Inhibition of Wnt Signaling Pathway Decreases Chemotherapy-resistant Side-population Colon Cancer Cells

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Abstract. *Background: The prognosis of advanced or recurrent colorectal cancer is still poor. Dye-effluxing side population (SP) colon cancer cells are reportedly resistant to chemotherapeutic agents. Most sporadic colorectal cancers involve constitutive activation of the Wnt signaling pathway. In this study, we examined the effect of the Wnt signaling on SP cells and the possibility that inhibition of Wnt signaling may decrease the resitance to chemotherapeutic drugs in the human colon cancer cells. Materials and Methods: Drug resistance of SP cells to 5-fluorouracil (5-FU) and irinotecan, decrease of SP cells by the Wnt signaling inhibition and activation of the Wnt signaling of the sorted SP cells were examined using the SW480 colon cancer cell line. mRNA expressions of ATP-binding cassette (ABC) transporters when Wnt signaling was inhibited were evaluated with real-time PCR using colon cancer cell lines (SW480, DLD-1, HCT116, HT29 and LOVO). The sensitivity to irinotecan and paclitaxel when the Wnt signaling was inhibited was investigated using SW480. Inhibition of Wnt signaling was performed by siRNA of β-catenin. Results: SP cells showed more resistance to 5-FU and irinotecan, and higher activation of the Wnt signaling pathway, than non-SP cells. Silencing of β-catenin decreased significantly more SP cells than non-SP cells. Expression of ABC transporter genes, such as ABCB1 and ABCG2, was significantly higher in SP cells than non-SP cells. Silencing of β-catenin decreased transcription of these ABC transporter genes; β-catenin-silenced cells became relatively sensitive to paclitaxel and irinotecan. Conclusion: These results indicate that inhibiting the Wnt signaling pathway may be a fruitful strategy for targeting chemotherapy-resistant colon cancer cells, including SP cells.*

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Colorectal cancer is one of the most common causes of cancer-related fatalities in the Western world (1) and its incidence is gradually increasing in Asia, including Japan. Treatment of advanced and metastatic cases is still poor, due to the response rates of to chemotherapeutic drugs being only 10–20% (2). It is therefore urgent that novel therapeutic strategies be developed for overcoming this low sensitivity of colorectal cancer to chemotherapeutic drugs.

Side population (SP) cells, which are robustly able to efflux fluorescent DNA-binding dye Hoechst 33342, were first identified in mouse bone marrow (3). Thereafter, SP cells have been identified in many tissues with high regenerative capacities. Recently, it has been also shown that SP cells exist in various types of tumors, including colorectal cancer (4, 5); and that ATP-binding cassette (ABC) transporter family members, such as ABCB1 (MDR1) and ABCG2 (BCRP1) contribute to the high Hoechst-dye effluxing capabilities of SP cells (6-8). Significantly, ABC transporters play an important role in effluxing other chemotherapeutic drugs (6, 9-13), which suggests that they are involved in drug resistance. For example, ABCB1 and ABCG2, involved in the efflux of paclitaxel and irinotecan respectively, are speculated to be candidates for adjuvant chemotherapeutic drugs for patients with advanced or metastatic colon cancer (12). Stem cell chemoresistance is thought to be provided through ABC transporters, and therefore these ABC transporters have been extensively studied in stem cells (14, 15).

The Wnt signaling pathway is evolutionarily conserved and controls many events during embryonic development. In the absence of Wnt signaling, β-catenin is contained within a large protein complex of axin and adenomatous polyposis coli (APC); β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK3β) and is subsequently degraded by ubiquitin-dependent proteolysis (16, 17). Following binding of Wnt to its frizzled receptor, GSK3β is inactivated, and

non-phosphorylated β-catenin is released from the complex (16, 17). Non-phosphorylated β-catenin accumulates within nuclei and forms complexes with the transcription factors T-cell factor (TCF) and lymphoid enhancer factor (LEF), resulting in transactivation of c-Myc and cyclin D1 (18, 19). Most sporadic colorectal tumors involve constitutive activation of the Wnt signaling pathway through mutations of *APC* or *β-catenin* (16, 20). For example, a human colon cancer cell line used in our study, SW480, has *APC* mutations (21). Interestingly, it has been shown that TCF/LEF activates the *MDR1* promoter (22, 23). Recently, two unrelated studies speculated that Wnt signaling affects MDR1 expression. One shows that β-catenin signaling may regulate P-glycoprotein expression in rat brain endothelial cells (24). The other shows that the frizzeled-1-mediated Wnt signaling pathway may contribute to chemoresistance of human neuroblastomas through MDR1 overexpression (25).

