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Original Paper

Retracted Article

Propofol Inhibits Proliferation, Migration, Invasion and Promotes Apoptosis Through Down-Regulating miR-374a in Hepatocarcinoma Cell Lines

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Key Words
Hepatocellular carcinoma • Propofol • [illegible] • Wnt/β-catenin • PI3K/AKT

Abstract
Background: Propofol is a c[illegible]ly used anaesthetic with controversial effects on cancer cells. We aimed to explo[illegible] the [illegible]al roles of propofol in hepatocellular carcinoma (HCC) cells as well as th[illegible]nderlying mechanisms. ***Methods:*** HepG2 and SMMC-7721 cells were used in this study. [illegible]tly, [illegible] effects of propofol on cell viability, migration, invasion, apoptosis, and inv[illegible] p[illegible]were assessed by Cell Counting Kit-8 assay, Transwell assay, flow cytometry [illegible] Western blot analysis, respectively. Subsequently, alteration of miR-374a after sti[illegible]la[illegible]ropofol was analyzed by qRT-PCR. miR-374a was overexpressed and the al[illegible]tio[illegible]oteins in the Wnt/β-catenin and PI3K/AKT pathways was detected by Wes[illegible]blot a[illegible]sis. The downstream factor of miR-374a was finally studied. ***Results:*** [illegible]op[illegible]fol [illegible]ited cell viability, migration and invasion but promoted apoptosis of HepG2 and [illegible]-772[illegible]. Meanwhile, cyclinD1, matrix metalloproteinase (MMP)-2 and MMP-9 were [illegible]gulated while Bax/Bcl-2, cleaved caspase-3 and cleaved caspase-9 were up-regulated [illegible]. Then, miR-374a level was reduced by propofol. Expression of Wnt3a, β-catenin, [illegible]I3K and p-AKT was decreased by propofol, whereas these decreases were reversed by mi[illegible]74a overexpression. Finally, TP53 was proven to be target of miR-374a in HepG2 cells. ***[illegible]clusion:*** Propofol inhibited cell proliferation, migration and invasion while promoted cell apoptosis of HepG2 and SMMC-7721 cells through inhibiting the Wnt/β-catenin and PI3K/AKT pathways via down-regulation of miR-374a. Besides, miR-374a affected propofol-treated HepG2 cells by targeting TP53.

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Introduction

Liver cancer, a fatal malignant tumor that is most commonly relative to virus infection, is the second leading cause of cancer-related death worldwide which leads to 745 thousand deaths annually all over the world [1, 2]. Hepatocellular carcinoma (HCC) resulting in hig[illegible] morbidity and mortality is categorized into primary liver cancer and accounts for 90% o[illegible] all cases with primary liver cancer [3]. On account of the high prevalence of hepatitis B virus-induced hepatitis, HCC in China accounts for >50% of world's burden [4, 5]. Mod[illegible] therapeutic strategies such as surgery, chemotherapy, radiotherapy and biotherapy [illegible]e been applied for the treatments of HCC, however, the outcome remains unsa[illegible]actory [illegible] The poor prognosis and high prevalence make the exploration of effective trea[illegible]ts [illegible] HCC become a hotspot.

Propofol (2, 6-diisopropylphenol) is an intravenous anesthetic that [illegible]monly [illegible]d for short-term sedation [7]. Numerous literatures have reported the i[illegible]odulatory action of propofol, especially for anti-inflammatory property [8, 9]. In [illegible]ditio[illegible]unting evidence has stated propofol exerts neuroprotection against foca[illegible]ebral ischemia in animal models [10, 11]. Recently, the functional roles of [illegible]ofol in [illegible]e cancer types are identified to be controversial. Cell proliferation and [illegible]f gastr[illegible]ancer cells are suppressed by propofol [12]. Conversely, cell proliferatio[illegible]nd [illegible]n of breast cancer cells are promoted by propofol via down-regulation of p53 [illegible]ctivation of Nrf2 pathway [13]. Thus, precise investigation with respect [illegible] explore the [illegible]e of propofol in a specific cancer is of great importance.

MicroRNAs (miRNAs/miRs) are small, no[illegible]g RNAs that post-transcriptionally participate in regulation of gene expression [illegible]]. [illegible]rous miRNAs are reported to be non-physiologically expressed in HCC, acting [illegible]onco[illegible]c or antitumor factor [15, 16]. A previous literature has reported that pr[illegible]fol [illegible]hibit osteosarcoma cell proliferation and invasion by modulating miR-143 [illegible]xp[illegible]ion [17]. Another study also illustrated that cell viability and apoptosis of lung [illegible] cel[illegible] were repressed by propofol through up-regulating miR-486 [18]. The[illegible]f[illegible], th[illegible] of propofol on physiological activity of cells may be related to its modula[illegible]n miRNAs. In our study, the functional roles of propofol in cell proliferation, migra[illegible]on, [illegible] and apoptosis of HCC cells including HepG2 and SMMC-7721 cells, as we[illegible]s the p[illegible]tially associated miRNAs, were thoroughly studied. Moreover, the involved s[illegible]aling pathways were also explored.

