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Blocking the NOTCH pathway can inhibit the growth of CD133-positive A549 cells and sensitize to chemotherapy

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ABSTRACT

Cancer stem cells (CSCs) are believed to play an important role in tumor growth and recurrence. These cells exhibit self-renewal and proliferation properties. CSCs also exhibit significant drug resistance compared with normal tumor cells. Finding new treatments that target CSCs could significantly enhance the effect of chemotherapy and improve patient survival. Notch signaling is known to regulate the development of the lungs by controlling the cell-fate determination of normal stem cells. In this study, we isolated CSCs from the human lung adenocarcinoma cell line A549. CD133 was used as a stem cell marker for fluorescence-activated cell sorting (FACS). We compared the expression of Notch signaling in both CD133+ and CD133− cells and blocked Notch signaling using the γ-secretase inhibitor DAPT (GSI-IX). The effect of combining GSI and cisplatin (CDDP) was also examined in these two types of cells. We observed that both CD133+ and CD133− cells proliferated at similar rates, but the cells exhibited distinctive differences in cell cycle progression. Few CD133+ cells were observed in the G_2/M phase, and there were half as many cells in S phase compared with the CD133− cells. Furthermore, CD133+ cells exhibited significant resistance to chemotherapy when treated with CDDP. The expression of Notch signaling pathway members, such as Notch1, Notch2 and Hes1, was lower in CD133+ cells. GSI slightly inhibited the proliferation of both cell types and exhibited little effect on the cell cycle. The inhibitory effects of DPP on these two types of cells were enhanced when combined with GSI. Interestingly, this effect was especially significant in CD133+ cells, suggesting that Notch pathway blockade may be a useful CSC-targeted therapy in lung cancer.

1. Introduction

The American Cancer Society estimates 228,190 new lung cancer cases and 159,480 lung cancer deaths will occur in the United States in 2013, accounting for 13.7% and 27.5% of the total cases worldwide. Lung cancers continue to be the most common causes of cancer death. Although chemotherapy has advanced considerably over last 30 years, the 5-year relative survival rate for lung cancer remains only 17% [1]. New therapeutic approaches to this highly invasive cancer are desperately needed.

The cancer stem cell hypothesis was proposed many years ago. This hypothesis suggests that tumors originate from tissue stem cells that exhibit dysregulation of the normal self-renewal process. Some tumor cells retain stem cell properties, including the ability to self-renew and differentiate [2]. These stem-like tumor cells, which are termed cancer stem cells (CSCs), are referred to as tumor-initiating cells. CSCs within a tumor are thought to drive disease progression and metastasis [3]. Remarkably, compared with normal cancer cells, therapies against CSCs are less effective. CSCs exhibit significant resistance to conventional anti-tumor therapy. Many ATP-binding cassette (ABC) transporters are highly expressed by CSCs. These transporters, such as ABCG1, ABCB1 and ABCC1, can actively efflux drugs from cells [4]. Therefore, although radiation and chemotherapy against tumors can cause complete regression, enough cancer stem cells could remain to cause tumor recurrence [5]. Thus, new treatments specifically targeting CSCs may significantly enhance the effect of chemotherapy. CSC markers such as CD133 are widely accepted and used for the isolation of CSCs in many tumor types, including non-small cell lung cancer [6].

If it is true that CSCs originate from normal stem cells, pathways regulating stem cell self-renewal and differentiation would also work on CSCs [5]. The Notch signaling pathway is considered to play an important role in the control of cell fates and developmental processes [7]. Several studies have reported the dysfunction of Notch pathways in the tumorigenesis of many cancers [8].

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Furthermore, a high percentage of lung cancer lines express Notch receptors and their target genes, such as Jagged1, Hes1 and Hey1. The inhibition of Notch signaling can reduce tumor cell proliferation [9].

To explore the function of the Notch signaling pathway in the regulation of CSCs, we isolated CD133+ cells from the human lung adenocarcinoma cell line A549. We observed the differential expression of Notch signaling between CD133+ and CD133− cells and blocked Notch signaling using the γ-secretase inhibitor DAPT. Furthermore, we also examined the resistance of CSCs to drugs and observed the enhanced anti-tumor effect of GSI/CDDP combination therapy. This combination therapy induced cell cycle arrest in both CD133+ and CD133− cells. These results suggest an important application of Notch pathway inhibitors in CSC-targeting therapy of lung cancer.

2. Materials and methods

2.1. Cell culture

The human lung adenocarcinoma cell line A549 was purchased from the China Center for Type Culture Collection, Wuhan University (Wuhan, China). The A549 cells were cultured in RPMI-1640 containing 10% fetal bovine serum (Hyclone, United States) in an incubator at 37 °C with 5% CO_2. C133+ cells sorted from A549 were maintained in serum-free stem cell media. Stem cell media was made in RPMI-1640 (Hyclone, United States), supplemented with 20 ng/ml epidermal growth factor (EGF) (R&D Systems, United States) and 20 ng/ml basic fibroblast growth factor (bFGF) (R&D).

