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Original Article

β-catenin inhibitors suppress cells proliferation and promote cells apoptosis in PC9 lung cancer stem cells

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Abstract: This study aimed to investigate the effect of β-catenin inhibitors on cells proliferation and apoptosis in lung cancer stem cells (LCSCs). Drug-resistance PC9 cells were induced by escalation of cisplatin repeated treatment, and then PC9 LCSCs were constructed by Sphere Formation methods. Membrane expression of OCT4, SOX2, CD44, CD133 and β-Catenin were detected by Immunofluorescent staining, and mRNA of CSCs marker genes and Wnt/β-Catenin target genes were determined by qPCR assay. PC9 LCSCs were nurtured for 5 days (Day 5) and then β-catenin inhibitor pyrvinium pamoate (PP) with IC_{50} concentration (0.221 μM) and ICG-100 with IC_{50} concentration (2.620 μM) were added and cultured for another 2 days (Day 7), respectively. CCK8 and AV/PI assays were performed to detect cells proliferation and apoptosis. We successfully constructed PC9 LCSCs and observed that OCT4, SOX2, CD44, CD133 and β-Catenin expressed on all cells, and stem-cell marker genes as well as Wnt/β-Catenin signaling pathway genes mRNA were all elevated in PC9 LCSCs compared to PC9 parent cells. Cells proliferation by CCK8 assay was decreased while apoptosis rate by AV/PI assay was increased in PP treatment group compared with control, C-Caspase 3 and Bcl-2 protein expression also supported the apoptosis results. Most of the stem-cell marker genes and Wnt/β-Catenin signaling pathway genes mRNAs were decreased accordingly. ICG-001 also inhibited cells proliferation while induced cells apoptosis in PC9 LCSCs. In conclusion, β-Catenin inhibitors suppressed the proliferation and promoted the apoptosis of LCSCs, which shed light on a new potential target for lung cancer treatment.

Keywords: β-Catenin, lung cancer stem cells (LCSCs), pyrvinium pamoate (PP), PC9

Introduction

Lung cancer, as one of malignant tumors with the highest mortality, is a critical threat to human health worldwide [1]. 2015 Global cancer statistics report reveals that 1.8 million people were diagnosed with lung cancer accounting for 13% of new cancer patients all over the world in 2012, while 1.6 million deaths were caused by lung cancer contributing to 20% patients died from all cancers [2]. In recent years, although great improvements of early diagnosis, targeted therapies, individual treatments and patients' care have been achieved, prognosis of lung cancer was still far from satisfactory result, especially for lung adenocarcinoma patients whose 5-year survival rates were only 4-17% worldwide [3-6].

Accumulating evidences disclosed that drug-resistance was considered as one of the most crucial cause of poor prognosis in lung adenocarcinoma patients [1, 7]. While cancer stem cells (CSCs), stem-like cancer cells which presented the property of self-renewal and differentiation into various types of cancer cells, are demonstrated to have high resistance to lots of cytotoxic agents and targeted medicine, which leads to bad prognosis in lung adenocarcinoma patients [8-10]. Recent studies illuminated the inhibition of CSCs could contribute to suppression of cancer cells and could be regarded as potential target for cancer treatment [10, 11].

Wnt/β-catenin signaling pathway, playing an important role in the etiology of a large amount of cancers, have been observed to be involved in the mechanism of regulation of CSCs, including lung cancer stem cells (LCSCs) [12-14]. And several studies have reveals the inhibition of

β-catenin suppresses the proliferation of lung adenocarcinoma cells and β-catenin expression associates with poor prognosis in NSCLC patients [15-17]. However, the effect of inhibition of β-catenin in repressing LCSCs proliferation were still unclear, thus this study was aimed to investigate the effect of β-catenin inhibitors on cells proliferation and apoptosis in LCSCs.

Materials and methods

Cell culture

PC9 lung adenocarcinoma cells were purchased from Chinese Academy of Science Shanghai Branch (Shanghai, China) and cultured in Dulbecco's modified eagle medium (DMEM) with 5% fetal bovine serum (FBS) (Thermo scientific, USA) and 1% penicillin-streptomycin (Thermo scientific, USA) according to the instructions of manufacturers, which was exchanged every 2 to 3 days. Cells were passaged when at high confluence (approximately 80%) as 1:3 ratios. The cell culture was performed in incubator with 37°C, 5% CO_2 and saturated humidity.

Drug-resistance cells induced by cisplatin escalation

Escalation of cisplatin repeated treatment was used to induce drug-resistance PC9 cells. PC9 cells were treated with cisplatin 1 μmol/L for 24 h, and then exchanged to non-cisplatin DMEM until recovery to 70-80% live cells by microscopy; and then repeatedly treated by cisplatin 1 μmol/L for another 24 h and exchange of DMEM were carried out as well. This operation was repeated until almost no cells were killed by the same concentration of cisplatin. Subsequently, the concentration of cisplatin was escalated to 2.5 μmol/L, 5.0 μmol /L and finally 10.0 μmol/L to repeatedly treat the PC9 cells until no cells were killed with the same concentration. The resistance of treated PC9 cells to cisplatin was finally determined according to cells proliferation by CCK8 assays compared to control (without cisplatin treatment). CCK8 assay was performed as follows: 10 μl CCK8 and 90 μl RPMI 1640 medium were added to each plate of cells, and were incubated under 5% CO_2 at 37°C. OD value was determined by microplate reader (Biotek, USA).

PC9 LCSCs

PC9 LCSCs were constructed by Sphere Formation methods as follows: Drug-resistance cells in logarithmic growth phase with 1×10^3/mL were plated in six-well plates, and serum free medium (DMEM-F12+insulin (4 U/L)+B27 (1X)+EGF (20 ng/mL)+bFGF (20 ng/mL)) was used for suspension culture. CSCs spheres were constructed after 2 to 3 weeks' culture and then passaged to third generation for the subsequent experiments.