Materials [illegible]nd [illegible]ds

Cell [illegible]e and [illegible]ents

Hum[illegible]C cell lines HepG2 and SMMC-7721 were obtained from the Cell Bank of the Chinese Academy [illegible]ces (S[illegible]hai, China). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, [illegible], MA, U[illegible]A) containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) in a humidified incub[illegible]7°C with a gas mixture of 5% CO_2 and 95% air. When monolayer cultures reached confluency, [illegible] were subcultured using the Trypsin (0.05%; Gibco). For stimulation of propofol, cells were incubated [illegible] culture medium containing diverse doses of propofol (0-20 μg/mL; Sigma, St. Louis, MO, USA) for 48 h [illegible]1].

Cell Counting Kit-8 (CCK-8) assay

Cell viability was tested by using a CCK-8 purchased from Dojindo Molecular Technologies (Gaithersburg, MD, USA). In brief, cells (1 × 10^3 cells/well) were seeded into 96-well plates and maintained at 37°C for 48 h. Then, 10 μL of CCK-8 solution was added into each well, and cells were cultured for additional 1 h at 37°C. Absorbance at 450 nm was read by using a Microplate Reader (Bio-Rad, Hercules, CA, USA).

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Migration and invasion assay

Cell migration and invasion were measured by Transwell migration/invasion assay, which was performed by using cell culture inserts carrying a membrane with 8 μm pores in 24-well plates (BD Biosciences, San Jose, CA, USA). Briefly, 5.0 × 10⁴ cells in 200 μL low serum DMEM medium (0.1% FBS) were plated into the upper compartment, whereas 600 μL DMEM supplemented with 10% FBS was added to [illegible] lower compartment. For Transwell migration assay, cells were incubated at 37°C for 20 h. For Transwe[illegible] invasion assay, the inserts were pre-coated with Matrigel (30 μg/insert; BD Biosciences) and the invasive procedure was lasted for 24 h at 37°C. Then, cells on the upper surface of the filter were removed by a cot[illegible] swab carefully. Migratory/invasive cells on the lower side of the filter were stained with 0.1% crystal v[illegible]t, and then cells in five randomly chosen fields were counted under a microscope (Olympu[illegible]tical, T[illegible] Japan).

Apoptosis assay

Cell apoptosis was determined by staining with fluorescein isothiocyanate (FITC)-co[illegible] Annexin V and propidium iodide (PI). Briefly, cells were trypsinized, washed with phosphate bu[illegible]ed sa[illegible]S), and resuspened in binding buffer. After being labeled by Annexin V-FITC and PI from [illegible]ITC Annexin V/Dead Cell Apoptosis Kit (Thermo Scientific, Waltham, MA, USA) following [illegible] recommen[illegible] of manufacturer. Labeled cells were analyzed using a flow cytometry (FACSCalibu[illegible]nces) w[illegible] FlowJo software (Tree Star, San Carlos, CA, USA).

Construction of recombined plasmids and cell tran[illegible]tion

Scramble miRNAs and miR-374a mimic were synt[illegible]d by GeneP[illegible]a Co. (Shanghai, China). Full-length TP53 sequences were amplified and ligated into p[illegible]1 plasmid (Invitrogen, Carlsbad, CA, USA), and the recombined plasmid was referred to as pc-T[illegible] mi[illegible] pcDNA3.1 or pc-TP53 were transfected into cells with the help of the Lipofectamine 3000 r[illegible]nt (I[illegible]ogen) according to the manufacturer's protocol.

Luciferase reporter assay

Wide-type (WT) TP53 3' un[illegible]ed [illegible]TR) containing the putative miR-374a binding site or a mutant sequence was inserte[illegible]he downstream of the firefly luciferase gene in the pmirGLO Dual-Luciferase miRNA Target Expre[illegible]ion v[illegible]omega Corporation, Fitchburg, WI, USA). The reconstructed plasmids were designated as [illegible]53-WT an[illegible]53-MUT. HEK-293T cells co-transfected with miR-374a mimic (scramble miRNA) and TP[illegible]VT (TP53-MUT) were collected at 48 h post-transfection, and luciferase activity was analyzed using [illegible]ciferase Reporter Assay System (Promega Corporation). Luciferase activity values we[illegible]ized to that of the Renilla luciferase internal control.

Quantit[illegible]ive re[illegible]e t[illegible]scription PCR (qRT-PCR)

As re[illegible]menda[illegible] supplier, total RNA of cells was extracted using Trizol reagent (Invitrogen). Then, 500 ng o[illegible] was reverse transcribed into cDNA by using the Taqman MicroRNA Reverse Transcription [illegible]pplied [illegible]stems, Foster City, CA, USA). The reaction condition was 16°C for 30 min, 42°C for 30 [illegible]owed by [illegible]5°C for 5 min. The following real-time PCR was performed with 50 ng cDNA as template usin[illegible]n Universal Master Mix II (Applied Biosystems) to analyze the expression of miR-374a. The [illegible]mal cycling parameters were 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C [illegible]or [illegible]min. The *Power* SYBR® Green RNA-to-CT™ 1-Step Kit (Applied Biosystems) was used for determination o[illegible]53 mRNA levels, according to the manufacturer's instructions. The relative expression was calculated [illegible]ccording to the $2^{-\Delta\Delta Ct}$ method [22]. U6 and GAPDH were acted as the internal controls for miR-374a and TP53 mRNA, respectively.