2.2. Flow cytometry and fluorescence-activated cell sorting

Cells were detached by trypsin-EDTA Solution (Beyotime, China) in the logarithmic phase of growth. Approximately 1×10^8 - cells were resuspended in 800 μl of buffer (containing phosphate buffered saline pH 7.2, 0.5% bovine serum albumin and 2 mM EDTA) (Miltenyi Biotec, Germany). The cell solution was mixed well with 200 μl of FcR Blocking reagent and 100 μl of CD133/2 (293C3)-PE antibody (Miltenyi Biotec) and incubated for 10 min in the dark 4 °C. After antibody incubation, the cells were washed and resuspended in 10 ml of buffer and sorted using the high-speed sorting flow cytometer FACS AriaTM III (BD, United States).

2.3. Cell proliferation assays

The Cell Counting Kit-8 (Beyotime, China) was used to quantify the proliferation of CD133+ and CD133− cells. The major constituent of the kit is 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-8). Cells were dispensed at a density of 3×10^5 cells/ml in medium and seeded at 100 μl/well in a 96-well plate. The plate was incubated for the appropriate length of time (24 h, 48 h, 3 d, 4 d, 6 d) in the incubator, and 10 μl of CCK-8 solution was added to each well of the plate. The absorbance was measured at 450 nm using a microplate reader after an hour. Growth curves were obtained by plotting the absorbance values over time.

2.4. Cell cycle analysis

Approximately 1×10^6 cells were collected and washed with PBS twice, centrifuged at 300*g* for 5 min, and incubated in 70% alcohol overnight at 4 °C. After incubation, the cells were washed with cold PBS twice. Approximately 1 ml of PBS containing 50 μg propidium iodide (PI, sigma, United States), 100 μg RNase A (sigma, United States) and 0.1% triton X-100 (sigma, United States) was added. The solution was incubated for 30 min at room temperature and analyzed by flow cytometry.

Table 1
Sequences of RT-PCR primers.

Target gene	Primer sequences	Size of targets (bp)
CD133	Sense: 5′-CCTCTGGTGGGGTATTTCTTT-3′ Antisense: 5′-CCAGTTTCCGACTCCTTTTG-3′	211
NOTCH1	Sense: 5′-GCGACAACGCCTACCTCTG-3′ Antisense: 5′-AAGCCATTGATGCCGTCC-3′	238
NOTCH2	Sense: 5′-TCAGCCGGGATACCTATGAG-3′ Antisense: 5′-CTGGCAGTGTCCTGGAATGT-3′	209
HES1	Sense: 5′-AACACTGATTTTGGATGCTCTG-3′ Antisense: 5′-CCTCGGTATTAACGCCCTC-3′	230
Actin	Sense: 5′-GTCCACCGCAAATGCTTCTA-3′ Antisense: 5′-TGCTGTCACCTTCACCGTTC-3′	190

2.5. Real-time fluorescence quantitative polymerase chain reaction (RT-PCR)

Total-RNA was extracted from cultured cells using TRIzol reagent (Invitrogen, United States). cDNA was synthesized from 1 μg of total RNA and reverse transcribed with RevertAid Reverse Transcriptase (Fermentas, Canada). RT-PCR was performed with ABI StepOne Plus (Applied Biosystems, United States). The reaction conditions were as follows: 95 °C for 10 min and 40 cycles at 95 °C for 10 s and 60 °C for 1 min. Gene expression was quantified using the comparative Ct method. The primer sequences utilized are listed in Table 1. Actin expression was used for normalization.

2.6. Protein isolation and Western blotting

Cells in each culture flask were washed three times by 4 °C PBS, and 400 μl of lysis buffer (50 mmol/L Tris·HCl, pH 8.0, 150 mmol/L NaCl, 1% TritonX-100, 100 μg/ml PMSF) for incubation for 30 min on ice. Approximately 30 μg of lysate per lane was separated by 10% SDS–PAGE and transferred onto a membrane (Millipore, United states). Membranes were blocked in 5% nonfat dry milk in TBST (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween-20) for 1 h at room temperature and then incubated with primary antibodies (anti-Notch1, anti-Notch2 and anti-Hes1 (Abcam, UK)). The protein expression was quantified using the Odyssey infrared imaging system (Li-Cor Biosciences). GAPDH was used for normalization.