Materials and Methods

Cell culture and reagents. Human colon carcinoma cell lines SW480, DLD-1, HT29, HCT116 and Lovo were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in RPMI-1640 medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (FBS; Biological Industries, Israel), 100 U/ml penicillin and 100 μg/ml streptomycin (Meiji Seika, Tokyo, Japan) at 37˚C in 5% CO_2. 5-Fluorouracil (5-FU; Sigma-Aldrich, St. Louis, MO, USA), paclitaxel (Sigma-Aldrich) and SN-38 (active form of irinotecan, Tocris Bioscience, Avonmouth, UK) were used as chemotherapeutic drugs.

SP analysis and cell sorting. The protocol was based on that of Goodell *et al*. (3) with slight modification. Briefly, cells (1×10^6 cells/ml) were incubated in prewarmed phosphate buffered saline (PBS) supplemented with 2% heat-inactivated fetal calf serum (HICS) containing freshly added Hoechst 33342 (5 μg/ml final concentration) for 60 minutes at 37˚C with intermittent mixing. In some experiments, cells were incubated with Hoechst dye in the presence of verapamil (50 μmol/l). At the end of the incubation, cells were spun down at 4˚C and resuspended in ice-cold PBS. Propidium iodide (2 μg/ml final concentration) was added 5 minutes before fluorescence-activated cell sorting (FACS) analysis, allowing the discrimination of dead cells from live ones. Samples were analyzed on an EPICS ALTRA system (Beckman Coulter). The Hoechst dye was excited with a UV laser at 351-364 nm; its fluorescence was measured with a 515-nm side population filter (Hoechst blue) and a 608 FELP optical filter (Hoechst red). A 540 DSP filter was used to separate the emission wavelengths. SP cells were routinely sorted twice and reanalyzed for purity, which was typically >95%. Cells that simply passed through EPICS ALTRA without Hoechst dye are described as whole cells in this paper.

Real-time reverse transcription-PCR. To assess expression levels of *cMYC*, *CTNNB1*, *ABCB1* and *ABCG2*, total RNA from sorted cells was purified with an RNeasy Mini -Kit (Qiagen, Valencia, CA, USA) and extracted RNA was reverse-transcripted to cDNA with Quantitect Reverse Transcription Kit (Qiagen) according to the manufacturer's protocol. Reactions were performed using SYBR Premix EX Taq (Takara Bio, Otsu, Japan) on a DNA Engine Opticon 2 System (MJ Research, Waltham, MA, USA). Each reaction was performed in triplicate. All primer sets amplified fragments <200 bp long. Primer sequences were as follows: *18s ribosomal RNA* (*18s rRNA*), forward, 5'-GAT ATG CTC ATG TGG TGT TG-3', reverse, 5'-AAT CTT CTT CAG TCG CTC CA-3'; *cMYC*, forward, 5'-CAG CTG CTT AGA CGC TGG ATT-3', reverse, 5'-GTA GAA ATA CGG CTG CAC CGA-3'; *CTNNB1*, forward, 5'-CCC ACT GGC CTC TGA TAA AGG-3', reverse, 5'-ACG CAA AGG TGC ATG ATT TG-3'; *ABCB1*, forward, 5'-TGA CAT TTA TTC AAA GTT AAA AGC A-3', reverse, 5'-TAG ACA CTT TAT GCA AAC ATT TCA A-3'; and *ABCG2*, forward, 5'-GCC GTG GAA CTC TTT GTG GTA G-3', reverse, 5'-ACA GC AAG ATG CAA TGG TTG T-3'. The amount of each target gene in a given sample was normalized to the level of *18s rRNA* in that sample.

