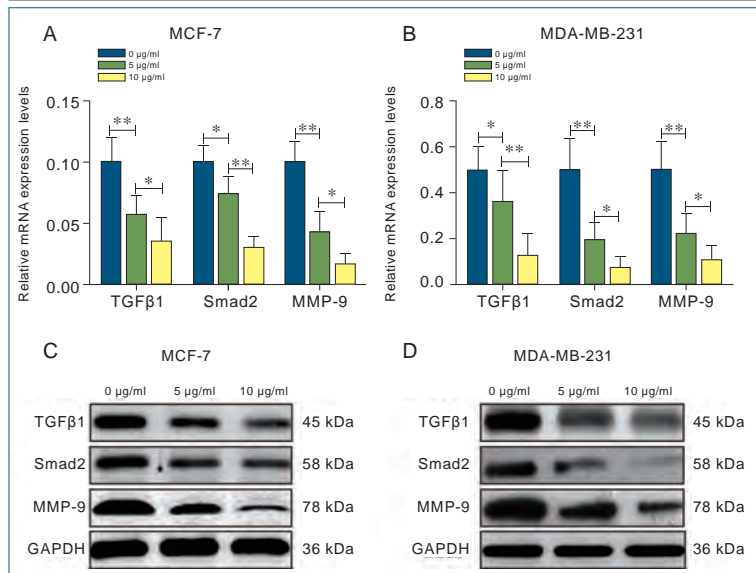Values were expressed as the means ± SD (n=3). The experiments were performed in duplicate and repeated three times, and a representative picture was shown (magnification, ×200). **P<0.01, *P<0.05.
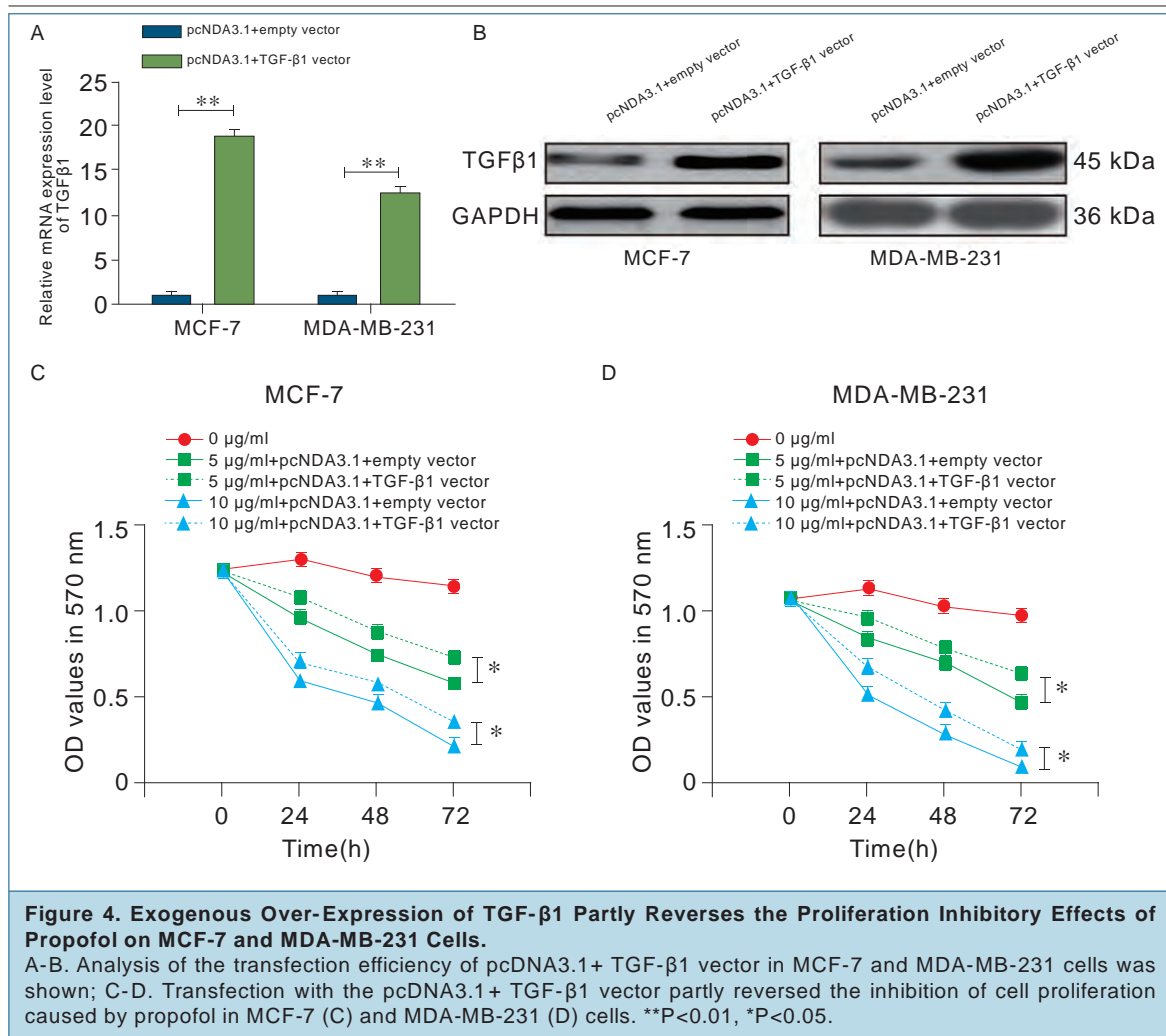