2.7. Statistical analysis

Statistical analyses were performed using SPSS Statistics V17.0. All data were presented as the mean ± standard error. A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. The expression of CD133 is low in A549 cells

We labeled A549 cells with monoclonal CD133/2 (293C3) antibodies conjugated to R-phycoerythrin (PE). These cells were separated into CD133+ and CD133− cell fractions by FACS for the expression of CD133. The stem cell marker CD133 was only expressed in 0.5 ± 0.12% of all A549 cells (Fig. 1A and B). After sorting, the percentage of CD133−expressing cells in the CD133+ group was 97.5 ± 1.72% (Fig. 1C and D). These results demonstrate that CD133+ cells are rare in A549 cells and that these cells could be well isolated and enriched by FACS.

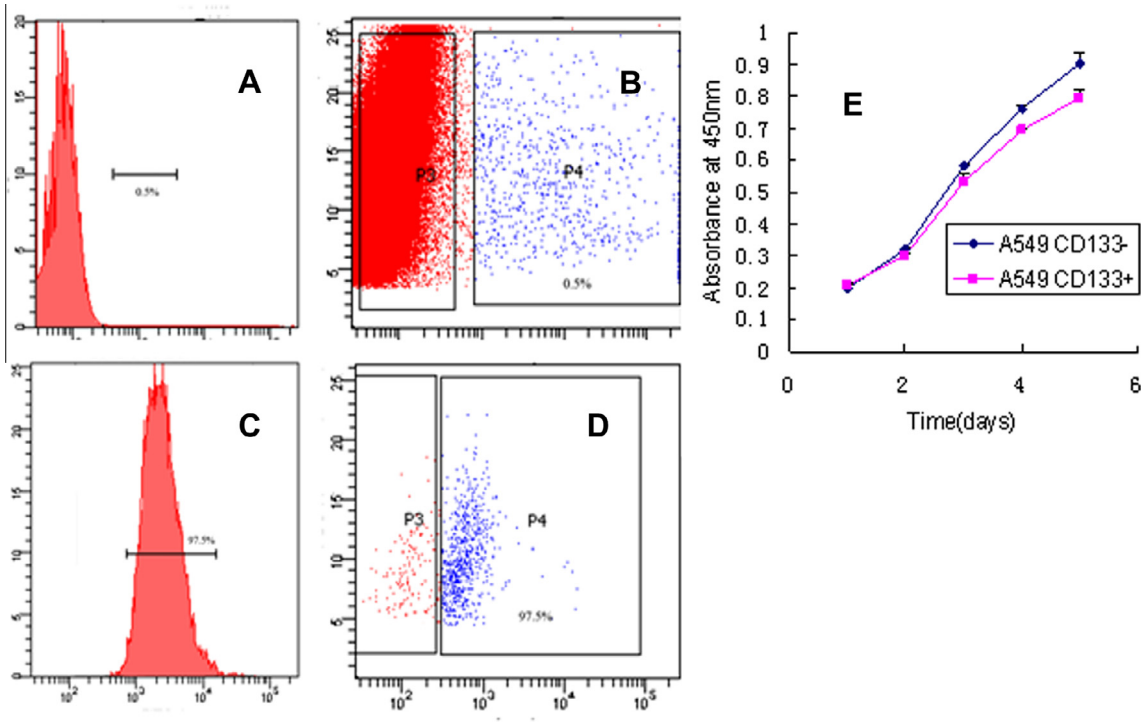

Fig. 1. (A–D) FACS analysis of CD133 expression and the cell sorting of A549 cells. (A) The stem cell marker CD133 was only expressed in 0.5 ± 0.12% of all A549 cells. (B) Cells in gate P3 were sorted and collected as CD133− cells. Gate P4 represented CD133+ cells. (C and D) In the CD133+ group after sorting, the expression of the stem cell marker CD133 was 97.5 ± 1.72%. (E) The growth curves of CD133+ and CD133− A549 cells. The difference was not significant.

3.2. The proliferation of CD133+ and CD133− cells in vitro

CD133+ and CD133− cells were seeded in 96-well plates at the same density and cultured under the same conditions. Cell proliferation assays over 7 days revealed that the difference between CD133+ and CD133− cells in the rate of proliferation was not significant (Fig. 1E).

3.3. Notch signaling pathway members are expressed in both CD133+ and CD133− cells

To examine the expression of the Notch pathway members in CD133+ and CD133− A549 cells, real-time fluorescent quantitative PCR and western blotting analyses were performed. RT-PCR revealed that both CD133+ and CD133− cells express Notch1 and Notch2, but CD133+ cells expressed lower levels than CD133− cells (0.069 ± 0.019 and 0.13 ± 0.026) (Fig. 2A and C). Hes1 was expressed in CD133− cells but not in CD133+ cells. Actin was used as an internal control (Fig. 2A). Western blotting analysis provided similar results to those obtained by RT-PCR (1.36 ± 0.13 vs 0.40 ± 0.08, 1.01 ± 0.20 vs 0.76 ± 0.11, $P < 0.05$) (Fig. 2B and D).