Western blot analysis

Proteins were extracted in RIPA lysis buffer (Beyotime, Shanghai, China) with the presence of protease inhibitors (Applygen Technologies Inc., Beijing, China). After quantification with the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA), proteins (30 μg/lane) were loaded and separated by SDS-PAGE. Then, proteins in the gels were transferred into polyvinylidene difluoride (PVDF) membranes, followed by blockage with 5% non-fat milk. Membranes were incubated with each antibody against cyclinD1 (ab134175),

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matrix metalloproteinase (MMP)-2 (ab97779), MMP-9 (ab73734), mammalian B cell lymphoma-2 (Bcl-2; ab194583), Bcl-2-associated X protein (Bax; ab32503), cleaved caspase-3 (ab2302), cleaved caspase-9 (ab2324), Wnt3a (ab81614), β-catenin (ab6302), phosphatidylinositol-3-kinase (PI3K; ab135952), phospho-PI3K (p-PI3K; ab182651), TP53 (ab131442), β-actin (ab8227) (all Abcam, Cambridge, UK), AKT (#9272) or phospho-AKT (p-AKT; #9271) (both Cell Signaling Technology, Beverly, MA, USA) at 4°C overnight. After rinsing, membranes were incubated with secondary antibodies marked by horseradish peroxidase (ab97051 and ab205719, Abcam) for 1 h at room temperature. After rinsing again, proteins in the membranes were visualized by chemiluminescence (ECL) system (Amersham Biosciences, Piscataway, New Jersey, USA).

Statistical analysis

All experiments were repeated three times. The results were presented as the mean ± SD. Statistical analysis was performed using Graphpad Prism 5 software (GraphPad, San Diego, CA, USA). The *P*-values were calculated using the one-way analysis of variance (ANOVA) or Student's *t*-test. A $P < 0.05$ was considered as a significant difference.

Results

Propofol inhibits cell proliferation of HepG2 and SMMC-7721 cells

The effects of propofol on cell viability were measured by CCK-8 assay in both HepG2 and SMMC-7721 cells. When compared to the 0 μg/mL group (control), cell viability was significantly reduced by propofol at 5 μg/mL ($P < 0.05$), 10 μg/mL ($P < 0.01$) and 20 μg/mL ($P < 0.001$; Fig. 1A) doses. However, the effects of 1 μg/mL propofol on cell viability were non-significant compared to the control. Considering that cell viability was reduced to almost

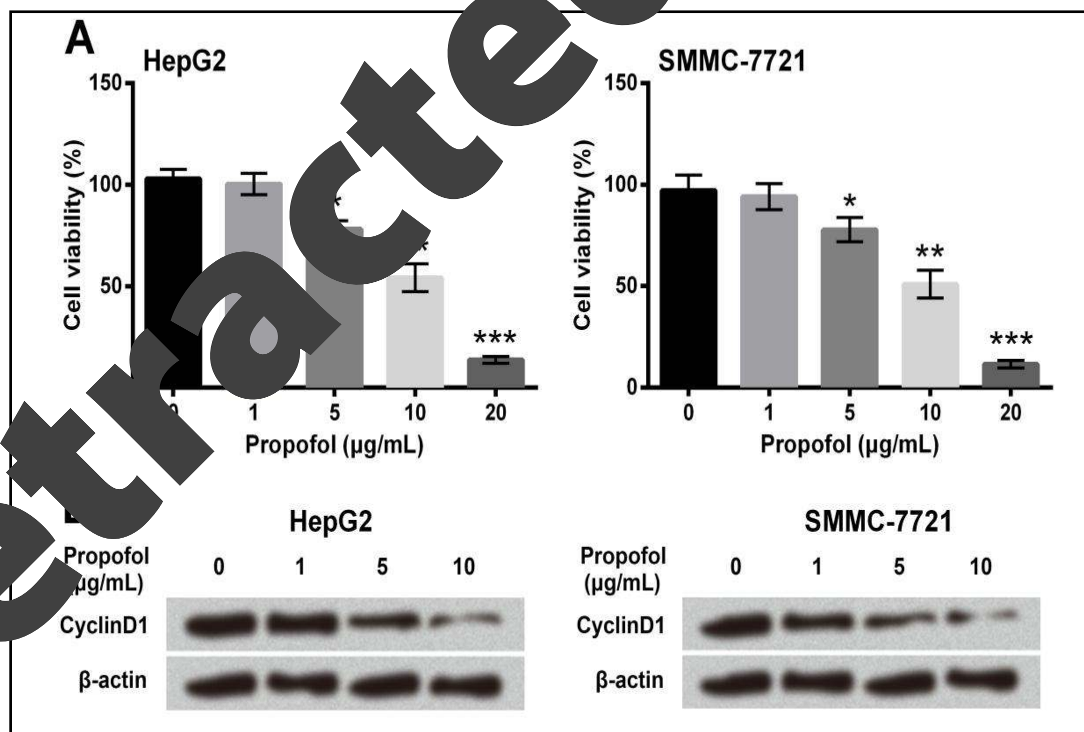

Fig. 1. Propofol inhibits cell viability of HepG2 and SMMC-7721 cells. Cells were stimulated with propofol under diverse concentrations (0, 1, 5, 10, 20 μg/mL) at 37°C for 48 h. Cells under 0 μg/mL propofol were acted as the control. A. Cell viability by a Cell Counting Kit-8 assay. B. Expression of cyclinD1 by Western blot analysis. Data are presented as the mean ± SD of at least three independent experiments. *, P<0.05; **, P<0.01; ***, P<0.001.