Figure 3. Propofol Reduces the TGF-β1, Smad2 and MMP-9 Expression in MCF-7 and MDA-MB-231 Cells.
A-B. RT-PCR assay revealed that the mRNA expression levels of TGF-β1, Smad2 and MMP-9 were significantly reduced in MCF-7 (A) and MDA-MB-231 (B) cells by propofol after 24 hours treatment; C-D. The protein expression levels of TGF-β1, Smad2 and MMP-9 were also significantly decreased in MCF-7 (C) and MDA-MB-231 (D) cells by propofol.
Data were expressed as the means ± SD (n=3). All experiments were performed in triplicate for three independent times. **P<0.01, *P<0.05.

Propofol Reduces the TGF-β1, Smad2 and MMP-9 Expression in MCF-7 and MDA-MB-231 Cells

It is well known that TGFβ1/Smad2/MMP-9 signal plays key roles in tumor growth and metastasis (18, 19). MCF-7 and MDA-MB-231 cells were treated with propofol (0, 5 or 10 μg/ml) for 48 hours and then cells were harvested for isolation of total RNA to examine TGF-β1, Smad2 and MMP-9 mRNA expression by RT-PCR assay. As shown in figure 3A and B, the mRNA expression levels of TGF-β1, Smad2 and MMP-9 were significantly reduced in MCF-7 and MDA-MB-231 cells by propofol after 48 h treatment (P< 0.05). Meanwhile, western blot analysis showed that markedly reduction in TGF-β1, Smad2 and MMP-9 protein expression levels were observed in MCF-7 and MDA-MB-231 cells treated with 5 and 10 μg/ml propofol, as compared with the control cells (Figure 3C and D).

Exogenous Over-Expression of TGF-β1 Partly Reverses the Inhibitory Effects of Propofol on MCF-7 and MDA-MB-231 Cells

Our previous results showed that propofol suppressed the TGF-β1, Smad2 and MMP-9 expression in MCF-7 and MDA-MB-231 cells. To further testify whether the inhibitory effect of propofol on cell migration and migration was executed through the TGFβ1/Smad2/MMP-9 signaling pathway, transfection of the cells with pcDNA3.1 + TGF-β1 vector was performed. The pcDNA3.1 + TGF-β1 vector significantly increased TGF-β1 expression level, suggesting the successful transfection of pcDNA3.1 + TGF-β1 vector into the MCF-7 and MDA-MB-231 cells (Figure 4A and B, P<0.01). Furthermore, transfection with the pcDNA3.1 + TGF-β1 vector partly reversed the inhibition of cell proliferation caused by propofol in MCF-7 and MDA-MB-231 cells (Figure 4C and D, P<0.05). Meanwhile, over-expression of TGF-β1 alleviated the migration inhibition effect of propofol on MCF-7 and MDA-MB-231 cells (Figure 5, P<0.05).