3.4. CD133+ cells are not as sensitive as CD133− cells to chemotherapy

Cisplatin (CDDP) is a classic chemotherapy drug for lung cancer. Therefore, we used CDDP in our drug intervention. CD133+ and CD133− cells were resuspended in RPMI-1640 and seeded in 96-well plates. The density was approximately 5000 cells per well. We treated these cells with CDDP at different doses (100, 10, 5, 1, and 0.1 mg/L). A CCK-8 assay was used to determine the viability of both CD133+ and CD133− cells at 48 h (Fig. 3A). CD133+ cells appeared more resistant to chemotherapy compared with CD133− cells at the same CDDP concentration. The IC_{50} was 5.9 ± 0.61 mg/L in CD133− cells and 21.1 ± 0.97 mg/L in CD133+ cells, representing a significant difference between the two types of cells ($P < 0.01$) (Fig. 3B).

3.5. Treatment with GSI could inhibit the growth of A549 cells and enhance the chemotherapeutic effect of CDDP

To study the role of the Notch signaling pathway in the growth of A549 cells, we used N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-2-phenylglycinetert-butyl ester (DAPT, GSI-IX) as a γ-secretase inhibitor. DAPT has been shown to cause a reduction in Aβ40 and Aβ42 levels and Notch signaling deficiencies at the morphological, molecular and biochemical levels in human cancer cells. We observed that DAPT could inhibit the growth of both CD133+ and CD133− cells, but the effect was not strong. After being treated with 2 μM DAPT for 48 h, CCK-8 assay revealed that the cell viability of CD133− cells was 79.2 ± 4.3% compared with control. In contrast, the cell viability of CD133+ cells was 68.2 ± 3.8%, ($P < 0.05$) which suggested that the suppression of growth by DAPT was more effective in CD133+ cells.

To further examine the effect of combining chemotherapy with GSI, we treated the two types of cells with 2 μM DAPT and 6 mg/L CDDP. We observed that this combination enhanced the antitumor activity of the chemotherapy. When cells were only treated with 6 mg/L CDDP, the viability of the cells was 43.5 ± 2.7% and 66.7 ± 4.2% in CD133− and CD133+ cells, respectively. However, when treated with GSI and CDDP combination therapy, the cell viability decreased to 35.3 ± 1.8% and 42.5 ± 2.1%, respectively (Fig. 3C). This observation supported our assumption that the Notch pathway blockade depresses the resistance of A549 cells to chemotherapy. Moreover, we also observed that the increase in the inhibitory effect of combination treatment was 14% in CD133− cells 55.6% in CD133+ cells. It is particularly noteworthy that the synergistic effect of CDDP/GSI was especially significant for CD133+ cells ($P < 0.01$).

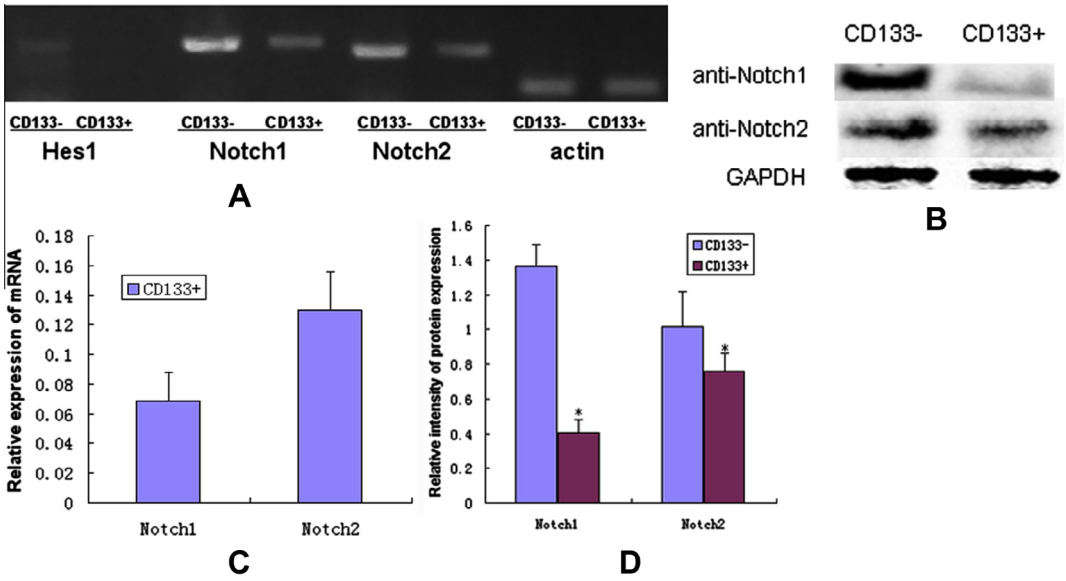

Fig. 2. The expression of Notch pathway members in CD133− and CD133+ cells. (A) RT-PCR analysis. Notch1 and Notch2 were expressed in CD133+ cells at lower levels. Hes1 was expressed only in CD133− cells. (B) Western blotting data. The result demonstrated that the expression of Notch signaling pathway proteins was lower in CD133+ cells than in CD133− cells. (C) The relative expression of mRNA (CD133+ cells/CD133− cells). (D) Analysis of the expression of proteins.