50% when cells were incubated with 10 μg/mL propofol, the concentration of propofol in subsequent experiments was 10 μg/mL. Meanwhile, the protein expression of cyclinD1 was obviously down-regulated as the increasing of the propofol concentration (Fig. 1B). As the first protein synthesized in the G1 phase, cyclinD1 acts a crucial role in cell cycle progression from G0/G1 phase to S phase [23]. Therefore, the down-regulation of cyclinD1 induced by propofol indicated an anti-proliferative role. Results indicated that cell proliferation was reduced by propofol in HepG2 and SMMC-7721 cells.

Propofol suppresses cell migration and invasion of HepG2 and SMMC-7721 cells

Then, the influences of propofol on migration and invasion of HepG2 and SMMC-7721 cells were tested by the Transwell assays. As compared to the control group, migration (Fig. 2A) and invasion (Fig. 2B) of HepG2 and SMMC-7721 cells were markedly decreased by propofol (all $P < 0.05$). Extracellular matrix (ECM) comprises complex components, including fibronectin and laminin, which are essential for the attachment of cells to structural skeleton [24]. MMPs are the most extensive ECM degrading enzymes that can degrade all important components of ECM [25]. Due to the specificity for degradation of the basement membrane, MMP-2 and MMP-9 are two most essential MMPs and had been reported to be correlated with cell migration and invasion of HCC cells [illegible]. [illegible] Western blot analysis showed the protein expression of MMP-2 and MMP-9 was [illegible] down-regulated after stimulation of propofol (Fig. 2C). Results described above indicated that migration and invasion of HepG2 and SMMC-7721 cells were reduced by propofol.

Propofol promotes cell apoptosis of HepG2 and SMMC-7721 cells

Next, the impacts of propofol on apoptosis of HepG2 and SMMC-7721 cells were tested by flow cytometry assay. In Fig. 3A, percentage of apoptotic cells was significantly elevated by treatment with propofol, as compared to the control group (both $P < 0.01$). Cell apoptosis is regulated by the Bcl-2 family and caspase family proteins [illegible 28]. In brief, [illegible] ratio of Bax/Bcl-2 [illegible] mitochondrial outer membrane permeabilization (MOMP) and release of pro-apoptotic proteins [29]. Then, caspase-9 and caspase-3 are cleaved in turns, resulting in the hallmarks of the cell apoptosis [27]. The following results of Western blot analysis showed that Bcl-2 level was decreased whereas levels of Bax, cleaved caspase-3 and cleaved caspase-9 were increased by

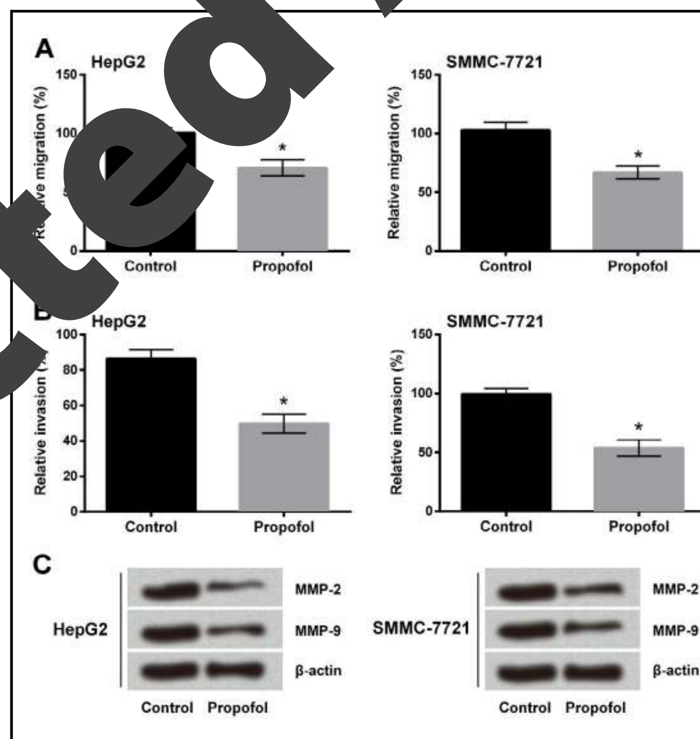

Fig. 2. Propofol suppresses cell migration and invasion of HepG2 and SMMC-7721 cells. Cells were stimulated with 10 μg/mL propofol at 37°C for 48 h. Non-treated cells were acted as the control. A. Cell migration by Transwell migration assay. B. Cell invasion by Transwell invasion assay. C. Expression of MMP-2 and MMP-9 by Western blot analysis. Data are presented as the mean ± SD of at least three independent experiments. *, P<0.05. MMP, matrix metalloproteinase.

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propofol (Fig. 3B). Results described above stated that cell apoptosis of HepG2 and SMMC-7721 cells were promoted by propofol.

miR-374a is down-regulated by propofol in HepG2 and SMMC-7721 cells

To explore whether miR-374a was involved in the modulation of propofol, the expression of miR-374a was determined by qRT-PCR. As compared to the 0 μg/mL group (control), miR-374a levels in the 5 μg/mL groups (both $P < 0.05$) and 10 μg/mL groups ($P < 0.05$ or $P < 0.01$) were significantly lower than the control group in HepG2 (Fig. 4A) and SMMC-7721 (Fig. 4B) cells. However, the alteration of miR-374a level after treatments with 1 μg/mL propofol was non-significant. Those data illustrated that propofol down-regulated miR-374a expression in HepG2 and SMMC-7721 cells.