Immunofluorescent staining of OCT4, SOX2, CD44, CD133 and β-Catenin

CSCs spheres werewashed by 10 ml phosphatebuffered saline (PBS) of pH 7.3, then 1 ml pancreatin and 5 ml 10% FCS-DMEM were added to collect the PC9 LCSCs. 500 μl 2×10^5/mL PC9 LCSCs by Countstar Cell Counter were added to Millicell Side (Millipore, USA) for 5 h culture (3 wells for target antibodies; 2 wells for controls). Then the supernatant was discarded and 100 μl Cytofix fixation buffer (BD, USA) was added and the cells were fixed at room temperature for 30 m. Subsequently, supernatant was discarded and 200 μl Perm/Wash buffer (dilution 1:10) was use to wash twice. The 100 μl Perm/Wash buffer (dilution 1:10) was added and stayed at room temperature for 10 m.

3 μl monoclonal mouse antibody against OCT4 with dilution 1:100, 3 μl monoclonal mouse antibody against SOX2 with dilution 1:100, 3 μl monoclonal rabbit antibody against CD44 with dilution 1:100, 3 μl of monoclonal mouse antibody against CD133 with dilution 1:100, and 3 μl monoclonal rabbit antibody against β-Catenin with dilution 1:100 (All from Santa Cruz, USA) were added to each target well, and no antibody was added to control wells. The wells were subsequently kept overnight in humidified chamber and then washed by 800 ml PBS, and then 1 μl Alexa Fluor 594 labeled (red) donkey antibodies against mouse IgG with dilution 1:500 (CST, USA), and 1 μl Alexa Fluor 488 (green) labeled donkey antibodies against rabbit IgG with dilution 1:500 (CST, USA) were added to OCT4/SOX2 well and β-Catenin well respectively. 2 μl 50x Hoechst 33342 were added to each well as well. All cells were then nurtured in humidified chamber for 1 h and next washed 3 times by 800 μl

Table 1. List of primers used in this study

Gene name	Sequence
OCT4	F: GAGAATTTGTTCCTGCAGTGCC
	R: ATCCTCTCGTTGTGCATAGTCG
SOX2	F: TGTACAACATGATGGAGACGGAG
	R: CTGATCTCCGAGTTGTGCATCTT
NANOG	F: AACTCTCCAACATCCTGAACCTC
	R: CTGATCTCCGAGTTGTGCATCTT
KLF5	F: TATATTCAGCTCACACCAGACCG
	R: TGTCTGATTTGTAGAACTGGGCA
MYC	F: CTCAACGTTAGCTTCACCAACAG
	R: AGCAGCTCGAATTTCTTCCAGAT
β-Catenin	F: CAACCAAGAAAGCAAGCTCATCA
	R: ACTTGGATCTGTCAGGTGAAGTC
WNT7B	F: GAAGGTTCTAGAGGACCGGATG
	R: CTGTCTCCATGGGCTTCTGATAG
FZD1	F: TCCTACCTCAACTACCACTTCCT
	R: TAACAGCCGGACAAGAAGATGAT
FZD10	F: GAACGCCTCAACATGGATTACTG
	R: TTCTTCTTTAACCTACGGCTGCA
LRP5	F: CTTGGCAAGCATCTCTACTGGAT
	R: ATCACCCTTGGCAATACAGATGT
Axin2	F: TGAAGAAGAGGAGTGGACTTGTG
	R: ACTGGATATCTCACTGTCGTTGG
CyclinD1	F: GGTGTCCTACTTCAAATGTGTGC
	R: GTCTCCTTCATCTTAGAGGCCAC

PBS. Fluorescence microscope was used for observation of immunofluorescent staining.

mRNA detection of PC9 LCSCs and PC9 parent cells

Total RNA from PC9 LCSCs and PC9 parent cells were extracted by TRIzol Reagent (TaKaRa, Japan) and then subjected to reverse transcription with the PrimerScript Real-time reagent kit (TaKaRa, Japan). Quantitative analysis of mRNA expression (OCT4, SOX2, NANOG, KLF5, MYC, β-Catenin, WNT7B, FZD1, FZD10, LRP5) was detected by SYBR Green PCR reaction mixture (Toyobo, Japan). All the operations were carried out in accordance with the instructions of manufacturers. Expression of mRNA was then calculated utilizing the $2^{-\Delta\Delta t}$ method and GAPDH as the internal reference. The primers were shown in **Table 1**.

β-Catenin inhibitors for PC9 LCSCs

In order to further investigate the effect of inhibition of β-Catenin for LCSCs, β-catenin nuclear transfer inhibitor pyrvinium pamoate (PP) (Sigma-Aldrich, USA) and β-catenin/CREB-binding protein (CBP) transcription complex inhibitor ICG-001 (Selleck, USA) were purchased and used to repress the PC9 LCSCs proliferation. Concentrations of PP and ICG-001 were escalated gradually to treat PC9 LCSCs, and CCK8 assay was performed to determine the cells viability. IC_{50} and IC_{90} were subsequently analyzed.

Effect of PP on suppressing proliferation of PC9 LCSCs

1 ml PC9 LCSCs with 1×10^5/mL and 9 ml 10% FCS-DMEM were plated in the 100 mm culture dish for 5 days (Day 5). Subsequently 0.221 μM PP (IC_{50} concentration) with 10 ml 10% FCS-DMEM were added for 48 h (Day 7) as PP treatment group, while 10 ml 10% FCS-DMEM were added for 48 h (Day 7) as control group. CCK8 assay was performed to determine cells proliferation on Day 0, Day 5 and Day 7 respectively.

