



The Mechanism of Propofol on Non-Small Cell Lung Cancer (NSCLC) through Modulating Mesenchymal Transition (EMT)

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Abstract

Background: Intensive investigations have focused on the effect of propofol on the malignant behaviours of cancer cells. However, much is still unknown about the effect of propofol on non-small cell lung cancer (NSCLC). Here we aimed to investigate the effect of propofol on NSCLC with cancer stem-like cells (CSCs) A549.

Methods: CCK-8 assays, flow cytometry, and transwell assay were used to assess the changes in the proliferation, migration, and invasion in A549 treated with propofol. By detecting hallmarks of mesenchymal transition (EMT), the mechanism of the effect of propofol on A549 was assessed.

Results: In A549, propofol exposure promoted cell proliferation, while inhibiting migration and invasion. By activating EMT using TGF- β pretreatment, propofol treatment downregulated hallmarks of EMT and led to inactivation of EMT.

Conclusion: Modulation of self-renewal capacity of CSCs by anesthetics may affect cancer malignant behaviors following surgery. The employment of propofol not only exerts inhibitory effects on cancer cells but also on CSCs in non-small cell lung cancer.

Keywords

Propofol, Cancer Stem-Like Cells (CSCs), Non-Small Cell Lung Cancer, Mesenchymal Transition, Self-Renewal Capacity

Introduction

Propofol (2,6-diisopropylphenol) has been widely accepted and clinically used as one of the most common intravenous anesthetic agents to produce smooth induction and rapid recovery from anesthesia since the late 80s [1]. Despite its multiple anesthetic advantages, propofol plays several unexpected effects un-related to the anesthetic role [2]. Accumulating evidence has been found that clinically relevant concentrations of propofol can inhibit the malignant

behaviors of human cancer cells [3]. Tsuchiya and colleagues reported that clinical concentrations of propofol exposure resulted in the apoptotic cell death of human promyelocytic leukemia cells by inducing mitochondrial injury and leakage of cytochrome C [4]. In human colon carcinoma cells, it has been found that exposure to propofol significantly decreased the migrating and invasive activity [5]. Liu found that, in human non-small cell lung cancer cell line A549, propofol treatment induced the expression of miR-

1284, lead the inactivation of mesenchymal transition (EMT) via upregulating E-cadherin, while down-regulating N-cadherin, Vimentin and Snail expressions [6]. In animal studies, it is still being found to exert antitumor activity, proliferation, and invasiveness by modulating the immune reaction [7].

Lung cancer is one of the leading causes of cancer-related deaths worldwide [8]. When it is diagnosed, frequently, it is in the late stage and the 5-year survival rate is discouraging. Although an interval improvement in survival rate and life quality has been achieved in other common malignancies, no efficient therapeutic strategies for lung cancer have been developed [9]. In recent years, the existence of cancer stem-like cells (CSCs) in different types of cancers has been recognized and accepted. The CSCs hypothesis demonstrated the existence of a population of rare, stem-like tumor cells maintaining stemness, exerting self-renewal capacity, and undergoing asymmetric division [10-12]. CSCs share molecular features with embryonic stem cells, including CD133 [13], Nanog [14], and Oct4 [15], which have been considered hallmarks of CSCs. In many forms of human cancers, CSCs have been isolated, including lung cancers [13,16]. By considering its central role of CSCs in tumorigenesis, inducing malignant behavior and chemoresistance, it might be considered as a therapeutic target to achieve effective cancer treatment [17]. Although the effects of propofol on tumor cells has been well studied, the role of propofol in regulating the malignant behaviors of CSCs is still largely unknown.

EMT is a complex series of morphological changes including the loss of epithelial characteristics and the acquisition of a mesenchymal phenotype. In solid cancer, EMT tightly regulates the processes of metastasis and is responsible for survival in the circulation and seed at secondary site [18]. It has been reported that, the activation of EMT via either the overexpression of hallmarks of EMT, or treatment with TGF- β , confers many of the properties of CSCs on otherwise epithelial carcinoma cells [19,20], indicating that activation of the EMT programmed is closely related to entrance into the CSC state in several different kinds of cancer cells.

The aims of the current study were to evaluate effects of propofol on the physiological processes of non-small cell lung cancer cell line A549 and the CSCs derived from A549. We then focused on the effects of propofol on maintenance of stemness and EMT programme in CSCs to evaluate the potential role of CSCs as a therapeutic target of non-small cell lung cancer cells.

Material and Methods

Cell Culture and CSCs Enrichment:

The human non-small cell lung cancer cell line A549 was purchased from ATCC Cell Bank and was cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) at 37°C, 5% CO₂. In order to enrich CSCs from A549 cells, 1×10⁶ A549 cells were cultured in DMEM/F12 (Gibco, Carlsbad, CA, USA) supplemented with 20 ng/mL of epidermal growth factor (EGF, PeproTech, Rocky Hill, NJ, USA), 10 ng/mL of basic fibroblast growth factor (bFGF, PeproTech, Rocky Hill, NJ, USA) and 2% B-27 (Life Technologies, Grand Island, NY, USA). Every four days, medium was half-refreshed. At day 10 and 20, cells were imaged under a X71 (U-RFL-T) fluorescence microscope (Olympus, Melville, NY, USA) and stored in liquid nitrogen.

Propofol Treatment:

Propofol (Sigma-Aldrich, MO, U.S.A.) was diluted with 10% intralipid. A549 cells or CSCs were incubated in DMEM supplemented with 10% FBS and without or without 10-100 μ mol/L propofol. For Edu staining, propofol exposure lasted for 24 h. For CCK-8 assay, cells were co-cultured with propofol for 24, 48 and 72 h. The intralipid treated cells were considered as negative control.