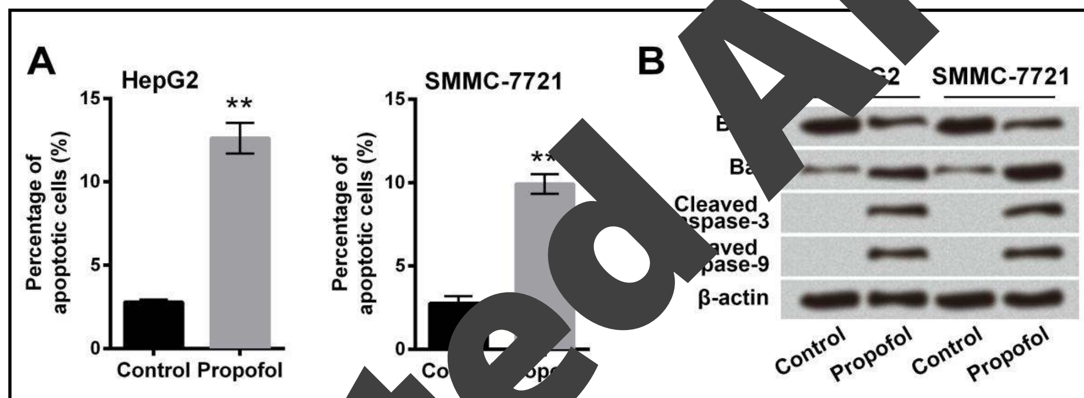

Fig. 3. Propofol promotes cell apoptosis of HepG2 and SMMC-7721 cells. Cells were stimulated with 10 μg/mL propofol at 37°C for 48 h. Non-treated cells were acted as the control. A. Percentage of apoptotic cells by flow cytometry assay. B. Expression of apoptosis-associated proteins by Western blot analysis. Data are presented as the mean ± SD of at least three independent experiments. **, P<0.01. Bcl-2, mammalian B cell lymphoma-2; Bax, Bcl-2-associated X protein.

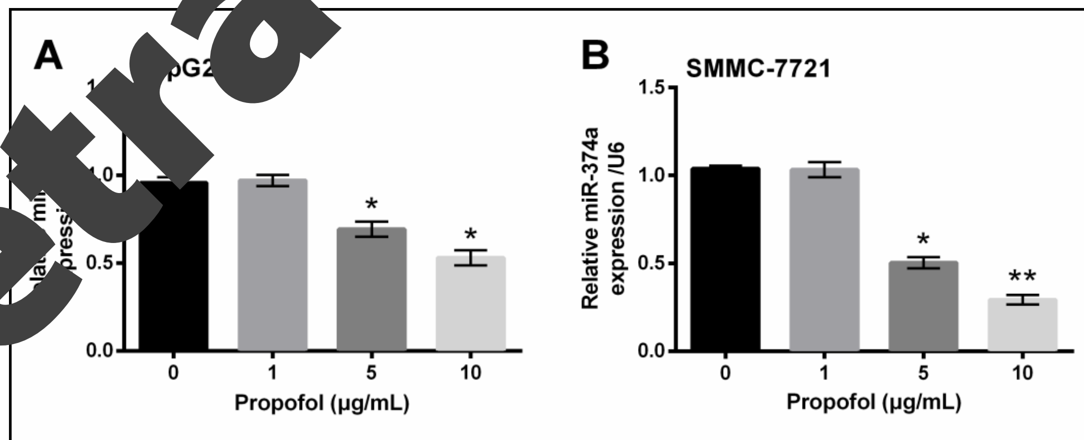

Fig. 4. MicroRNA (miR)-374a is down-regulated by propofol in HepG2 and SMMC-7721 cells. Cells were stimulated with propofol under diverse concentrations (0, 1, 5, 10 μg/mL) at 37°C for 48 h. Cells under 0 μg/mL propofol were acted as the control. Expression of miR-374a in HepG2 (A) and SMMC-7721 (B) cells was measured by quantitative reverse transcription PCR. Data are presented as the mean ± SD of at least three independent experiments. *, P<0.05; **, P<0.01.

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Propofol inhibits the Wnt/β-catenin and PI3K/AKT pathways through down-regulating miR-374a

The possible regulatory mechanisms of propofol in HepG2 and SMMC-7721 cells were investigated. After cell transfection, expression of miR-374a was remarkably up-regulated by transfection with miR-374a mimic compared with the scramble group (both $P < 0.01$), which indicated that miR-374a was successfully overexpressed by transfection with miR-374a mimic (Fig. 5A). Then, expression of key kinases in the Wnt/β-catenin and PI3K/AKT pathways was assessed. In Fig. 5B, expression of Wnt3a and β-catenin was down-regulated by propofol, whereas the down-regulation was reversed by overexpression of miR-374a. Likewise, phosphorylation of PI3K and AKT was reduced by propofol while the reduction could be reversed by overexpression of miR-374a (Fig. 5C). Taken together, these results indicated that propofol inhibited the Wnt/β-catenin and PI3K/AKT pathways through down-regulating miR-374a in HepG2 and SMMC-7721 cells.