Effect of PP on promoting cells apoptosis of PC9 LCSCs

AV/PI assay at Day 7 was performed to detect the cells apoptosis in PP treatment group and control group as follows: Cells were digested by pancreatin and subsequently washed by PBS. After the cells were suspended in 100 μl Blinding Buffer, 5 μl Annexin V-FITC (AV) was added and stood in the darkness for 15 mins at room temperature. 5 μl Propidium Iodide (PI) was added just before flow cytometry assay. And flow cytometry was used to analyze the results. Besides, C-Caspase 3 expression and Bcl-2 expression were also detected by Western Blot for evaluation of cells apoptosis at Day 7 as follows: Total protein was extracted from cells with 1 ml RIPA buffer (Thermo Fisher Scientific, USA) and then the concentration was measured using the bicinchoninic acid (BCA) kit (Pierce Biotechnology, USA) and adjudged according to the standard curve. Subsequently 20 μg protein samples with equal concentration were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, USA). After blocking with 5% skim milk for 2 h, membranes were incubated with the corresponding primary antibodies overnight at 4°C:

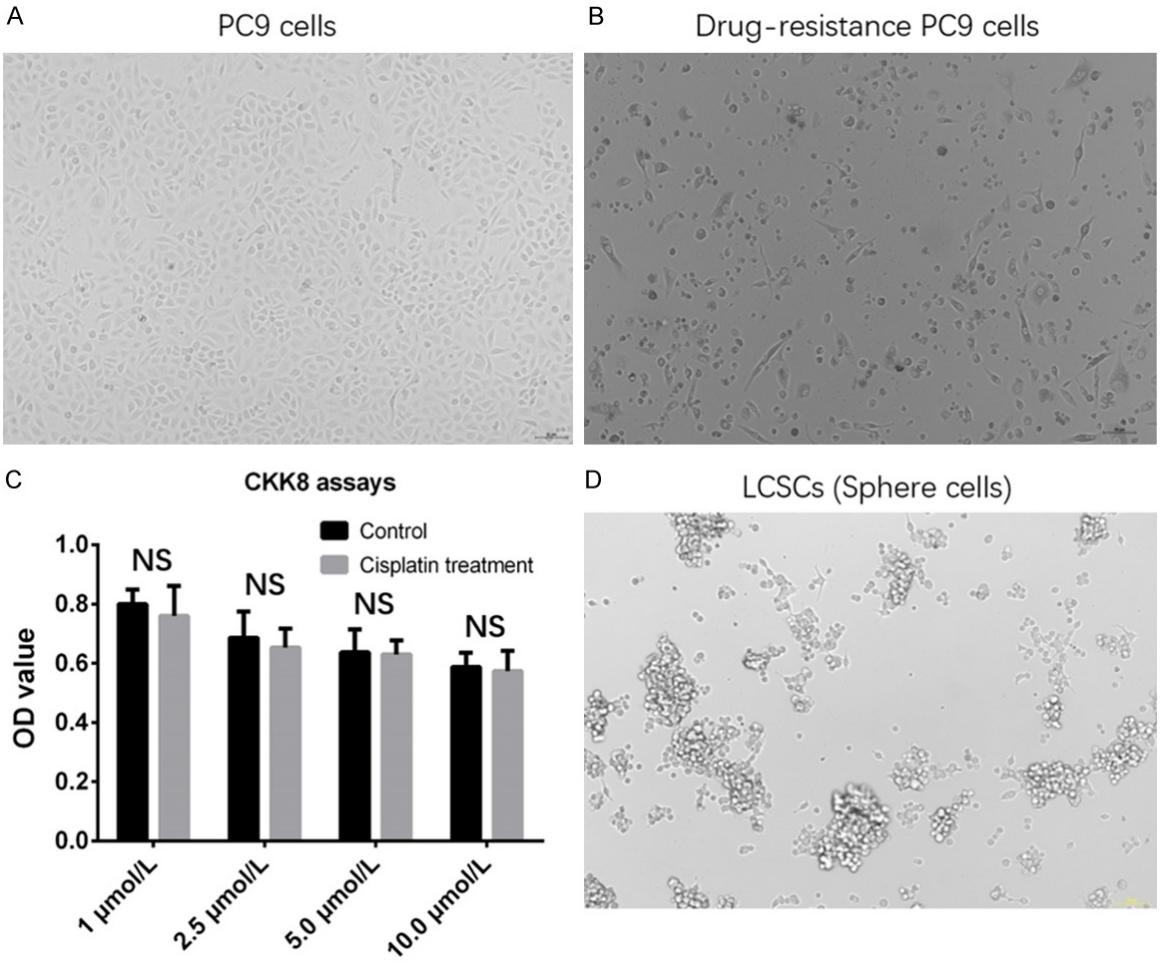

Figure 1. Construction of PC9 LCSCs. A. PC9 cells. B. Drug-resistance PC9 cells induced by escalation of cisplatin repeated treatment. C. CKKh8 assay disclosed no difference of cells proliferation between cisplatin treatment and control was discovered, suggesting cells were resistant to cisplatin. D. PC9 LCSCs by Sphere Formation method.

Cleaved caspase-3 rabbit mAb with 1:1000 dilution (CST, USA), caspase-3 rabbit antibody with 1:1000 dilution (CST, USA), Bcl-2 rabbit antibody with 1:1000 dilution (CST, USA) and GAPDH rabbit mAb with 1:1000 dilution (CST, USA). Then, membranes were incubated with the secondary antibody anti-rabbit IgG, HRP-linked Antibody with 1:1000 dilution (CST, USA) for 1 h at room temperature. The bands were visualized using an enhanced chemiluminescence (ECL) kit (Millipore, Bedford, USA) followed by exposure to X-ray film.

Effect of PP on β-Catenin protein expression and stem-cell marker genes as well as Wnt/β-Catenin pathway genes in PC9 LCSCs

In addition, β-Catenin protein expression at Day 7 was detected by immunofluorescent staining. And stem-cell marker genes as well as Wnt/β-Catenin related genes mRNA expression (OCT4, SOX2, NANOG, KLF5, MYC, β-Catenin, WNT7B, FZD1, FZD10, LRP5, Axin2 and cyclinD1) were detected in PP treated cells and control cells as well by qPCR methods. All the experiments were performed three times.