Immunoblotting. Whole-cell extraction was obtained by lysing cells with M-PER Reagents (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's instructions. Whole-cell extract (50 μg) was separated by electrophoresis on 12.5% SDS-polyacrylamide gel, and transferred to Protran nitrocellulose membranes (Dassel, Germany). Membranes were incubated with primary antibodies against β-catenin (1:1000; BD Biosciences Pharmingen) and α-tubulin (clone DM1A, 1:1000; Sigma-Aldrich) overnight at 4˚C. After washing in PBS, membranes were incubated with appropriate horseradish peroxidase conjugated secondary antibody (ECL Plus™ Western Blotting Reagent Pack, 1:5000; Amersham Biosciences, Piscataway, NJ, USA) at room temperature for one hour. Blots were developed with ECL Plus™ Western Blotting Detection System (Amersham Biosciences) and were visualized with a Molecular Imager FX (Bio-Rad, Hercules, CA, USA).

Luciferase assay. Cells in six-well plates were transfected with plasmids using TransFast Transfection Reagent™ (Promega) according to the manufacturer's instructions. Cells in each well were co-transfected with 500 ng pTOPFLASH or pFOPFLASH (kindly provided by Dr. Akihide Ryo, Yokohama City University, Kanagawa, Japan) and 10 ng pRL-SV40 (Promega, Madison, WI, USA) for 24 hours. Luciferase assays were performed with a Dual-Luciferase Assay System (Promega) according to the manufacturer's instructions. The luciferase activities were normalized to that of *Renilla* luciferase. Each experiment was carried out in triplicate. The activity of controls was set at 1.0.

Small interfering RNA (siRNA) against CTNNB1. Cells were transfected with 50 nM of *CTNNB1* siRNA (On-Target plus SMART Pool, L-003482; Dhramcon RNA Technologies Chicago, IL, USA) or non-targeting control siRNA (ON-TARGET plus siCONTROL Non-targeting pool, D-001810; Dharmacon), using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA). Forty-eight hours after the transfection, cells were harvested and were used for experiments.

Statistical analysis. Student's *t*-test was used for statistical analysis. *P*-values less than 0.05 were considered significant.

Results

SP cells are more resistant to chemotherapeutic drugs than non-SP cells. When the ratio of SP cells to total cells was calculated, the SP cell ratio in SW480 cells was 0.5-1.2%

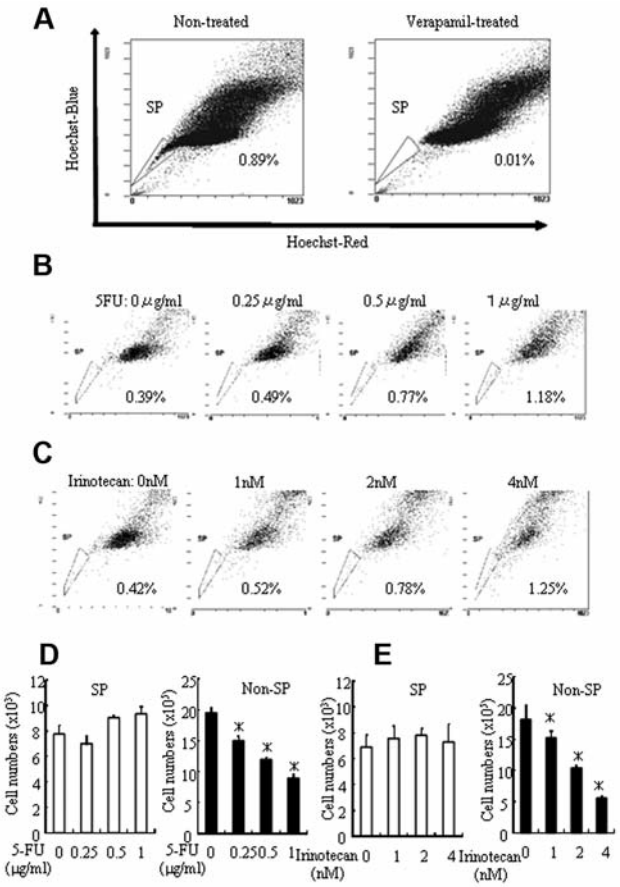

Figure 1. *SP cells are more resistant to chemotherapeutic drugs than non-SP cells. A: SW480 cells (1×10^6 cells/ml) were incubated in PBS/2% HICS containing freshly added Hoechst 33342 (5 μg/ml final concentration) in the presence or absence of verapamil (50 μmol/l) for 60 minutes at 37˚C with intermittent mixing. SP/Non-SP cell numbers in SW480 cells were determined by FACS. B, C: After the treatment of the chemotherapeutic drugs, 5-FU (B) or SN-38; irinotecan (C) at the indicated concentrations for 48 hours, the SP cell ratio in SW480 cells was analyzed by FACS. D, E: After treatment with chemotherapeutic drugs, 5-FU (D) or SN-38 (E) at the indicated concentrations for 48 hours, the number of SP cell and non-SP cells in SW480 cells was estimated using FACS. Bars show mean±SD. *p<0.05.*