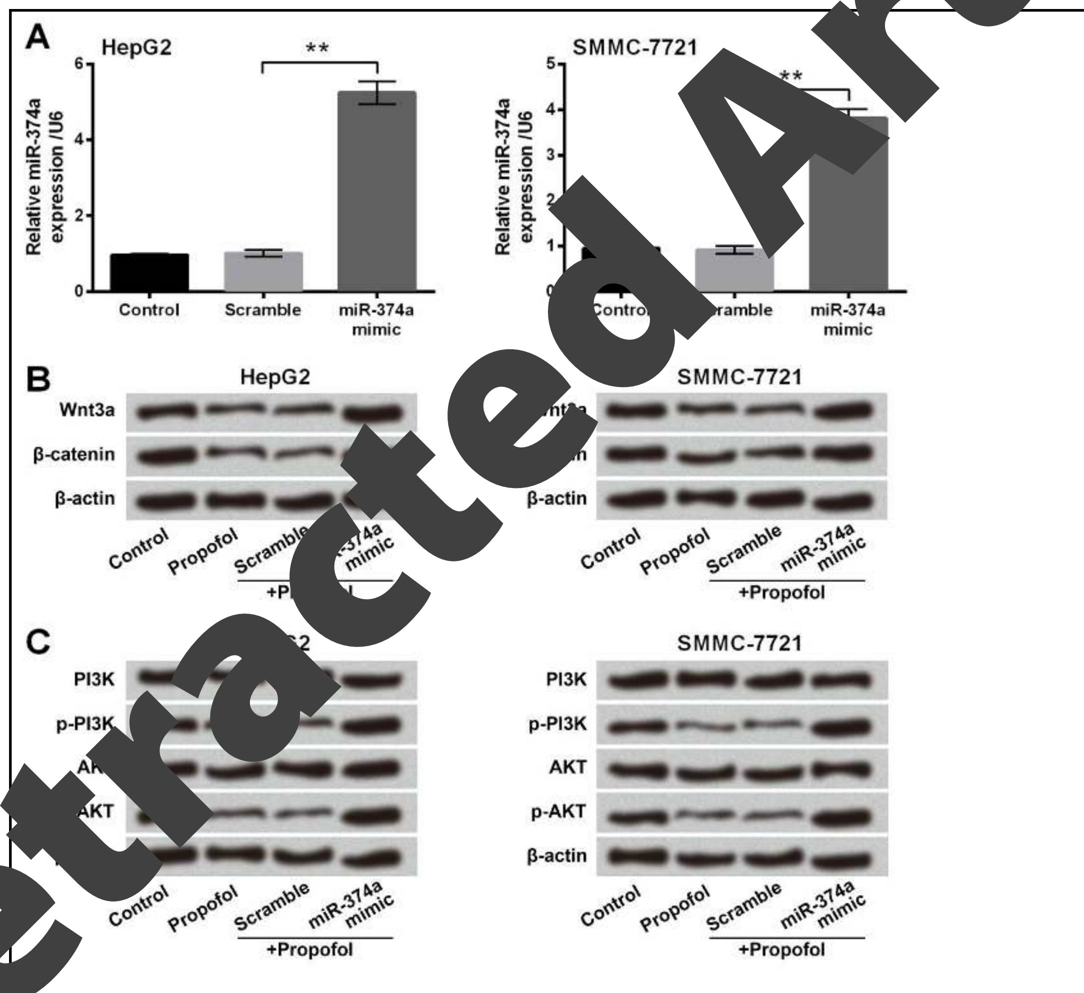

Fig. 5. Propofol inhibits the Wnt/β-catenin and PI3K/AKT pathways through down-regulating microRNA (miR)-374a. Cells were transfected with scramble miRNAs and miR-374a mimic, respectively. Non-treated cells were acted as the control. A. Expression of miR-374a by quantitative reverse transcription PCR. Cells transfected with scramble miRNAs or miR-374a and non-transfected cells were stimulated with 10 μg/mL propofol at 37°C for 48 h. Non-treated cells were acted as the control. Expression of key kinases in the Wnt/β-catenin (B) and PI3K/AKT pathways (C) was assessed by Western blot analysis. Data are presented as the mean ± SD of at least three independent experiments. **, P<0.01. PI3K, phosphatidylinositol-3-kinase; p-, phospho.