Effect of ICG-001 on cells proliferation and apoptosis in PC9 LCSCs

1 ml PC9 LCSCs with 1×10^5/mL and 9 ml 10% FCS-DMEM were plated in the 100 mm culture dish for 5 days (Day 5). Subsequently 2.620 μM ICG-001 (IC_{50} concentration) with 10 ml 10% FCS-DMEM were added for 48 h (Day 7) as ICG-001 treatment group, while 10 ml 10% FCS-DMEM were added for 48 h (Day 7) as control group. CCK8 assay was performed to

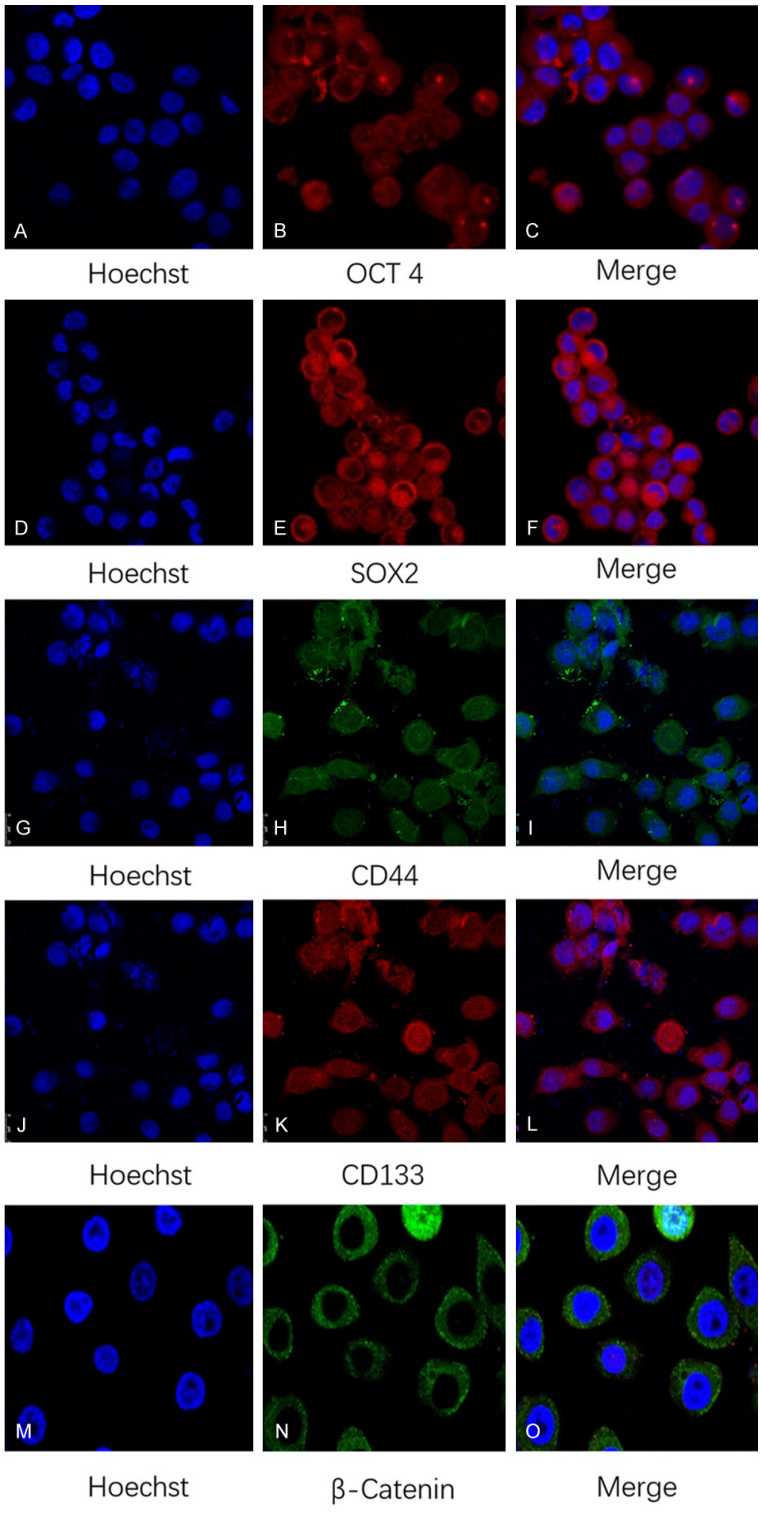

Figure 2. Immunofluorescent staining of OCT4, SOX2, CD44, CD133 and β-Catenin expressions on PC9 LCSCs. Immunofluorescent staining assays revealed that OCT4 (A-C), SOX2 (D-F), CD44 (G-I), CD133 (J-L) and β-Catenin (M-O) were all highly expressed on PC9 LCSCs.

determine cells proliferation on Day 0, Day 5 and Day 7 respectively. In addition, AV/PI assay at Day 7 was performed to detect the cells apoptosis in ICG-001 treatment group and control group

Statistics

Data was presented as mean ± standard deviation (SD). Comparison between two groups was determined by t test. IC_{50} and IC_{90} were analyzed by Probit regression. All the statistical analysis was performed using SPSS 22.0 (IBM, USA) and GraphPad 6.01 (GraphPad, USA). $P<0.05$ was considered significant.

Results

Construction of PC9 LCSCs

PC9 cells were presented in **Figure 1A**, after escalation of cisplatin repeated treatment, drug-resistance PC9 cells were collected (**Figure 1B**). The resistance to cisplatin was demonstrated by that no difference of cells proliferation between cisplatin treatment and control was discovered (**Figure 1C**). And then Sphere Formation method was used to construct PC9 LCSCs (CSCs spheres, **Figure 1C**).

OCT4, SOX2, CD44, CD133 and β-Catenin expression on membrane of PC9 LCSCs

To confirm whether the PC9 LCSCs were constructed successfully, LCSCs specific membrane expressed proteins were detected by immunofluorescent staining, and we found OCT4, SOX2, CD44 and CD133 were all highly expressed on cells (**Figure 2A-L**), which indicates CSCs were successfully built. Besides, β-Catenin was observed to expressed on all cells as well (**Figure 2M-O**), this indicates its potential role in CSCs development and progression.