DISCUSSION

Increasing evidences suggest that propofol has the ability of influencing the growth and metastasis of human cancer cells (12, 14). In gastric cancer, pro-
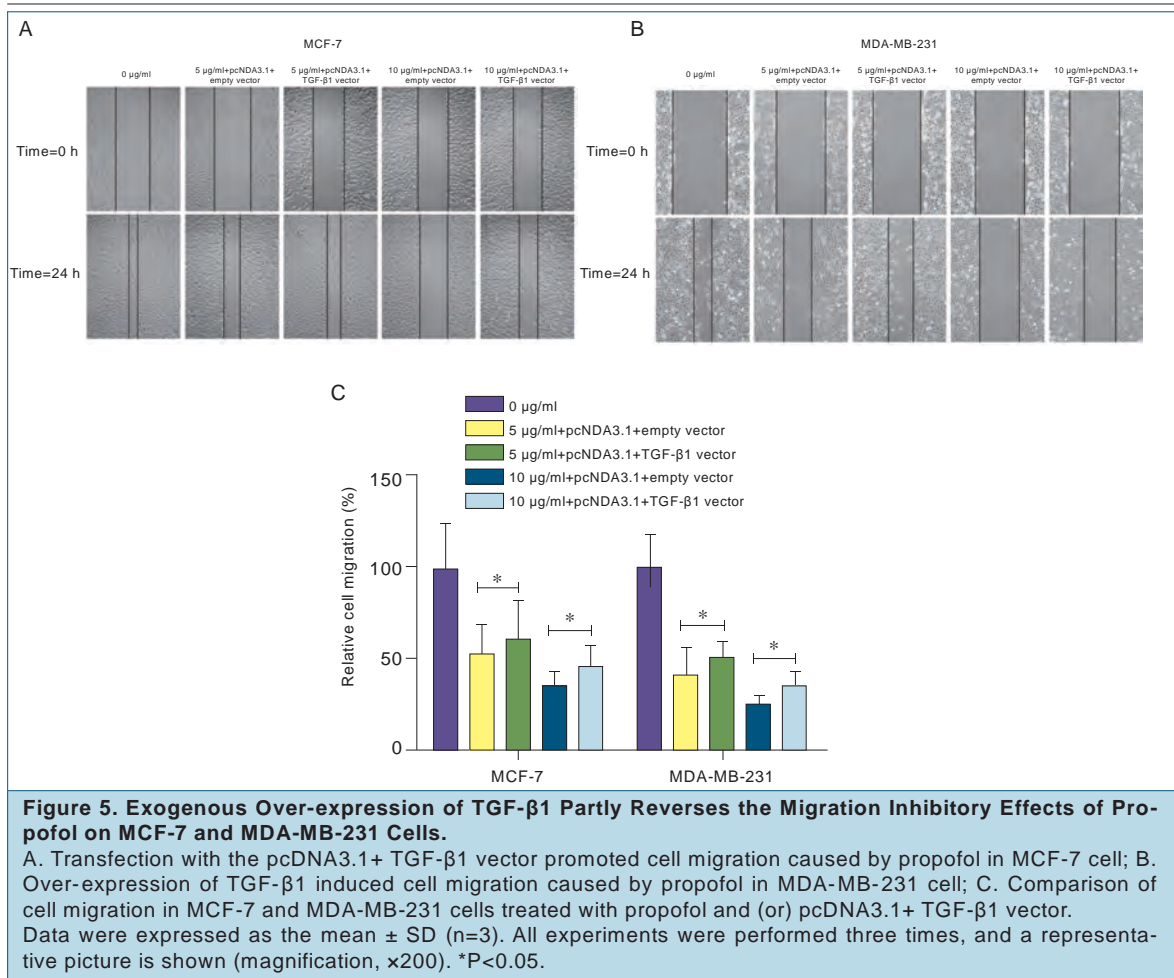
Figure 4. Exogenous Over-Expression of TGF-β1 Partly Reverses the Proliferation Inhibitory Effects of Propofol on MCF-7 and MDA-MB-231 Cells.
A-B. Analysis of the transfection efficiency of pcDNA3.1+ TGF-β1 vector in MCF-7 and MDA-MB-231 cells was shown; C-D. Transfection with the pcDNA3.1+ TGF-β1 vector partly reversed the inhibition of cell proliferation caused by propofol in MCF-7 (C) and MDA-MB-231 (D) cells. **P<0.01, *P<0.05.