Cell Cycle Assay:

The cell cycle distribution of cells was checked using Propidium Iodide (PI, Sigma-Aldrich, MO, U.S.A.) staining on a flow cytometer. The cells were fixed with 4% paraformaldehyde, and stained with 5 μ g/mL PI for 10min in darkness. Then cells were washed three times with PBS and PI absorbance was determined by FACS on a flow cytometry (FACSCalibur, Becton Dickinson, U.S.A.).

EdU Staining:

For EdU staining, diluted EdU-labeling reagent (Life Technologies, Grand Island, NY, USA) was added and incubated with cells for 4 h. Then the medium was removed and cells were washed with PBS for three times. Cells were fixed with 4% paraformaldehyde for 15 min at room temperature. Click-iT kit (Life Technologies, Grand Island, NY, USA) was employed for EdU detection following the manufacturer's instruction. Then the cells were counterstained with 4', 6-diamidino-2-phenylindole (DAPI). Images were taken and analyzed using Image J software.

Transwell Assay:

Cell migration and invasion was evaluated using an 8-mm pore size transwell system (Costar Corp, Cambridge, MA) without or with pre-coated Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Briefly, cells were dissociated into single cells and resuspended in DMEM medium at a density of 1×10^5 cells/ml. The top chamber of the transwell was loaded with 200 μ L cell suspension, and 800 μ L DMEM medium supplemented with 10% FBS was added to each lower chamber. Following incubation in the incubator for 24 h at 37°C, the cells remain on the upper surface of upper chamber were removed, and the cells on the lower surface of upper chamber were fixed in 4% paraformaldehyde at room temperature for 10 min and subsequently stained with 0.25% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 10 min followed by three washes with PBS. Images of the stained cells from five random views were taken under a X71 (U-RFL-T) fluorescence microscope (Olympus, Melville, NY).

Western Blot:

Cells were collected by centrifugation at 800g, 4°C for 10 min and washed with PBS for three times. Total protein was extracted using NP-40 lysis buffer (Beyotime, Shanghai, China), according to the manufacturer's instructions. The concentration of total protein was measured using a BCA detection kit (Sigma-Aldrich, St. Louis, MO, USA). 20 μ g of total protein was fractionated via 10% sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) electrophoresis, followed by blot transferring onto polyvinylidene fluoride (PVDF, Life Technologies,

Grand Island, NY, USA). After blot transferring, PVDF membrane was blocked in 5% skim milk. Then the PVDF membrane was incubated with the following primary antibodies at 4°C overnight: Rabbit anti-human polyclonal antibody against CD24 (1:1000 diluted; cat.no. ab179821); rabbit anti-human polyclonal antibody against CD44 (1:1000 diluted; cat.no. ab157107); rabbit anti-human polyclonal antibody against CD133 (1:1000 diluted; cat.no. ab19898); rabbit anti-human polyclonal antibody against Oct4 (1:1000 diluted; cat.no. ab181557); rabbit anti-human polyclonal antibody against Nanog (1:1000 diluted; cat.no. ab21624); rabbit anti-human polyclonal antibody against β -actin (1:5000 diluted; cat.no. ab8227); rabbit anti-human polyclonal antibody against E-cadherin (1:1000 diluted; cat.no. ab40772); rabbit anti-human polyclonal antibody against Vimentin (1:1000 diluted; cat.no. ab92547); rabbit anti-human polyclonal antibody against Slug (1:1000 diluted; cat.no. ab27568); rabbit anti-human polyclonal antibody against Snail (1:1000 diluted; cat.no. ab82846). All antibodies were purchased from Abcam (Cambridge, England). After three washes with PBS supplemented with 0.1% Tween-20, the membranes were subsequently incubated with horseradish peroxidase-linked goat anti-rabbit IgG (1:5000 diluted, cat. no. ab7090) at room temperature for 2h. Enhanced chemiluminescence solution (Life Technologies, Grand Island, NY, USA) was added for luminescent image development. The amount of β -actin was considered as a reference.

RNA Extraction and qRT-PCR:

TRIzol reagent (Life Technologies, Grand Island, NY, USA) was purchased for RNA extraction. In brief, cDNA was synthesized by using the Reverse Transcriptional Kit (RIBOBIO, Guangzhou, China) from 1 μ g of total RNA. The RT reaction was carried under the following conditions: 10 min at 25°C, 60 min at 42°C and 10 min at 85°C in total 20 μ L of reaction mixture. Quantitative real-time (qRT)-PCR was performed in a ABI7500 (Applied Biosystems, Foster City, CA, USA). The reaction mixture consists of 2 μ L of forward and reverse primers in 10 μ L of SYBR Green mastermix (Life Technologies, Grand Island, NY, USA) to a total volume of 20 μ L. The qRT-PCR cycle conditions are 2 min at 50°C, 10 min at

95°C, followed by 40 cycles of 15 s at 95°C and 1min at 60°C. The primer sequences were described as the following: E-cadherin Forward: 5'-CGAGAGCTACACGTTTCACGG -3', E-cadherin Reverse: 5'-GGGTGTCGAGGGAATAAGG -3'; Vimentin Forward: 5'- GACGCCATCAACACCGAGTT -3', Vimentin Reverse: 5'- CTTTGTCTGTTGGTTAGCTGGT -3'; Slug Forward: 5'- CGAACTGGACACACATACAGTG -3', Slug Reverse: 5'- CTGAGGATCTCTGGTTGTGGT -3'; Snail Forward: 5'- GCGACCAACATCGAGCAGATT -3', Snail Reverse: 5'- CTGGTTTCCTTCAATGGGTGT -3'; β -actin Forward: 5'- CATGTACGTTGCTATCCAGGC -3; and β -actin Reverse: 5'- CTCCTTAATGTACGCACGAT -3. The relative quantification of LIN28B gene was determined by using the comparative CT ($\Delta\Delta CT$) method as recommended by the manufacturer.