(data not shown). In the present study, the SP cell ratio was 0.89% (Figure 1A). Since SP cells have a high dye-effluxing ability, we first investigated whether SP cells are more resistant to chemotherapeutic drugs than non-SP cells. The chemotherapeutic drugs, 5-FU and irinotecan were selected due to the fact that these drugs are commonly used for treatment of colon cancer. Both 5-FU and irinotecan were found to increase the SP/non-SP cell ratio in a dose-dependent manner (Figures 1B, 1C). The resulting decrease in the non-SP cell population was higher than that in the SP cell population (Figures 1D, 1E). These findings indicate that the increased SP/non-SP cell ratio is mainly the result of fewer non-SP cells, and that SP cells are more resistant to these drugs than non-SP cells.

Activation of Wnt signaling in SP cells is higher than in non-SP cells. A crucial contribution of the Wnt signaling pathway to colon cancer progression has been reported (26-29). Here, the activation status of Wnt signaling in SP cells and non-SP cells, collected separately with a cell sorter, was investigated. The activation status of Wnt signaling was estimated *via* the β-catenin/TCF responsive luciferase reporter activity and *cMYC* mRNA expression, a canonical target of Wnt signaling. Both luciferase activity (Figure 2A) and *cMYC* mRNA expression (Figure 2B) were higher in SP cells than in non-SP cells, indicating a higher activation of Wnt signaling in the SP cells. To exclude the possibility that the Hoechst-dye itself affects luciferase activity, whole SW480 cells were collected with a cell sorter without the addition of the Hoechst-dye. Hoechst-dye hardly affects luciferase activity in non-SP cells (Figure 2C).

Silencing β-catenin decreases SP cells more selectively than non-SP cells. Activation of the Wnt signaling of SP cells was found to be increased (Figure 2). It has been shown that Wnt signaling pathway plays an important role in the proliferation of colon cancer cells. Therefore, we investigated whether inhibition of Wnt signaling affects the proliferation of SP cells. In the present study, inhibition of Wnt signaling was performed by silencing *β-catenin* mRNA using RNA interference. Transfection with siRNA that targets β-catenin resulted in 90% or greater decrease in *β-catenin* (Figure 3A) and β-catenin protein (Figure 3B) expressions in SW480 cells. Silencing β-catenin decreased the SP/non-SP cell ratio (Figure 3C). When the numbers of SP *versus* non-SP cells were calculated separately, silencing *β-catenin* decreased SP cells more than non-SP cells (Figure 3D). These results indicate that the low SP/non-SP cell ratio was due to a decrease in SP cells, which suggests that the Wnt signaling pathway affects SP cells more than non-SP cells.

To directly confirm that silencing of *β-catenin* can decrease the numbers of SP cells, SP cells instead of whole cells were used as targets. The SP/non-SP cell ratio of SW480 used in this experiment was originally 0.58% (Figure 4A, left panel). The SP/non-SP cell ratio immediately after cell sorting became 92.3% (Figure 4A, right panel). However, non-SP cells rapidly begin to proliferate from SP cells, suggesting a high differentiation capability of the SP cells. As a result, the SP cell ratio remarkably decreases from 92.3% to 19.9% two days after the culture (Figure 4B, left panel). Silencing of *β-catenin* in SP cells further decreased the SP/non-SP cell ratio to 5.71 % (Figure 4B, right panel). In conclusion, we could not directly prove that the decrease in SP cells was caused by silencing *β-catenin*.