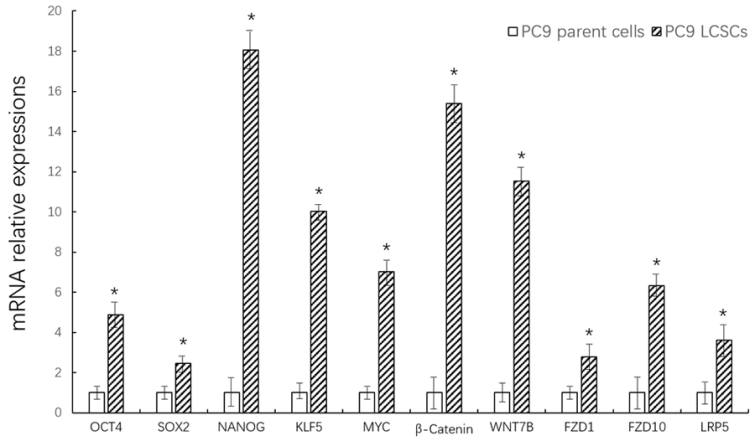

Figure 3. Stem-cell specific genes and Wnt/β-Catenin pathway genes mRNAs expression in PC9 LCSCs and PC9 parent cells. Stem-cell markers (OCT4, SOX2, NANOG, KLF5 and MYC) and Wnt/β-Catenin pathway genes (β-Catenin, WNT7B, FZD1, FZD10 and LRP5) mRNAs were all dramatically increased in PC9 LCSCs compared to PC9 parent cells.

Expression of stem-cell specific genes and Wnt/β-Catenin pathway genes in PC9 LCSCs

qPCR was performed to explore the expression of stem-cell specific genes and Wnt/β-Catenin pathway genes in PC9 LCSCs compared with PC9 parent cells (**Figure 3**). And we found stem-cell markers OCT4, SOX2, NANOG, KLF5 and MYC were dramatically increased in PC9 LCSCs compared to PC9 parent cells (all $P<0.05$), which further confirmed the stem-cell property of PC9 LCSCs. In the meanwhile, genes related to Wnt/β-Catenin signaling pathway including β-Catenin, WNT7B, FZD1, FZD10 and LRP5 were elevated in PC9 LCSCs as well, which suggested Wnt/β-Catenin signaling pathway might influence the regulation of LCSCs.

IC_{50} of β-Catenin inhibitors (PP and ICG-001) for PC9 LCSCs

As presented in **Figure 4**, we found both the two reagents (PP and ICG-001) were effective in killing PC9 LCSCs. IC_{50} of PP was 0.221 μM, 95% CI 0.176-0.277 μM and IC_{90} was 6.565 μM, 95% CI 4.205-11.533 μM (**Figure 4A**). While IC_{50} and IC_{90} were both numerically higher of ICG-001 than PP, with IC_{50} 2.620 μM, 95% CI 2.127-3.232 μM and IC_{C} 53.765 μM, 95% CI 36.362-87.854 μM (**Figure 4B**).

PP suppressed the proliferation of PC9 LCSCs

In order to further investigate the inhibiting effect of PP for PC9 LCSCs, PC9 LCSCs were nurtured for 5 days and then 0.221 μM PP (IC_{50}) was added for another 2 days' culture. As presented in **Figure 5**, PP markedly inhibited the proliferation of PC9 LCSCs compare to control at Day 7.

PP promoted the apoptosis of PC9 LCSCs

AV/PI assay was performed at Day 7 to investigate the effect of PP on cell apoptosis in PC9 LCSCs. As presented in **Figure 6A**, **6B**, cells apoptosis rate was greatly increased in PP treatment group compare with control group. In the meanwhile, apoptosis-related protein C-Caspase 3 was increased while Bcl-2 was decreased in PP treatment group compared to control group (**Figure 6C**), which also indicated PP enhanced the cells apoptosis in PC9 LCSCs

PP downregulated β-Catenin protein expression and stem-cell marker genes as well as Wnt/β-Catenin pathway genes

As presented in **Figure 7**, β-Catenin protein expression by immunofluorescent staining was decreased in PP treatment group cells compared with control cells. In the meanwhile, expression of stem-cell marker genes as well as Wnt/β-Catenin pathway genes were determined in PP treatment cells and control cells at Day 7, and OCT4, NANOG, KLF5, MYC, β-Catenin, WNT7B, FZD1, FZD10, LRP5, Axin2 and cyclinD1 mRNA expressions were observed to be decreased in PP treatment group compared with control group (all $P<0.05$, **Figure 8**).

ICG-001 inhibited cells proliferation and induced apoptosis of PC9 LCSCs

In order to further validate the effect of β-Catenin inhibitors on PC9 LCSCs, we next used ICG-001 with IC_{50} concentration (2.620 μM) to treat PC9 LCSCs, which illuminated that cells proliferation by CCK8 assay was decreased in ICG-001 treatment group compared to control group (Supplementary Figure 1A). Besides, AV/PI assay revealed that cells apoptosis rate was increased in ICG-001 treatment group com-