Serial Replating Assay:

For evaluating self-renewal capacity, we assessed serial replating assay. Briefly, 1000 cells were plated in DMEM/F12 medium supplemented with 20 ng/mL of epidermal growth factor, 10 ng/mL of basic fibroblast growth factor and 2% B-27 and incubated for 10 days. Then the same number of dissociated cells were replated. 4 passages were replated.

CCK-8 Assay:

For evaluating the effects of propofol exposure on cell viability, CCK-8 assay was performed. Cells were seeded in 96-well plates (5×10^4 cells/well) and 10 μ L CCK-8 solution (Sigma-Aldrich, St. Louis, MO, USA) was added to each well and incubated at 37°C for 2 h in a CO₂ incubator. The absorbance value was measured at 620 nm wavelength on a Multiskan spectrum microplate reader (Thermo Electron Corporation, Waltham, MA, USA). The experiments were repeated three times.

CFSE/PI Double Staining Assay:

The cytotoxicity of propofol was evaluated by CFSE/propidium iodide (PI, Sigma-Aldrich, MO, U.S.A.) double staining. Briefly, CFSE-pre-stained cells were incubated with propofol for 24h, then cells were stained with 5 μ g/mL PI for 10 min in darkness. Then cells were washed three times with PBS and PI absorbance was determined by FACS on a flow

cytometry (FACSCalibur, Becton Dickinson, U.S.A.).

Statistical Analysis:

All data in this study were presented as mean $\pm$ SD. T-test was applied to compare between two groups. Two-way analysis of variance (ANOVA) was used to analyse the statistical significance. P values less than 0.05 was considered as statistically significant.

Results

Physiological Processes of A549 were Inhibited by Propofol Treatment:

A549 cells were treated with 0, 10, 25, 50, 75 or 100 μ mol/L propofol over 24, 48 or 72 h, and cell survival rate was determined by CCK-8 assay. Treatment with all these concentrations of propofol exerted no detectable effects on cell death over 72 h (**Fig-1A**). Then cell viability was determined after propofol exposure. The results were found that, with 75 or 100 μ mol/L propofol exposure for 24 h, significant decrease in cell viability was observed compared to mock group, respectively (**Fig-1B**). Similar results were found with exposure to 75 or 100 μ mol/L propofol for 24h by performing EdU staining (**Fig-1C**). For evaluating the effects of propofol exposure on malignant behaviors including cell migration and invasion, transwell assay without or with Matrigel was performed after 24-hour pretreatment of different concentration of propofol. As expected, relative low concentration of propofol decreased both migration and invasion activity in A549 (**Fig-1D**), which is consistent with previous finding [6].

Enrichment and Identification of CSCs from A549 Cells:

The inhibitory effects of propofol on A549 cells promoted us wonder whether propofol exerts similar effects on CSCs derived from A549 cells. By considering the widely accepted method for enriching the stem-like cells by sphere-forming ability of cancer cells in serum-free medium, A549 cells were cultured in serum-free DMEM/F12 supplemented with EGF, bFGF and B27 for 10 and 20 days. Morphologically, unattached spheres were observed at both 10 and 20 days (**Fig-2A**). To confirm whether enriched spheres present self-renewal capacity, additional serial replating experiments were performed. By passaging

for 5 times, no detectable decrease in the number of formed spheres in each 1000 cells, indicates the remarkable self-renewal capacity of stem-like cells enriched from A549 (**Fig-2B** and **Fig-2C**). Further, several hallmarks of stemness, including CD24, CD44, CD133, Oct4 and Nanog, were determined by semi-quantitative western blot. As expected, compared to parental A549 cells, CD44, CD133, Oct4 and Nanog were found significantly upregulated in both 10-day and 20-day spheres (**Fig-2D**).

The Effects of Propofol Treatment on Physiological Processes of CSCs Derived from A549 Cells:

By considering the inhibitory effects of different concentration of propofol on A549's malignant behaviors including proliferation, migration and invasion (**Fig-1**), we wonder whether propofol exerts similar effects to CSCs enriched from A549. By performing CCK-8 assay, surprisingly, it is found that 50, 75 and 100 $\mu\text{mol/L}$ propofol exposure obviously promoted cell viability after 24 and 48 h (**Fig-3A**). For further confirming its effects on proliferation of CSCs, 50 $\mu\text{mol/L}$ propofol was employed. As shown in **Fig-3B**, it is observed that propofol exposure significantly promoted cell viability, which is opposite with our expectation. In order to rule out the possibility of