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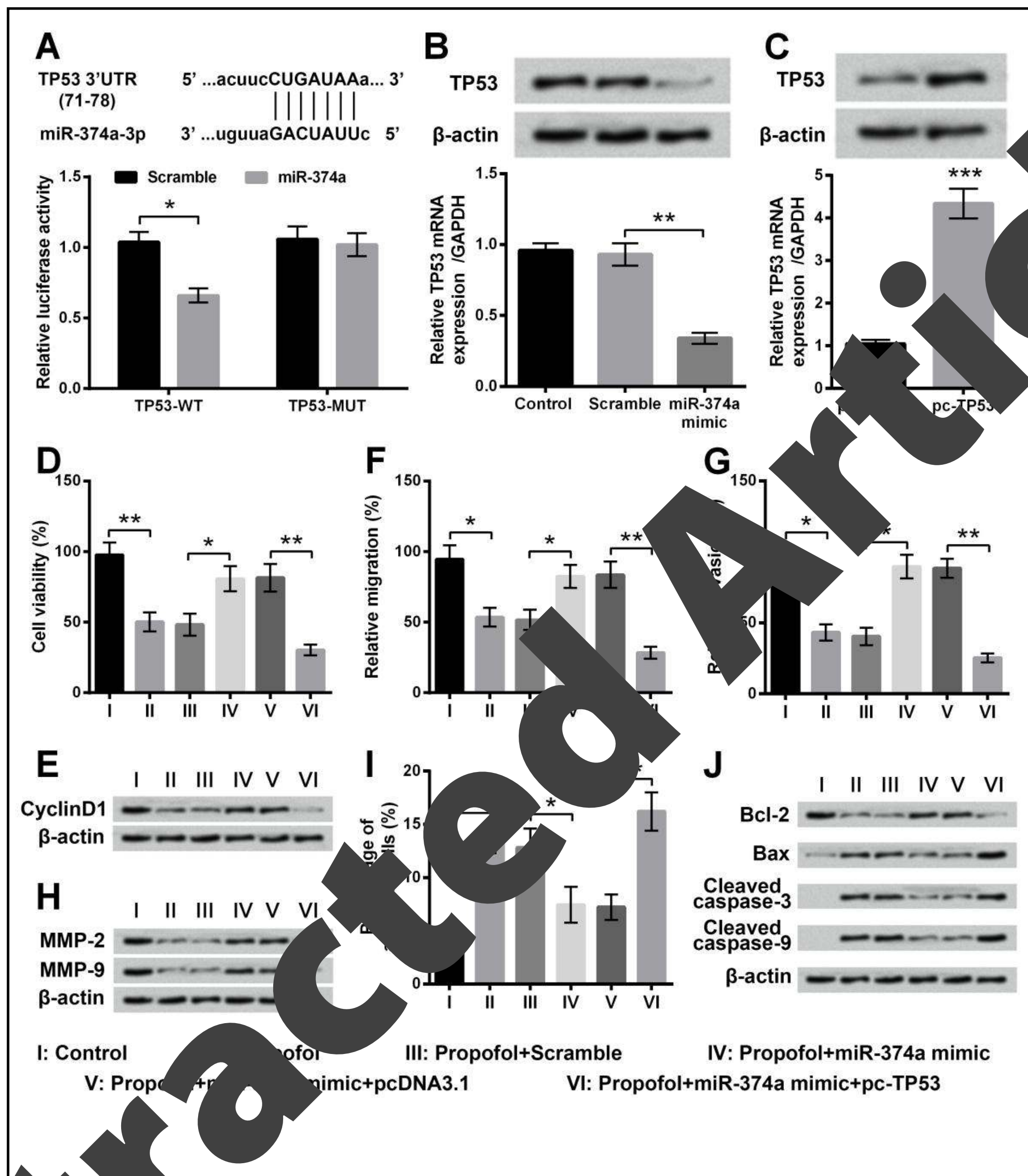

TP53 i[illegible]rget gene of miR-374a. HEK-293T cells were co-transfected with TP53-WT (TP53-MUT) a[illegible]374a mimic (scramble miRNA). A. Complementary sequences between miR-374a and TP53 3'UTR and lu[illegible] activity by luciferase reporter assay. HepG2 cells were transfected with scramble miRNAs [illegible]miR-374a mimic, and non-treated cells were acted as the control. B. Expression of TP53 by quantitative reve[illegible]e transcription PCR and Western blot analysis. HepG2 cells were transfected with pcDNA3.1 or [illegible]NA3.1. C. Expression of TP53 by quantitative reverse transcription PCR and Western blot analysis. Transfected or untransfected HepG2 cells were treated with 10 μg/mL propofol at 37°C for 48 h. Non-treated cells were acted as control. D. Cell viability by a Cell Counting Kit-8 assay. E. Expression of cyclinD1 by Western blot analysis. F. Cell migration by Transwell migration assay. G. Cell invasion by Transwell invasion assay. H. Expression of MMP-2 and MMP-9 by Western blot analysis. I. Percentage of apoptotic cells by flow cytometry assay. J. Expression of apoptosis-associated proteins by Western blot analysis. Data are presented as the mean ± SD of at least three independent experiments. *, P<0.05; **, P<0.01; ***, P<0.001. miR/miRNA, microRNA; 3'UTR, 3' untranslated region; MMP, matrix metalloproteinase; Bcl-2, mammalian B cell lymphoma-2; Bax, Bcl-2-associated X protein.

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TP53 might be a downstream factor of miR-374a in propofol-treated cells

Finally, the possible downstream facotr of miR-374a in HepG2 and SMMC-7721 cells was studied to elucidate the regulatory mechanism of propofol. By using the online TargetScan software, TP53 was predicated to be a target of miR-374a and the complementary sequences were shown in the Fig. 6A. Luciferase reporter assay showed that transfection with miR-374a mimic could significantly reduce luciferase activity in TP53-WT-transfected cells ($P < 0.05$), while it could not alter luciferase activity in TP53-MUT-transfected cells. In the meantime, mRNA and protein expression levels of TP53 were markedly down-regulated by miR-374a overexpression ($P < 0.01$, Fig. 6B). Results suggested that TP53 might be a target gene of miR-374a. In Fig. 6C, mRNA and protein expression of TP53 was remarkably up-regulated in cells transfected with pc-TP53 compared with pc-DNA3.1-transfected cells ($P <$ [illegible]), indicating TP53 was overexpressed successfully after transfection with pc-TP53. Following results illustrated that effects of miR-374a overexpression on HepG2 cells were reversed by TP53 overexpression, showing significant reduction of cell viability ($P <$ [illegible], Fig. 6D), down-regulation of cyclinD1 (Fig. 6E), decrease of migration and invasion (both $P <$ [illegible]01, Fig. 6F-6G), down-regulation of MMP-2 and MMP-9 (Fig. 6H), increase of apoptotic cells ($P < 0.01$, Fig. 6I), and down-regulation of Bcl-2 as well as up-regulation of Bax, cleaved caspase-3 and cleaved caspase-9 (Fig. 6J). Results illustrated that miR-374a could affect propofol-treated cells through down-regulating TP53.