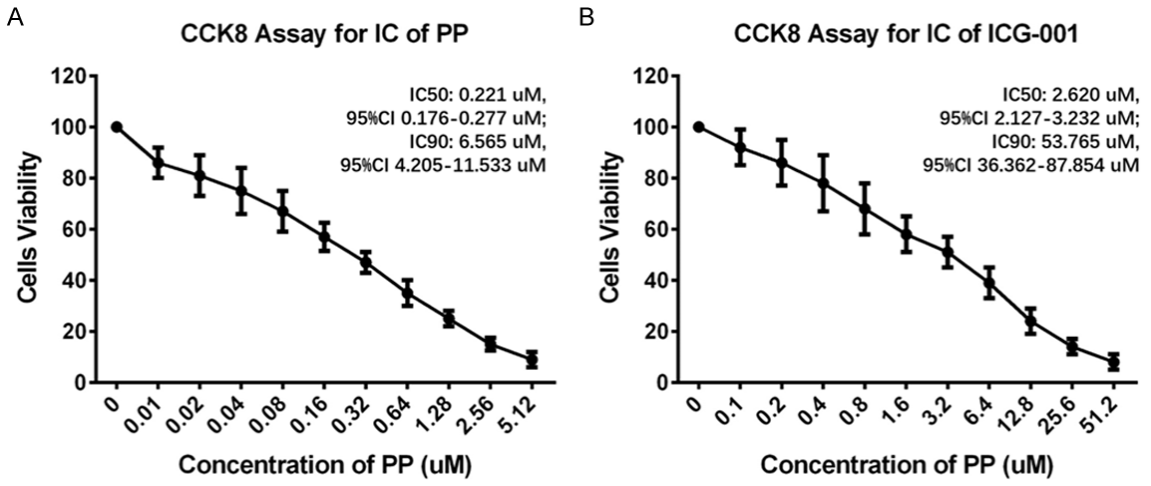

Figure 4. IC_{50} and IC_{90} of β-Catenin inhibitors (PP and ICG-001) for PC9 LCSCs by CCK8 assays. Concentrations of PP and ICG-001 were escalated gradually to treat PC9 LCSCs, and CCK8 assay was performed to determine the cells viability. IC_{50} and IC_{90} were subsequently analyzed by Probit regression which disclosed that IC_{50} of PP was 0.221 μM, 95% CI 0.176-0.277 μM and IC_{90} was 6.565 μM, 95% CI 4.205-11.533 μM (A). While IC_{50} of ICG-001 was 2.620 μM, 95%CI 2.127-3.232 μM and IC_{90} 53.765 μM, 95%CI 36.362-87.854 μM (B).

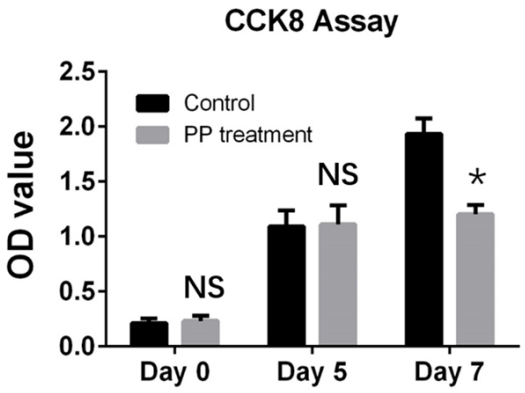

Figure 5. PP suppressed the proliferation of PC9 LCSCs compared to control by CCK8 assay.

pared with control group (Supplementary Figure 1B, 1C)

Discussion

In this present study, we successfully constructed PC9 LCSCs and observed that OCT4, SOX2, CD44, CD133 and β-Catenin expressed on all cells, and stem-cell marker genes (OCT4, SOX2, NANOG, KLF5 and MYC) as well as Wnt/β-Catenin signaling pathway genes (β-Catenin, WNT7B, FZD1, FZD10 and LRP5) were elevated in PC9 LCSCs compared to PC9 parent cells. While after PP or ICG-001 treatment to inhibit β-Catenin, the proliferation of LCSCs was dramatically suppressed, while the cells apoptosis was promoted. In addition, most of the stem-cell marker genes and Wnt/β-Catenin signaling pathway genes were decreased accordingly.

CSCs, a fresh definition firstly brought out in late last decade, was firstly proposed in acute myeloid leukemia (AML), which are a rare population of cancer cells possess stem-like features characterized by self-renewal and differentiation potency [18]. CSCs was considered not only a tumor initiation factor, but also a tumor relapsing factor due to its high resistance to various anti-cancer treatments including chemotherapy, targeted therapy and radiotherapy [8, 19-21]. On account of that, presence of CSCs was also proved to correlates with poor prognosis in patients with various cancers [22-24].

Although many membrane markers have been discovered toidentify CSCs, such as CD44, CD117, CD133, HER2 and so on [8-11], the specific markers for LCSCs were still controversial, and OCT4 as well as SOX2 were reported to specifically expressed on LCSCs compared to normal lung cancer cells in recent studies [25-27], and CD44 as well as CD133 were demonstrated in many studies to be markers of CSCs. Thus we used the OCT4, SOX2, CD44 and CD133 as the markers of successful construction of PC9 LCSCs in this study.

Wnt/β-Catenin pathway, as a critical singling in the development and progression of a great