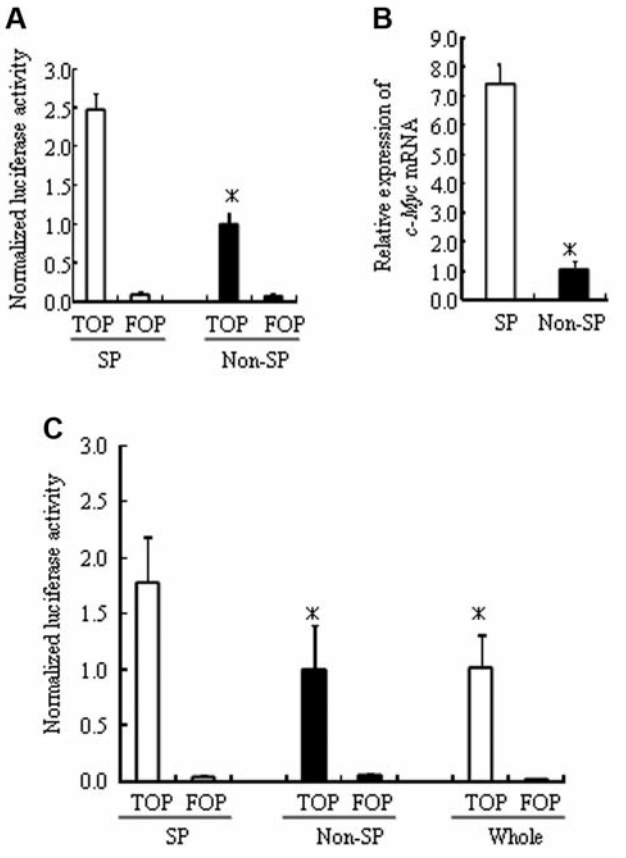

Figure 2. *Activation of Wnt signaling is higher in SP cells than non-SP cells. A: After SP cells and non-SP cells in SW480 cells were separately collected by cell sorting, activation status of Wnt signal in each populations was estimated by β-catenin/TCF responsive luciferase reporter activity. Luciferase activity was normalized to that of Renilla luciferase. B: cMYC mRNA expressions in sorted SP cells and non-SP cells were evaluated by real time RT-PCR. cMYC mRNA levels were normalized to 18s rRNA levels. Bars show mean±SD. C: The activation status of Wnt signaling in whole SW480 cells was analyzed by β-catenin/TCF responsive luciferase reporter activity and was compared with those in sorted SP cells and non-SP cells. Bars show mean±SD. *p<0.05.*