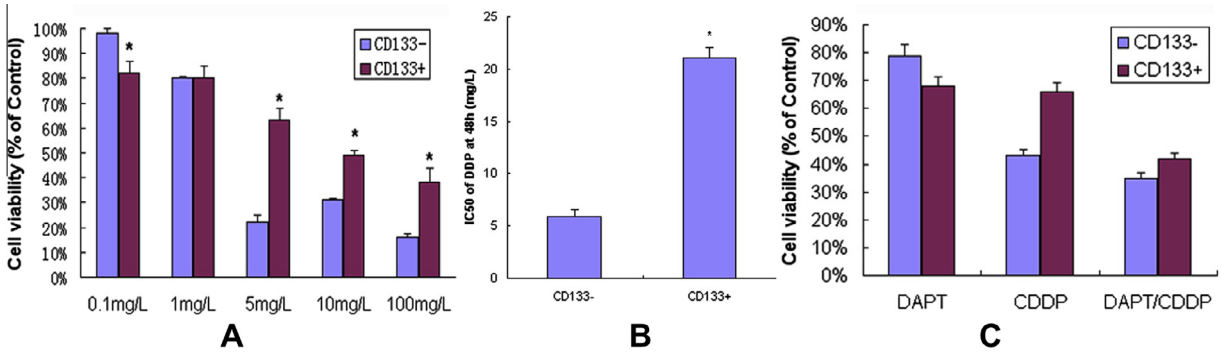

Fig. 3. (A and B) CD133+ cells exhibited resistance to chemotherapy compared with CD133− cells. (A) The cell viability of CD133− and CD133+ cells when treated with CDDP for 48 h at the same doses (100, 10, 5, 1, 0.1 mg/L). (B) The difference of the IC_{50} of CDDP was significant between the two types of cells. (C) The effects of monotherapy of CDDP or DAPT and co-therapy of CDDP/DAPT for 48 h on CD133− and CD133+ cells. DAPT could inhibit the growth of both CD133+ and CD133− cells, but the effect was not strong. The suppression of growth by DAPT was more effective in CD133+ cells. Notch pathway blockade could depress the resistance of A549 cells to chemotherapy. Impressively, the synergistic effect of CDDP/GSI was especially significant for CD133+ cells.

3.6. Cell cycle analysis

In addition to depressing the growth of A549 cells, GSI and CDDP also affect the cell cycle. We used PI to stain the DNA of A549 cells for cell cycle analysis. Before FACS analysis, cells were treated with 2 μM DAPT, 6 mg/L CDDP or a combination therapy for 48 h. In the control group, we observed that 5.75 ± 0.35% of CD133− cells and 0.1 ± 0.02% of CD133+ cells were in the G_2/M phase (Fig. 4). This finding remarkably demonstrated that in most CD133+ cells, DNA replication was not active ($P < 0.01$). This inactivity of DNA replication may be the reason why cancer stem cells exhibit increased resistance to chemotherapy compared with normal cancer cells.

CDDP can bind to DNA and interfere with the cell's repair mechanisms, which eventually leads to cell death. In our study, CDDP arrested cells in the G_2/M phase. When treated with 6 mg/L CDDP for 48 h, the proportion of cells in the G_2/M phase increased to 39.46 ± 1.84% in CD133− cells and 17.50 ± 0.77% in CD133+ cells (Fig. 4). This result confirms that CD133+ cells were not as sensitive as CD133− cells to chemotherapy ($P < 0.05$).

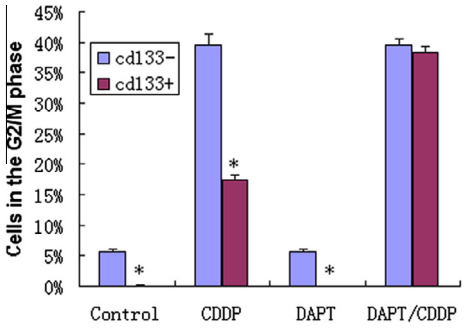

Fig. 4. The results of cell cycle analysis. In the control group, approximately 5.75 ± 0.35% CD133− cells and 0.1 ± 0.02% CD133+ cells were in the G_2/M phase. This means most CD133+ cells were quiescent ($^*P < 0.01$). Cell cycle analysis of A549 cells in the CDDP group, demonstrating cell cycle arrest in the G2/M phase. The effect of arrest on CD133+ cells was not as significant as CD133− cells. In the DAPT group, there was little change in G_2/M in both types of cells. In the DAPT/CDDP group, this co-therapy significantly potentiated the cell cycle arrest in the G_2/M phase in CD133+ cells. The proportion of CD133+ cells at G_2/M increased very close to the value of CD133− cells.