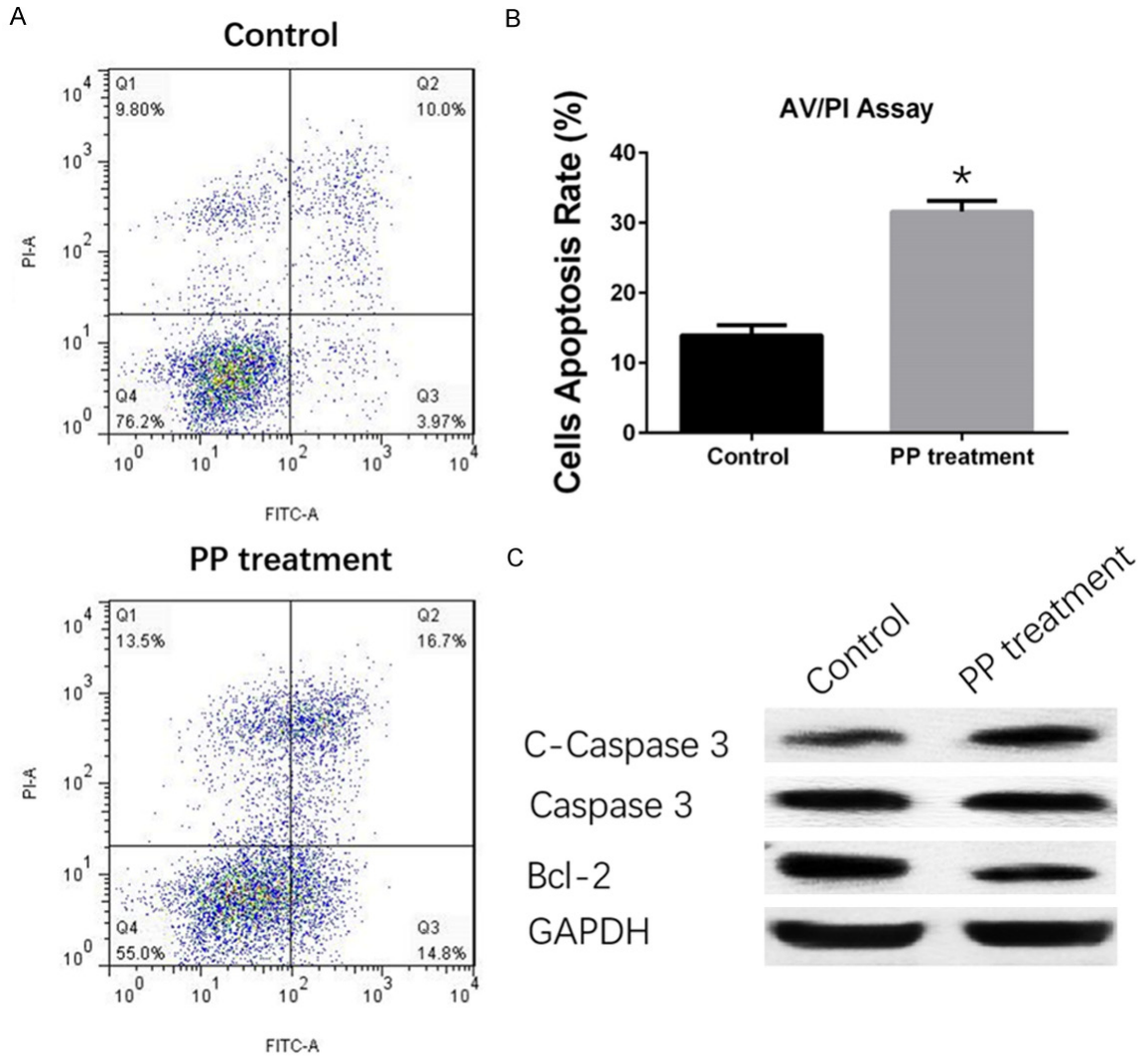

Figure 6. PP promoted cells apoptosis of PC9 LCSCs compared to control. AV/PI assay revealed PP promoted PC9 LCSCs apoptosis (A, B), and the protein expression of C-Caspase 3 and Bcl-2 also suggested PP induced cells apoptosis (C).

amount of cancers, was recently reported to regulate activity and properties of CSCs [12]. In colorectal CSCs, Wnt/β-Catenin pathway was observed to stimulate hypoxia effect on hierarchy of the CSCs [28]. In prostate cancer, Wnt/β-Catenin pathway could regulate self-renewal symmetric cells division of $hTERT^{high}$ CSCs [29]. In addition, telomerase was stimulated by Wnt/β-Catenin signalingin various stem cells and cancer cells [30]. In addition, the most predominant CSCs markers used to identify this rare population are direct Wnt/β-Catenin targeting genes including CD44, LGR5, MYC, CD133 and so on [12, 31]. These indicate the important role of Wnt/β-Catenin signaling in CSCs.

As to lung cancer, a recent study illustrated that lung cancer A549 cell lines stimulated by lithium chloride leads to accumulated concentration of β-Catenin expressions and up-regulates cyclin D1 gene (a typical Wnt/β-Catenin target gene), which in the meanwhile not only induces proliferation, clone formation and drug resistance ability but also increases the stem-cell-marker OCT4 expressed cells [14]. And another study further confirms that Wnt/β-catenin mediated the increasing expression of β-catenin, Oct4 and cyclin D in side population (SP) cells which presented properties of stem-like cancer cells but not in non-SP cells [13]. In our study, we constructed the PC9 LCSCs by Sphere Formation method, and OC-

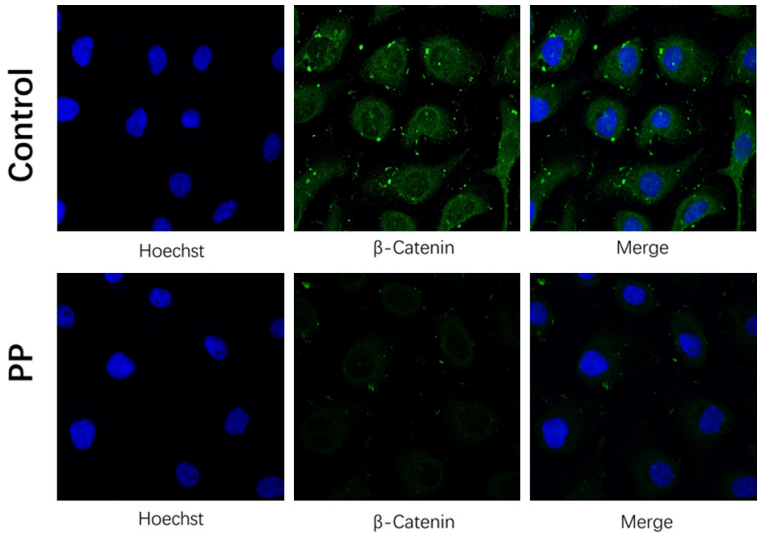

Figure 7. PP suppressed β-Catenin expression by immunofluorescent staining assay.