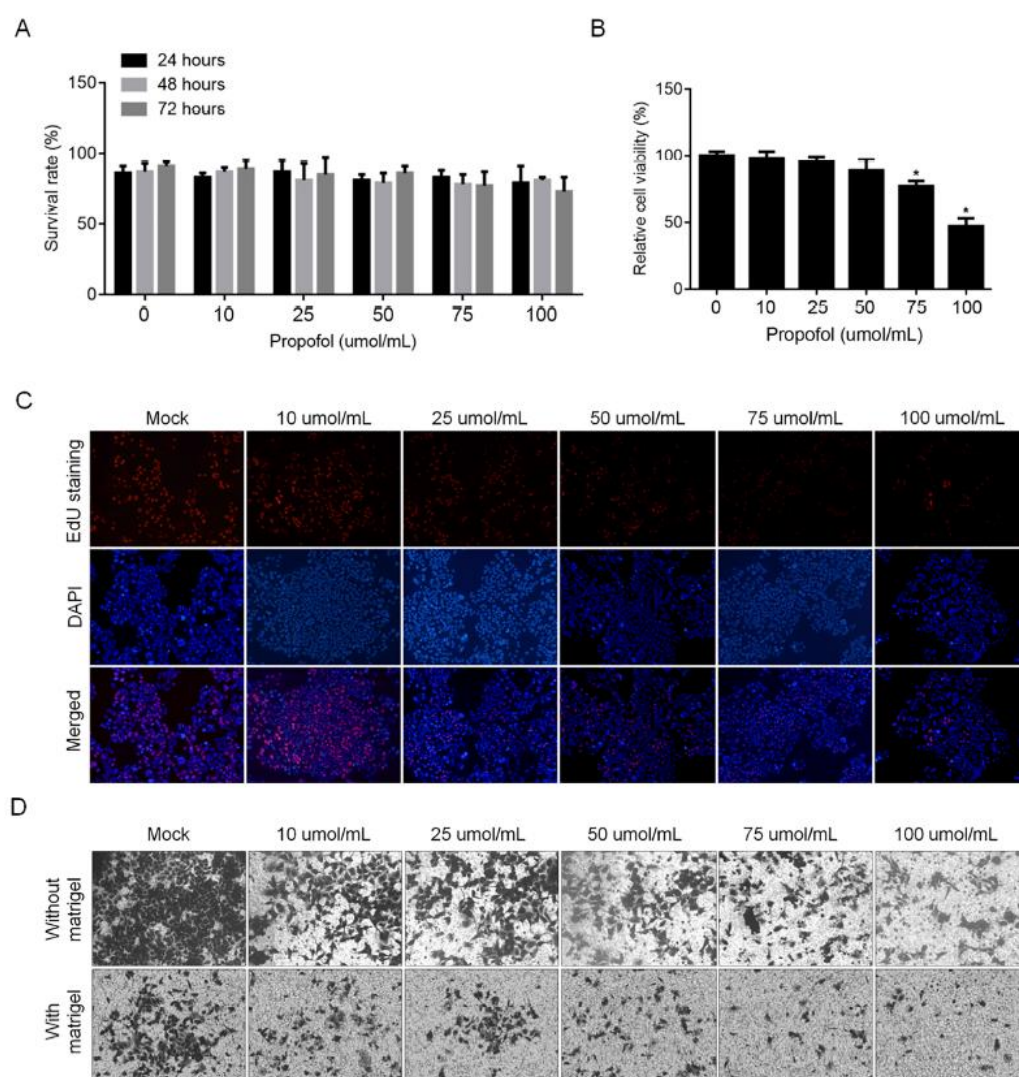


Fig-1: Propofol treatment inhibited A549 malignant behaviors in a dose-dependent way

(A) CFSE/PI double staining was performed to identify the effects of 10, 25, 50 or 100 μM propofol on cell survival rate. (B) CCK-8 assay was performed to evaluate cell viability after propofol treatment. (C) EdU staining assay was performed to identify the effects of propofol on cell proliferation. (D) Transwell assay without or with Matrigel were performed to evaluate propofol's effects on migration and invasion, respectively.