pofol inhibits cell proliferation and invasion by down-regulation of miR-221 expression (20). Propofol suppresses proliferation and invasion of pancreatic cancer cells by up-regulating miR-133a expression (3). In addition, propofol represses proliferation and invasion of glioma cells by up-regulating microRNA-218 expression (21). These results all suggest that propofol may be an extraordinary suitable anesthetic for cancer surgery.

Although there have been advances in the treatment of breast cancer, the total survival rate of patients with breast cancer remains unsatisfactory. This is mainly attributed to the high incidence rate of malignant metastatic spread and multi-factorial drug-resistant cancer cells. In this study, we evaluated the effects of propofol on the behavior of human breast cancer cells and found that propofol inhibited the proliferation and migration of MCF-7 and MDA-MB-231 cells in a dose- and time-dependent manner. Our results were consistent with the data in previous researches.

TGF-β1 is a prototypical member of a multi-functional cytokine family that regulates a wide variety of cellular functions (22). TGF-β1 has been identified to promote tumor progression through increasing tumor cells growth and metastasis (23, 24). Intracellular Smad2 plays a crucial role in mediating anti-mitogenic effects of TGF-β1 in cellular growth (25). MMPs are involved in tumor angiogenesis, metastasis and stimulation of growth factor release from the ECM (26). Increased expression of MMPs is associated with tumor invasion and metastasis (27). Inhibition of MMP expression or MMP activity have been recognized as early targets in preventing cancer metastasis (28). MMP-9 is the key enzyme, which is involved in degrading type-I and -IV collagens and extracellular matrix

Figure 5. Exogenous Over-expression of TGF-β1 Partly Reverses the Migration Inhibitory Effects of Propofol on MCF-7 and MDA-MB-231 Cells.
A. Transfection with the pcDNA3.1+ TGF-β1 vector promoted cell migration caused by propofol in MCF-7 cell; B. Over-expression of TGF-β1 induced cell migration caused by propofol in MDA-MB-231 cell; C. Comparison of cell migration in MCF-7 and MDA-MB-231 cells treated with propofol and (or) pcDNA3.1+ TGF-β1 vector.
Data were expressed as the mean ± SD (n=3). All experiments were performed three times, and a representative picture is shown (magnification, ×200). *P<0.05.

(29). In particular, MMP-9 has been reported to be related to the invasive metastatic potential of tumor cells (30, 31). TGF-β1 initiates signaling by binding to type I and type II receptor serine/threonine kinases on the cell surface and the receptor-mediated downstream Smad2 and MMP-9 activation, which is a classical pathway for TGFβ1/Smad2/MMP-9 signal transduced from the cell membrane to the nucleus, and resulting in breast cancer suppression (18, 19).

The present study also analyzed the underlying mechanisms of the effects of propofol on MCF-7 and MDA-MB-231 cells growth and migration. We observed that treatment with propofol inhibited the mRNA and protein levels of TGF-β1, Smad2 and MMP-9 in MCF-7 and MDA-MB-231 cells in a dose-dependent manner. More importantly, over-expression of TGF-β

1 with transfection of pcDNA3.1+ TGF-β1 vector partly reversed the effects of propofol in breast cancer cells. These results suggested that the anti-tumor function of propofol may be partly owing to the down-regulation of TGFβ1/Smad2/MMP-9 signal.

In conclusion, this present study demonstrates that treatment with propofol inhibited proliferation and migration of breast cancer cells partly by down-regulating TGFβ1/Smad2/MMP-9 signal. However, further studies are required to illuminate its clinical relevance.

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