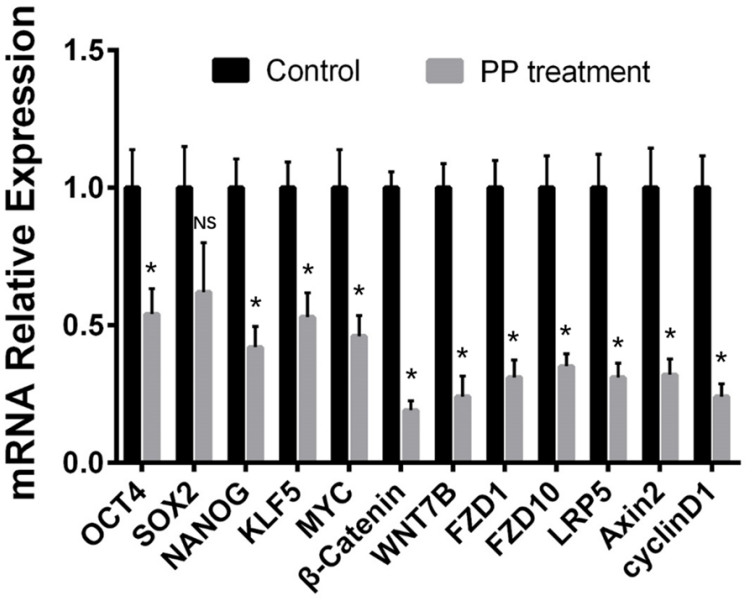

Figure 8. PP decreased stem-cell marker genes as well as Wnt/β-Catenin pathway genes. PP decreased OCT4, NANOG, KLF5, MYC, β-Catenin, WNT7B, FZD1, FZD10, LRP5, Axin2 and cyclinD1 mRNA expression compared to control.

T4, SOX2 and β-Catenin were highly expressed on cell membrane, while CSCs marker genes and Wnt/β-Catenin target genes were critically elevated in LCSCs. After inhibition of β-Catenin by PP or ICG-001 treatment, the proliferation of LCSCs was greatly suppressed while cells apoptosis was enhanced and majority of CSCs related and Wnt/β-Catenin target genes were decreased as well. These implied the inhibition of β-Catenin might be a potential treatment target for lung cancer treatment, especially for drug-resistance patients or relapsed patients.

In conclusion, we found β-Catenin inhibitors suppressed the proliferation and promoted the apoptosis of LCSCs, which shed light on a new potential target for lung cancer treatment.

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Disclosure of conflict of interest

None.

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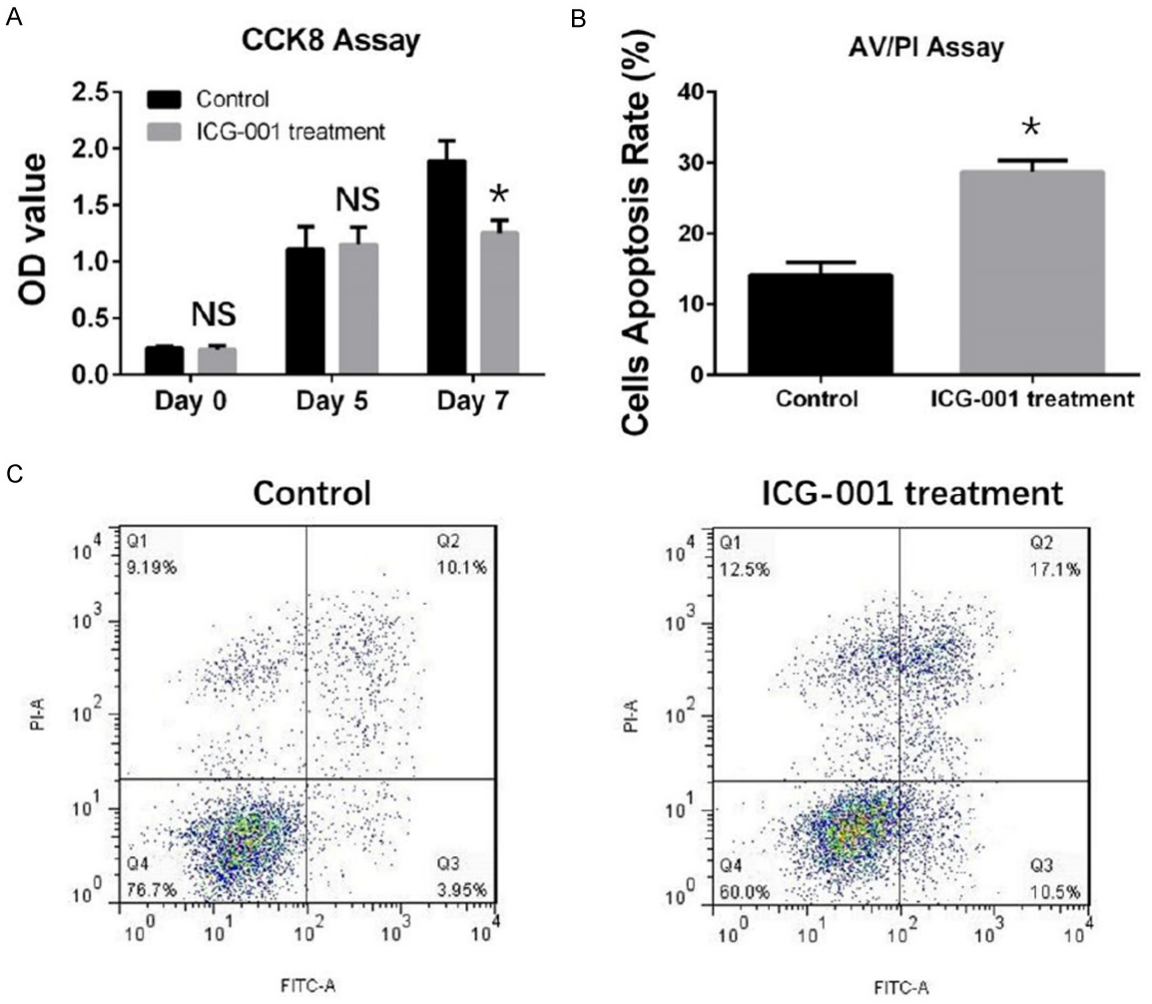

Supplementary Figure 1. ICG-001 inhibited cells proliferation and increased cells apoptosis in PC9 LCSCs. CCK8 assay illuminated that ICG-001 decreased cells proliferation of PC9 LCSCs, while AV/PI assay presented that ICG-001 increased cells apoptosis of PC9 LCSCs compared to control.