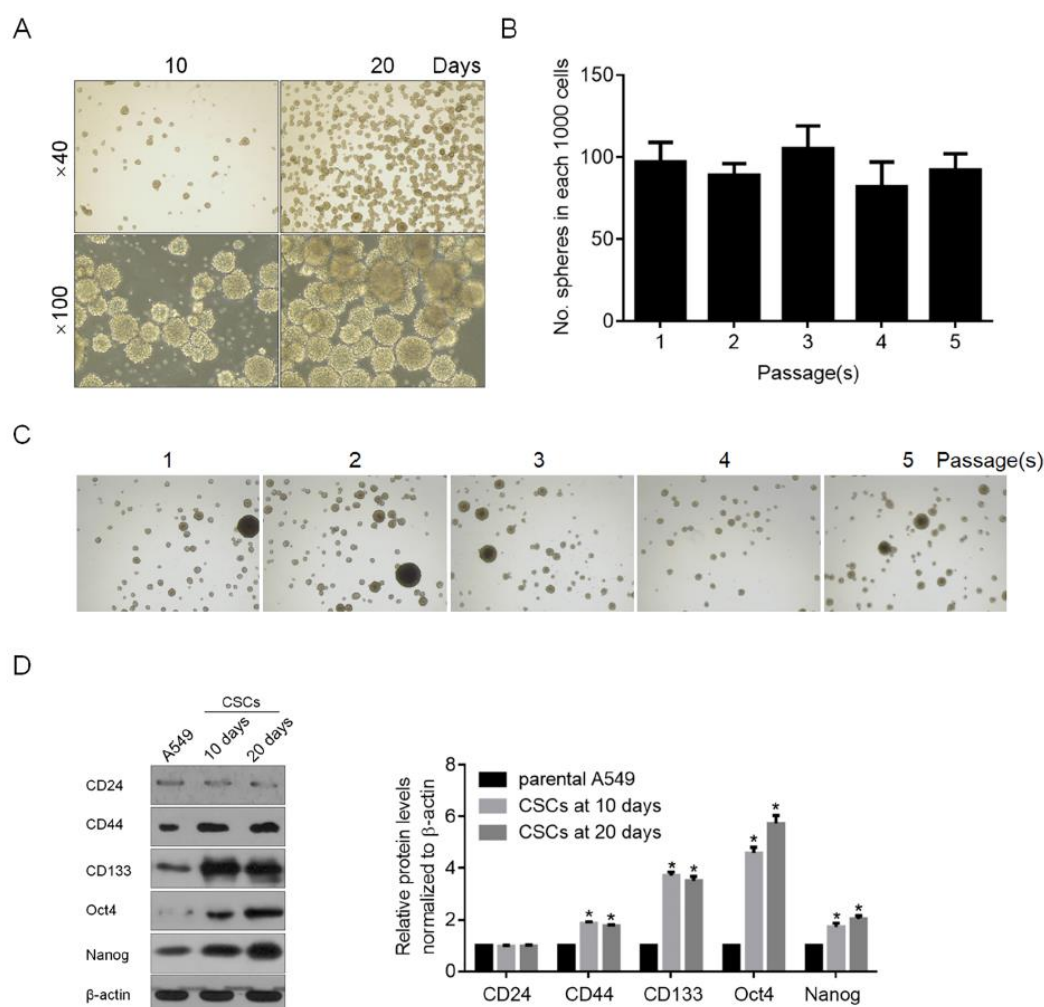


Fig-2: Enrichment and identification of CSCs from parental A549 cells

(A) Morphology of A549 CSCs after 10 and 20-day culture. (B) Serial replating assay was performed to identify the self-renewal capacity of A549 CSCs. (C) Semi-quantitative Western blot was performed to identify the expressing levels of hallmarks of CSCs, including CD24, CD44, CD133, oct4 and nanog normalized to β-actin.

promoting viability instead of promoting proliferation, propofol-exposed CSCs were fixed with 4% paraformaldehyde and stained with 5μg/ml PI followed by cytometric analysis. The results confirmed that, propofol exposure decreased the proportion of G1/Go phase in CSCs, and oppositely, arrested cell phase at G1/Go in A549 cells, which explain that the opposite effects in CSCs and A549 cells (**Fig-3C**). We then evaluate the effects of propofol on migration and invasion in CSCs. Surprisingly, propofol treatment inhibited migration and invasion in CSCs, which is similar in A549 cells (**Fig-3D**).

Propofol Treatment Inactivated EMT Program and Decreased Stemness of CSCs:

EMT has been accepted as a key and reversible

process, which allows cancer cells to be activated during the metastasis process [21,22]. By considering the effects of propofol on migration and invasion in CSCs enriched from A549 cells, we measured the relative expressing levels of EMT hallmarks, including E-cadherin, Vimentin, Slug and Snail. As shown in **Fig-4A** and **Fig-4B**, both mRNA and protein levels of E-cadherin, were upregulated and which of Vimentin, Slug and Snail were downregulated in propofol-treated CSCs, compared to untreated CSCs, demonstrating that propofol treatment inactivated EMT program. It has been reported that the changes in expressing levels of these hallmarks of EMT made the cells acquire the morphological changes [23]. After being cultured in propofol- contained medium for 3-6 days, spheres attempted to attached cells (**Fig-4C**), indicates that

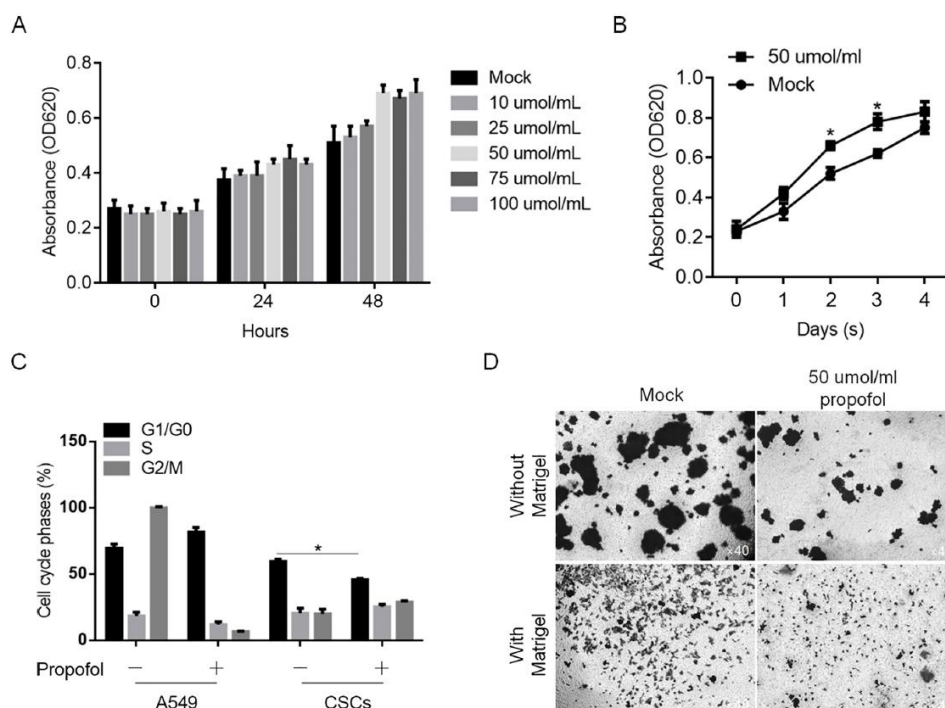


Fig-3: The effects of propofol on physiological processes of CSCs derived from A549 cells

(A & B) CCK-8 assay was performed to detect the effect of propofol exposure on proliferation. (C) PI stained cells were analyzed using flow cytometry assay to evaluate the distribution of cell cycle phases. (D) Transwell assay without or with Matrigel was performed to evaluate the effects of propofol exposure on migration or invasion.