DAPT seemed to have little effect on cell cycle changes in CD133− cells. After 48 h of DAPT treatment, the percentage of cells in the G_2/M phase was 5.65 ± 0.44%, which was not significantly different from the control group at 5.75 ± 0.35% ($P > 0.05$). The situation was similar for CD133+ cells; the proportion of cells in the G_2/M phase was 0.00% in the DAPT group and 0.1 ± 0.02% in control group. However, the proportion of cells in S phase increased from 11.96 ± 0.89% to 18.48 ± 0.64% ($P < 0.05$) in CD133+ cells.

We observed that the proportion of G_2/M cells in CD133− cells exhibited no significant difference between CDDP and DAPT/CDDP combination therapy (39.46 ± 1.84% and 39.53 ± 1.08%, $P > 0.05$) (Fig. 4). However, we found that DAPT/CDDP combination therapy significantly potentiated the cell cycle arrest in the G_2/M phase in CD133+ cells (38.41 ± 0.93% vs 0.1 ± 0.02%, 38.41 ± 0.93% vs 17.50 ± 0.77%, $P < 0.05$). Compared with the CDDP group, the percentage of G_2/M cells in CD133+ cells increased from 17.50 ± 0.77% to 38.41 ± 0.93% ($P < 0.01$), very close to the value of CD133− cells.

4. Discussion

Cancer stem cells have been shown to possess the ability of self-renewal and differentiation in many studies [10]. Compared with normal cancer cells, CSCs are considered more tumorigenic. These cells can easily cause tumor relapse and metastasis by generating new tumors [11]. CD133 is one of the conventional CSC markers used to identify CSCs in a number of cancer cells [12,13]. In our study, we used FACS to isolate CSCs from the adenocarcinoma cell line A549 by the expression of the surface marker CD133. We observed that this population of CD133+ cells represented only a small proportion of all A549 cells.

CSCs are known to exhibit resistance to chemotherapy [14]. Like the stem cells in normal tissues, CSCs exhibit many specific characteristics, such as drug transporter expression, apoptosis inhibitors and DNA methylation. These features may be the reason why current cancer chemotherapy can promote remission but often fails to cure cancer [15,16]. We observed that CD133+ A549 cells appeared more resistant to chemotherapy compared with CD133− cells in the same CDDP concentration. When treated with CDDP, the CD133+ cells were enriched. In the post-treatment group, the percentage of CD133+ cells was increased from 0.5% to 2.7%.

The Notch signaling pathway is known to regulate the differentiation of epithelial progenitors during lung development [17,18]. Because CSCs exhibit similar properties to normal stem cells, the Notch pathway may also play an important role in lung tumorigenesis. Notch signaling is altered in many lung cancers, and the deregulation of the Notch pathway may be a frequent event in non-small cell lung cancers [19]. The upregulation of Notch signaling can increase survivin expression and contribute to lung cancer clonogenic capacity in vitro [20,21]. Furthermore, the downregulation of Notch signaling inhibits the growth, migration, and invasiveness of cancer cells and induces cell apoptosis [22]. Interestingly, some research has demonstrated the opposite outcome; the overexpression of Notch signaling can cause cell cycle arrest and depress the growth of cancer cells [23]. In the current study, we found that Notch signaling was expressed in both CD133+ and CD133− cells. However, compared with CD133− cells, the expression in CD133+ cells was reduced. Hes1 was expressed in CD133− cells but not in CD133+ cells. We blockaded the Notch pathway in CD133− and CD133+ cells using the γ-secretase inhibitor DAPT. As anticipated, we observed a depression of growth in both types of cells. However, the cell cycle arrest in the G_1 phase was not observed in any group.