Discussion

HCC is a lethal cancer with increasing incidence and poor prognosis. Innovative and effective drugs are of great importance for the treatments of HCC. In our study, we focused on the effects of propofol, a commonly used anaesthetic, on HCC cells. Interestingly, propofol acted as an anti-tumor factor in both HepG2 and SMMC-7721 cells, showing decreased cell proliferation, migration and invasion, and increased cell apoptosis. The level of miR-374a was down-regulated by propofol and further experiments illustrated that propofol inhibited the Wnt/β-catenin and PI3K/AKT pathways through down-regulating miR-374a. Besides, TP53 was proven to be target of miR-374a, and miR-374a could affect propofol-treated cells through down-regulating TP53.

Overwhelming numbers of studies focused on the significant effects of propofol on tumor cells. In lung cancer cells, propofol exerts inhibitory effects on cell viability and inductive effects on cell apoptosis [18]. In C6 glioma cells, propofol inhibits invasion and proliferation [30]. In esophageal squamous cell carcinoma cells, propofol inhibits cell migration and invasion through down-regulating Sex-determining region Y-box 4 (SOX4) [31]. Consistent with the results described above, in our study, propofol suppressed cell viability, migration and invasion but promoted cell apoptosis in both HepG2 and SMMC-7721 cells.

To support the influence of propofol on HepG2 and SMMC-7721 cells, the alteration of proteins associated with proliferation, migration, invasion and apoptosis after propofol stimulation was also assessed. As an important protein in proliferation, down-regulation of cyclinD1 induced by DYC-279 is accompanied by inhibited proliferation of HepG2 cells [32]. Melittin exerts an anti-proliferative role in HepG2 cells through PTEN-induced down-regulation of histone deacetylase 2 (HDAC2) and cyclinD1 [33]. Therefore, down-regulation of cyclinD1 also indicated an anti-proliferative role of propofol. Expression of MMP-2 and MMP-9 was down-regulated by propofol in esophageal squamous cell carcinoma cells, along with inhibited migration and invasion [31]. Invasion of glioma cells was inhibited by propofol, along with down-regulation of MMP-2 [34]. Herein, significant down-regulation of MMP-2 and MMP-9 was observed in propofol-treated cells, supporting an anti-migratory and anti-invasive role of propofol. A previous study has reported that Bcl-2 level was decreased while levels of Bax and cleaved casapse-3 were increased in response to propofol, which was consistent with the results of apoptosis [35]. Herein, the alteration of proteins related to

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apoptosis after propofol treatments was consistent with the previous literature, supporting the pro-apoptotic role of propofol.

miR-374a has been reported as an oncogene in diverse tumor types, including HCC [36, 37]. In the literature by He *et al.*, miR-374a could promote osteosarcoma cell proliferation through down-regulating FOXO1 expression [38]. Meanwhile, propofol has been reported to up-regulate FOXO1 expression in a dose-dependent manner [39]. Hence, we hypothesized there might be potential correlation between miR-374a and propofol. In our study, the expression of miR-374a was significantly down-regulated by propofol, verifying the hypothesis described above.

Activation of the Wnt/β-catentin and PI3K/AKT pathways is frequently observed in HCC [40, 41]. Moreover, propofol has been reported to inhibit hepatocellular carcinoma growth and invasion through the Wnt/β-catenin pathway [21]. To reveal the underlying mechanisms of the propofol-associated modulation, the alteration of the Wnt/β-catentin and PI3K/AKT pathways was further explored. In our study, these two signaling cascades were inhibited in propofol-treated cells, suggesting these two pathways were involved in the regulation of propofol. Furthermore, the propofol-induced inhibition of the Wnt/β-catentin and PI3K/AKT pathways was reversed by miR-374a overexpression, demonstrating that propofol might inhibit the Wnt/β-catentin and PI3K/AKT pathways through down-regulating miR-374a.

To enrich the regulatory mechanism of propofol, the downstream factor of miR-374a had been explored since miRNAs commonly functioned through repressing expression of target genes [42]. TP53 mutation is one of the common genetic alterations in HCC, suggesting the critical role of TP53 in HCC [43]. Restoration of TP53 has been reported to suppress growth of HCC *in vitro* and *in vivo* [44]. Therefore, the interaction between miR-374a and TP53 was further studied among those predicted targets. Results in this study illustrated that up-regulation of TP53 after propofol-induced down-regulation of miR-374a might be a rational regulatory mechanism in propofol treatments.

Conclusion

In conclusion, propofol was [illegible]ified to inhibit cell proliferation, migration and invasion of HepG2 and SMMC-7721 cells. Cell apoptosis of these two cell lines was promoted via the mitochondrial- and caspase-dependent pathways. miR-374a was down-regulated by propofol, and the Wnt/β-catentin and PI3K/AKT pathways were inhibited by propofol via down-regulating miR-374a. Besides, TP53 was proven to be a target of miR-374a. Our study provide [illegible]ively regulatory mechanism about the propofol in HCC cells and points out [illegible] novel direction for the treatment of HCC. More experiments performed *in vivo* are needed to pro[illegible] the regulatory mechanism in the future.

Disclosure Statement

The authors declare that no potential conflicts of interest exist.

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