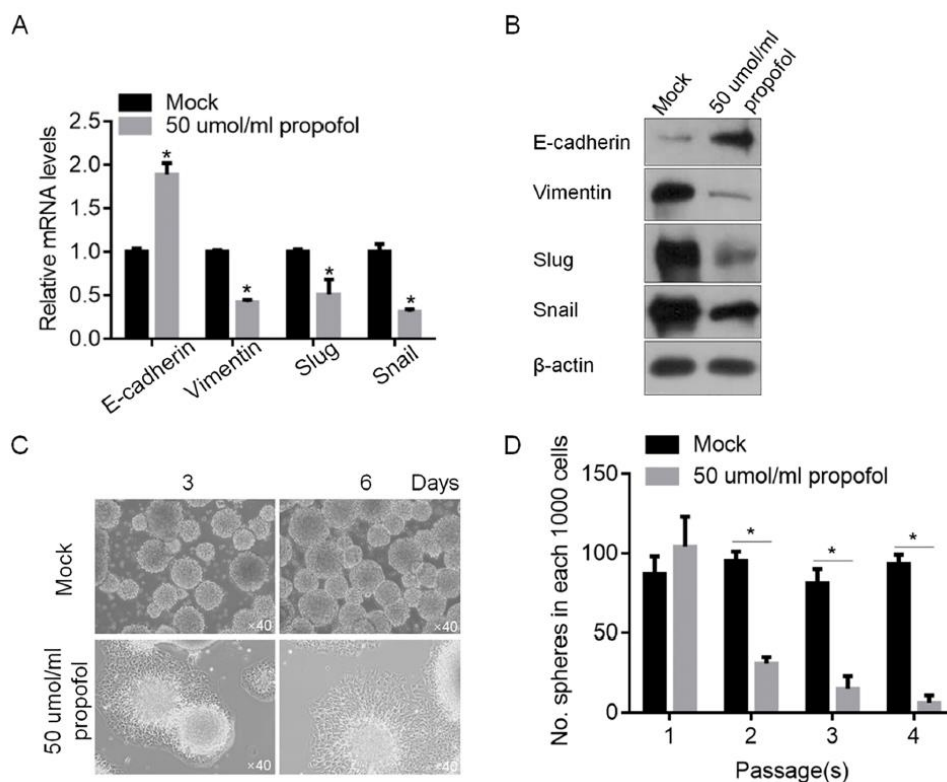


Fig-4: Propofol exposure inactivated EMT program and decreased stemness of CSCs derived from A549

RT-qPCR (A) and semi-quantitative Western blot (B) were performed to evaluate the expressing levels of hallmarks of EMT. (C) morphological changes after propofol exposure was imaged. (D) Serial replating assay was performed to detect the maintenance of stemness of CSCs derived from A549.

propofol treatment promoted cells differentiation or inhibited maintenance of stemness. By performing self-replating experiment, it is confirmed that propofol exposure decreased self-renewal capacity of CSCs (Fig-4D).

Propofol Treatment Regulated Physiological Processes of CSCs Potentially Via Inactivating EMT Program:

Transforming growth factor- β is the most studied growth factor which plays a central regulating role in activating EMT. In this study, by employing TGF- β as an EMT-activator before propofol exposure, we tried to clarify whether the effects of propofol exposure on physiological processes of CSC by inactivating EMT-program. By measuring the hallmarks of EMT with semi-quantitative Western blot, it is illustrated that upregulation of EMT hallmarks by TGF- β stimulation is inhibited after propofol exposure, indicates that propofol exposure not only morphologically inhibited

migration and invasion, but also affected EMT signaling in CSCs (Fig-5A). By performing Transwell assay with Matrigel, the consistent results were obtained with the expressing levels of EMT hallmarks (Fig-5B). TGF- β promoted cell proliferation was also inhibited by propofol exposure in CSCs (Fig-5C). By performing serial replating experiments, as expected, propofol decreased self-renewal capacity in CSCs and exerted antagonistic effect on maintaining stemness by TGF- β stimulation (Fig-5D).

Discussion

Although rapid advances in diagnostic and operative techniques have been developed, due to the advanced stage of lung cancer, it remains one of the most difficult human malignancies to treat, which leads to low survival rate and poor life quality. Recently, attention has been attracted on the potential roles of CSCs on regulating malignant behaviors, including metastasis and recurrent. Anesthesia represents one of