Cancer treatment can be made more effective by using a combination of drugs that kill both replicating cancer cells and more quiescent cancer stem cells [16]. GSI can deplete CSCs through reduced proliferation and increased apoptosis in heterogeneous glioblastoma neurospheres. Notch blockade in conjunction with other therapies may maximize the efficacy of anti-CSC agents such as GSI [24]. In addition, GSI not only inhibits the growth of CSCs but also sensitizes tumors to chemotherapy [25]. In lung cancer, the addition of GSI could enhance the cytotoxicity of radiation, and this enhancement has been demonstrated both in vitro and in vivo [26]. In our study, cells were treated with DAPT and CDDP as combination chemotherapy. Compared with monotherapy of CDDP, this co-therapy demonstrated a significant enhancement of antitumor effect. Interestingly, this effect was particularly evident for CD133+ cells. It has been shown that the main effect of CDDP is G_2/M arrest. Cisplatin-induced cell death is highly dependent on cell-cycle phase. Some crucial events, such as DNA-damage and the apoptotic process, are executed in G_2/M [27]. We observed that when treated with DAPT alone, there was little change in the cell cycle. CD133− cells exhibited no significant change in the proportion of G_2/M cells between CDDP and CDDP/GSI therapy. In contrast, in the CD133+ cells, DAPT/CDDP combination therapy could significantly potentiate the cell cycle arrest in G_2/M phase. This may be the reason why CDDP/GSI co-therapy exhibited a particularly synergetic effect on CD133+ cells.

Our discovery demonstrated a depression of growth in CD133+ A549 cells caused by GSI. Blockade of Notch signaling pathway enhanced the effect of chemotherapy with CDDP. Furthermore, this DAPT/CDDP co-therapy caused a G_2/M arrest and effectively eliminated both CD133− and CD133+ cells. This synergetic effect was especially significant on CD133+ cells. Further studies are needed to demonstrate the mechanisms of GSI-induced enhancement with CDDP. Nevertheless, targeting the Notch pathway has exhibited great potential to be an improved cancer treatment that could kill both replicating cancer cells and more quiescent cancer stem cells and reduce tumor relapse.

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Reference

[1] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2013, CA Cancer J. Clin. 63 (1) (2013) 11–30.
[2] M.S. Wicha, S.L. Liu, G. Dontu, Cancer stem cells: an old idea–a paradigm shift, Cancer Res. 66 (4) (2006) 1883–1890.
[3] J.P. Sullivan, J.D. Minna, J.W. Shay, Evidence for self-renewing lung cancer stem cells and their implications in tumor initiation, progression, and targeted therapy, Cancer Metastasis Rev. 29 (1) (2010) 61–72.
[4] M. Dean, T. Fojo, S. Bates, Tumour stem cells and drug resistance, Nat. Rev. Cancer 5 (4) (2005) 275–284.
[5] T. Reya, S.J. Morrison, M.F. Clarke, I.L. Weissman, Stem cells, cancer, and cancer stem cells, Nature 414 (6859) (2001) 105–111.
[6] G. Bertolini, L. Roz, P. Perego, M. Tortoreto, E. Fontanella, et al., Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment, Proc. Natl. Acad. Sci. USA 106 (38) (2009) 16281–16286.
[7] S. Artavanis-Tsakonas, M.D. Rand, R.J. Lake, Notch signaling: cell fate control and signal integration in development, Science 284 (5415) (1999) 770–776.
[8] S.J. Bray, Notch signallng: a simple pathway becomes complex, Nat. Rev. Mol. Cell Biol. 7 (9) (2006) 678–689.
[9] J. Konishi, K.S. Kawaguchi, H. Vo, N. Haruki, A. Gonzalez, D.P. Carbone, T.P. Dang, Gamma-secretase inhibitor prevents Notch3 activation and reduces proliferation in human lung cancers, Cancer Res. 67 (17) (2007) 8051–8057.
[10] R. Pardal, M.F. Clarke, S.J. Morrison, Applying the principles of stem-cell biology to cancer, Nat. Rev. Cancer 3 (12) (2003) 895–902.
[11] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison, M.F. Clarke, Prospective identification of tumorigenic breast cancer cells, Proc. Natl. Acad. Sci. USA 100 (7) (2003) 3983–3988.
[12] T. Baba, P.A. Convery, N. Matsumura, R.S. Whitaker, E. Kondoh, T. Perry, Z. Huang, R.C. Bentley, S. Mori, S. Fujii, J.R. Marks, A. Berchuck, S.K. Murphy, Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells, Oncogene 28 (2) (2009) 209–218.

[13] H. Kawamoto, T. Yuasa, Y. Kubota, M. Seita, H. Sasamoto, J.M. Shahid, T. Hayashi, H. Nakahara, R. Hassan, M. Iwamuro, E. Kondo, S. Nakaji, N. Tanaka, N. Kobayashi, Characteristics of CD133(+) human colon cancer SW620 cells, Cell Transplant. 19 (6) (2010) 857–864.

[14] R. Nadal, F.G. Ortega, M. Salido, J.A. Lorente, M. Rodríguez-Rivera, M. Delgado-Rodríguez, M. Macià, A. Fernández, J.M. Corominas, J.L. García-Puche, P. Sánchez-Rovira, F. Solé, M.J. Serrano, CD133 expression in circulating tumor cells from breast cancer patients: potential role in resistance to chemotherapy, Int. J. Cancer 133 (10) (2013) 2398–2407.