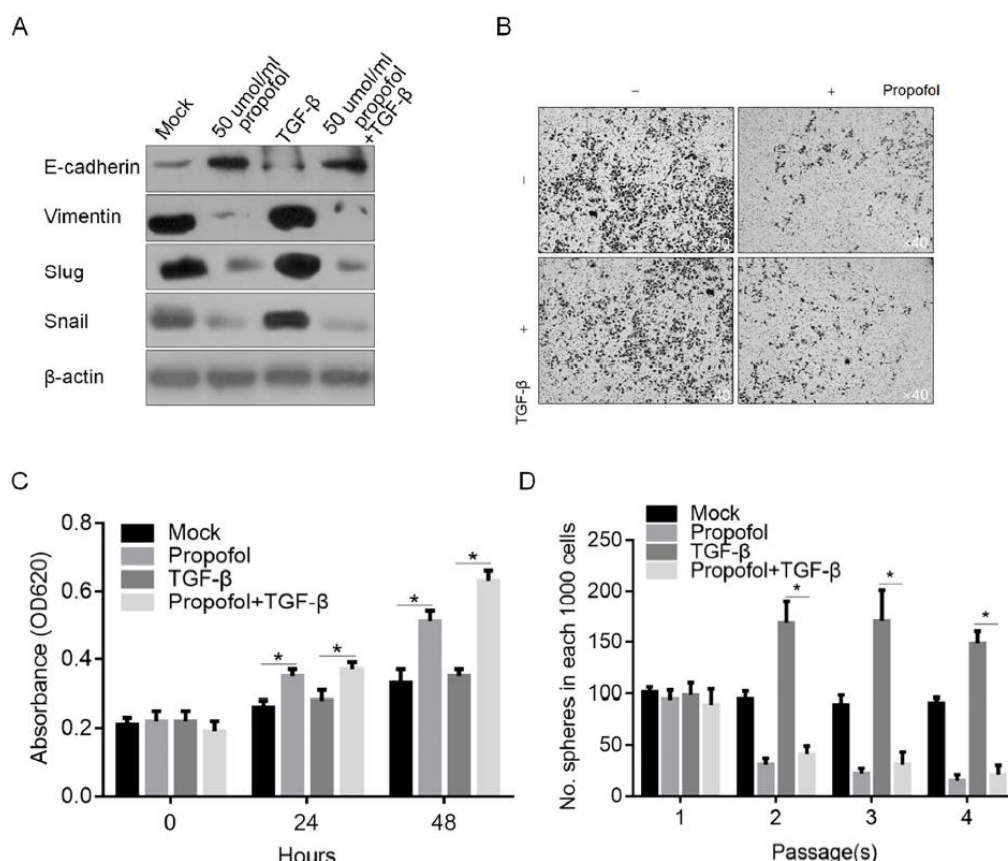


Fig-5: The inhibition of EMT by propofol exposure potentially leads to cell cycle arrest

(A) Hallmarks of EMT in untreated (mock), propofol treated (Propofol), TGF- β treated (TGF- β) and propofol/ TGF- β co-treated (propofol/ TGF- β) cells were evaluated. (B) Transwell with or without matrigel were performed to detect the migration and invasion ability. (C) Cell viability of above-mentioned cells was measured. (D) Serial replating assay was performed.

the most critical medical advances in human history and widely used for cancer resection, which made its regulatory role on cancer cells important. Although intensive investigation on the effects of anesthetic agent, including propofol, have been performed, little is known about the effects of anesthetic agent on CSCs, which contributes to malignant behaviors in cancer cells.

Propofol is one of the most well-known and widely used anesthetics for decades. Numerous reports have demonstrated its regulatory roles on different kinds of cancer cells. Garib and colleagues found that clinical concentration of propofol (34 $\mu\text{mol/L}$) activated migrating activity of breast carcinoma cells MDA-MB-468 via promoting EMT-programme [24]. Interestingly, in several human cancer cells, including Hela, HT1080, HOS and RPMI-7951, the clinical concentration of propofol (5.6-28.0 $\mu\text{mol/L}$) exposure decreased the migrating and invasive abilities by inhibiting EMT programme [3]. Also, Liu et al. reported that propofol stimulation promoted invasion of A549 cells [6]. By considering the contradictory results, we set a concentration range of propofol (10-100 $\mu\text{mol/L}$) exposure to test its effect on the malignant behaviors of A549 and CSCs derived from A549. Our results showed that A549 and CSCs derived from A549 present different reacting sensitivity to propofol. Propofol exposure inhibited migrating and invasive abilities in both A549 and CSCs derived from A549. However, instead of inhibiting proliferation in A549, propofol exposure promoted proliferation in CSCs.

In this study, we exposed non-small cell lung cancer cells A549 and CSCs derived from A549 to propofol, and tested its efficacy against the physiological processes, including self-renewal capacity, proliferation, migration and invasion. This preclinical study documented that propofol exposure exerted inhibitory effects to malignant behavior in A549 cells. In CSCs, despite of promoting proliferation, propofol exposure also inhibited malignant behaviors. We found that propofol treatment significantly decreased the maintenance of self-renewal capacity in CSCs by inactivating EMT-programme. The inducing effects of EMT by TGF- β was confirmed in CSCs, and propofol treatment abolished the induction of TGF- β on EMT.

This indicated the potential mechanism of propofol's regulating effects on CSCs via affecting EMT programme. By considering that all our experiments were carried out in vitro, the exact roles of propofol on lung epithelial cancer cells and CSCs should be detected in vivo.

In conclusion, our findings are consistent with previous report showing that propofol treatment inhibited physiological processes in A549 cells. It is the first time to evaluate the effects of propofol exposure on CSCs derived from non-small cell lung cancer cells and its inhibitory effects towards to CSCs potentially via regulating EMT was confirmed. Taken together, our results suggest that propofol can be an anesthetic agent that play inhibitory effects not only to non-small cell lung cancer cells, but also the CSCs, which is a critical sub-population contributes to poor prognosis. All our data may provide therapeutic suggestions on the usage of propofol in non-small cell lung cancer-related surgery.

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Conflict of Interest

The authors have read and approved the final version of the manuscript. The authors have no conflicts of interest to declare.

References

- [1] Marik PE. Propofol: therapeutic indications and side-effects. *Curr Pharm Des.* 2004;10(29):3639-49. [PMID: 15579060]
- [2] Vasileiou I, Xanthos T, Koudouna E, Perrea D, Klonaris C, Katsargyris A, Papadimitriou L. Propofol: a review of its non-anaesthetic effects. *Eur J Pharmacol.* 2009 Mar 1;605(1-3):1-8. [PMID: 19248246]
- [3] Mammoto T, Mukai M, Mammoto A, Yamanaka Y, Hayashi Y, Mashimo T, Kishi Y, Nakamura H.