[15] R. Besançon, S. Valsesia-Wittmann, A. Puisieux, C. Caron de Fromentel, V. Maguer-Satta, Cancer stem cells: the emerging challenge of drug targeting, Curr. Med. Chem. 16 (4) (2009) 394–416.

[16] J. Zhou, Y. Zhang, Preclinical development of cancer stem cell drugs, Expert Opin. Drug Discov. 4 (7) (2009) 741–752.

[17] J.S. Guseh, S.A. Bores, B.Z. Stanger, Q. Zhou, W.J. Anderson, D.A. Melton, J. Rajagopal, Notch signaling promotes airway mucous metaplasia and inhibits alveolar development, Development 136 (10) (2009) 1751–1759.

[18] P.N. Tsao, F. Chen, K.I. Izvolsky, J. Walker, M.A. Kukuruzinska, J. Lu, W.V. Cardoso, Gamma-secretase activation of notch signaling regulates the balance of proximal and distal fates in progenitor cells of the developing lung, J. Biol. Chem. 283 (43) (2008) 29532–29544.

[19] B. Westhoff, I.N. Colaluca, G. D'Ario, M. Donzelli, D. Tosoni, S. Volorio, G. Pelosi, L. Spaggiari, G. Mazzarol, G. Viale, S. Pece, P.P. Di Fiore, Alterations of the Notch pathway in lung cancer, Proc. Natl. Acad. Sci. USA 106 (52) (2009) 22293–22298.

[20] Y. Chen, D. Li, H. Liu, H. Xu, H. Zheng, F. Qian, W. Li, C. Zhao, Z. Wang, X. Wang, Notch-1 signaling facilitates survivin expression in human non-small cell lung cancer cells, Cancer Biol. Ther. 11 (1) (2011) 14–21.

[21] J. Osanyingbemi-Obidi, I. Dobromilskaya, P.B. Illei, C.L. Hann, C.M. Rudin, Notch signaling contributes to lung cancer clonogenic capacity in vitro but may be circumvented in tumorigenesis in vivo, Mol. Cancer Res. 9 (12) (2011) 1746–1754.

[22] X. Ji, Z. Wang, A. Geamanu, F.H. Sarkar, S.V. Gupta, Inhibition of cell growth and induction of apoptosis in non-small cell lung cancer cells by delta-tocotrienol is associated with notch-1 down-regulation, J. Cell. Biochem. 112 (10) (2011) 2773–2783.

[23] Q. Zheng, H. Qin, H. Zhang, J. Li, L. Hou, H. Wang, X. Zhang, S. Zhang, L. Feng, Y. Liang, H. Han, D. Yi, Notch signaling inhibits growth of the human lung adenocarcinoma cell line A549, Oncol. Rep. 17 (4) (2007) 847–852.

[24] X. Fan, L. Khaki, T.S. Zhu, M.E. Soules, C.E. Talsma, N. Gul, C. Koh, J. Zhang, Y.M. Li, J. Maciaczyk, G. Nikkhah, F. Dimeco, S. Piccirillo, A.L. Vescovi, C.G. Eberhart, NOTCH pathway blockade depletes CD133−positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts, Stem Cells 28 (1) (2010) 5–16.

[25] S.M. McAuliffe, S.L. Morgan, G.A. Wyant, L.T. Tran, K.W. Muto, Y.S. Chen, K.T. Chin, J.C. Partridge, B.B. Poole, K.H. Cheng, J. Daggett Jr, K. Cullen, E. Kantoff, K. Hasselbatt, J. Berkowitz, M.G. Muto, R.S. Berkowitz, J.C. Aster, U.A. Matulonis, D.M. Dinulescu, Targeting Notch, a key pathway for ovarian cancer stem cells, sensitizes tumors to platinum therapy, Proc. Natl. Acad. Sci. USA 109 (43) (2012) E2939–48.

[26] H. Mizugaki, J. Sakakibara-Konishi, Y. Ikezawa, J. Kikuchi, E. Kikuchi, S. Oizumi, T.P. Dang, M. Nishimura, Γ-Secretase inhibitor enhances antitumour effect of radiation in Notch-expressing lung cancer, Br. J. Cancer 106 (12) (2012) 1953–1959.

[27] S. Mueller, M. Schittenhelm, F. Honecker, E. Malenke, K. Lauber, S. Wesselborg, J.T. Hartmann, C. Bokemeyer, F. Mayer, Cell-cycle progression and response of germ cell tumors to cisplatin in vitro, Int. J. Oncol. 29 (2) (2006) 471–479.