Intravenous anesthetic, propofol inhibits invasion of cancer cells. *Cancer Lett.* 2002 Oct 28;184(2):165-70. [PMID: [12127688](#)]

[4] Tsuchiya M, Asada A, Arita K, Utsumi T, Yoshida T, Sato EF, Utsumi K, Inoue M. Induction and mechanism of apoptotic cell death by propofol in HL-60 cells. *Acta Anaesthesiol Scand.* 2002 Oct;46(9):1068-74. [PMID: [12366500](#)]

[5] Miao Y, Zhang Y, Wan H, Chen L, Wang F. GABA-receptor agonist, propofol inhibits invasion of colon carcinoma cells. *Biomed Pharmacother.* 2010 Nov;64(9):583-88. [PMID: [20888181](#)]

[6] Liu WZ, Liu N. Propofol Inhibits Lung Cancer A549 Cell Growth and Epithelial-Mesenchymal Transition Process by Upregulation of MicroRNA-1284. *Oncol Res.* 2018 Dec 27;27(1):1-8. [PMID: [29402342](#)]

[7] Inada T, Kubo K, Shingu K. Possible link between cyclooxygenase-inhibiting and antitumor properties of propofol. *J Anesth.* 2011 Aug;25(4):569-75. [PMID: [21611862](#)]

[8] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer.* 2010 Dec 15;127(12):2893-17. [PMID: [21351269](#)]

[9] Lynch TJ, Adjei AA, Bunn PA Jr, Eisen TG, Engelman J, Goss GD, Haber DA, Heymach JV, Jänne PA, Johnson BE, Johnson DH, Lilenbaum RC, Meyerson M, Sandler AB, Sequist LV, Settleman J, Wong KK, Hart CS. Summary statement: novel agents in the treatment of lung cancer: advances in epidermal growth factor receptor-targeted agents. *Clin Cancer Res.* 2006 Jul 15;12(14 Pt 2):4365S-71S. [PMID: [16857812](#)]

[10] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature.* 2001 Nov 1;414(6859):105-11. [PMID: [11689955](#)]

[11] Wang JC, Dick JE. Cancer stem cells: lessons from leukemia. *Trends Cell Biol.* 2005 Sep;15(9):494-501. [PMID: [16084092](#)]

[12] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL, Wahl GM. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res.* 2006 Oct 1;66(19):9339-44. [PMID: [16990346](#)]

[13] Tirino V, Camerlingo R, Franco R, Malanga D, La Rocca A, Viglietto G, Rocco G, Pirozzi G. The role of CD133 in the identification and characterisation of

tumour-initiating cells in non-small-cell lung cancer. *Eur J Cardiothorac Surg.* 2009 Sep;36(3):446-53. [PMID: [19464919](#)]

[14] Chiou SH, Wang ML, Chou YT, Chen CJ, Hong CF, Hsieh WJ, Chang HT, Chen YS, Lin TW, Hsu HS, Wu CW. Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation. *Cancer Res.* 2010 Dec 15;70(24):10433-44. [PMID: [21159654](#)]

[15] Yuan P, Kadara H, Behrens C, Tang X, Woods D, Solis LM, Huang J, Spinola M, Dong W, Yin G, Fujimoto J, Kim E, Xie Y, Girard L, Moran C, Hong WK, Minna JD, Wistuba II. Sex determining region Y-Box 2 (SOX2) is a potential cell-lineage gene highly expressed in the pathogenesis of squamous cell carcinomas of the lung. *PLoS One.* 2010 Feb 9;5(2):e9112. [PMID: [20161759](#)]

[16] Eramo A, Lotti F, Sette G, Pilozi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C, De Maria R. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ.* 2008 Mar;15(3):504-14. [PMID: [18049477](#)]

[17] Justilien V, Regala RP, Tseng IC, Walsh MP, Batra J, Radisky ES, Murray NR, Fields AP. Matrix metalloproteinase-10 is required for lung cancer stem cell maintenance, tumor initiation and metastatic potential. *PLoS One.* 2012;7(4):e35040. [PMID: [22545096](#)]

[18] Zhang L, Wang D, Li Y, Liu Y, Xie X, Wu Y, Zhou Y, Ren J, Zhang J, Zhu H, Su Z. CCL21/CCR7 Axis Contributed to CD133+ Pancreatic Cancer Stem-Like Cell Metastasis via EMT and Erk/NF-κB Pathway. *PLoS One.* 2016 Aug 9;11(8):e0158529. [PMID: [27505247](#)]

[19] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Briskin C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell.* 2008 May 16;133(4):704-15. [PMID: [18485877](#)]

[20] Morel AP, Lièvre M, Thomas C, Hinkal G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. *PLoS One.* 2008 Aug 6;3(8):e2888. [PMID: [18682804](#)]

[21] Spaderna S, Schmalhofer O, Hlubek F, Berx G, Eger A, Merkel S, Jung A, Kirchner T, Brabletz T. A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal

- cancer. *Gastroenterology.* 2006 Sep;131(3):830-40. [PMID: [16952552](#)]
- [22] Prall F. Tumour budding in colorectal carcinoma. *Histopathology.* 2007 Jan;50(1):151-62. [PMID: [17204028](#)]
- [23] Tiwari N, Gheldof A, Tatari M, Christofori G. EMT as the ultimate survival mechanism of cancer cells. *Semin Cancer Biol.* 2012 Jun;22(3):194-207. [PMID: [22406545](#)]
- [24] Garib V, Lang K, Niggemann B, Zänker KS, Brandt L, Dittmar T. Propofol-induced calcium signalling and actin reorganization within breast carcinoma cells. *Eur J Anaesthesiol.* 2005 Aug;22(8):609-15. [PMID: [16119598](#)]