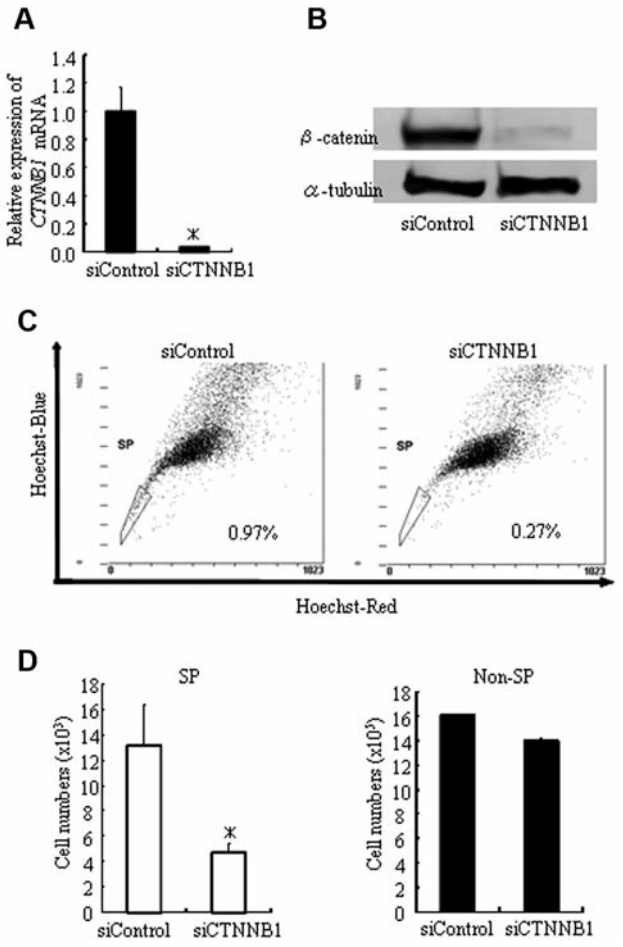

Figure 3. *Silencing β-catenin decreases SP cells more selectively than non-SP cells. A: β-Catenin (CTNNB1) mRNA expressions in SW480 cells transfected with control or β-catenin siRNA were evaluated by real-time RT-PCR. Bars show mean±SD. B: β-Catenin protein expressions in SW480 cells transfected with control or β-catenin siRNA was evaluated by Western blot analysis. α-Tubulin was used for loading control. C: After SW480 cells were transfected with control or β-catenin siRNA, SW480 cells were incubated in PBS/2% HICS containing freshly added Hoechst 33342 (5 μg/ml final concentration) for 60 minutes at 37˚C with intermittent mixing. SP cell ratio in SW480 cells transfected with control or β-catenin siRNA was evaluated by FACS. D: SW480 cells were transfected with control or β-catenin siRNA. Forty-eight hours after the transfection, the numbers of SP cells and non-SP cells in each transfected SW480 cells were estimated by FACS. Bars show mean±SD. *p<0.05.*

Silencing of β-catenin decreases mRNA expression of ABC transporters in SW480 cells. To speculate on the molecular mechanism by which silencing *β-catenin* induces the decrease of both SP/non-SP cell ratio and numbers of SP cells, we again analyzed in detail the flow cytometry of SW480 cells. Silencing of *β-catenin* slightly shifted the fluorescence intensity of Hoechst dye to the upper right (Figure 5A). This finding suggests that silencing of *β-catenin* induces the decrease of dye-efflux or the increase of dye-influx. Since an efflux of Hoechst dye is mainly regulated by ABC transporters, we examined whether silencing *β-catenin* affects transcription of ABC transporter genes *ABCB1* and *ABCG2*. *ABCB1* and *ABCG2* mRNA expressions were significantly higher in SP cells than in non-SP cells (Figure 5B). When *β-catenin* mRNA expression of SW480 cells was silenced by RNA interference, mRNA expression of these *ABC transporters* was decreased (Figure 5C). Decreased *ABCB1* mRNA expression was observed in 3 cell lines (DLD-1, HT-29, HCT116) out of 4 different colon cancer cell lines (Figure 5D, upper panel). However, a significant

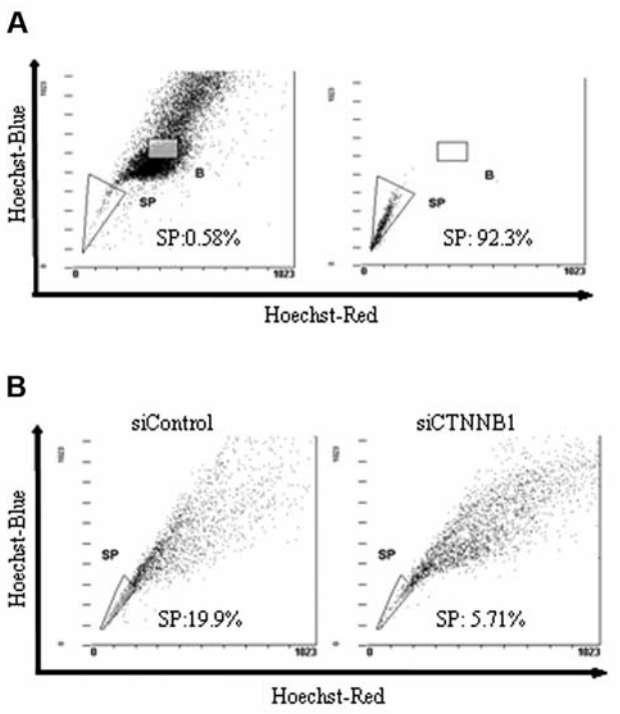

Figure 4. *The silencing of β-catenin did not directly decrease the numbers of SP cells. A: SW480 cells were incubated in PBS/2% HICS containing freshly added Hoechst 33342 (5 μg/ml final concentration) for 60 minutes at 37˚C with intermittent mixing. SP/non-SP cell ratio in SW480 cells (left panel) and SP/non-SP cell ratio right after the cell sorting (right panel) were analyzed by FACS. B: Sorted SP cells were transfected with control or β-catenin siRNA. Forty-eight hours after the transfection, cells were incubated in PBS/2% HICS containing freshly added Hoechst 33342 (5 μg/ml final concentration) for 60 minutes at 37˚C and SP cell ratio was estimated by FACS.*

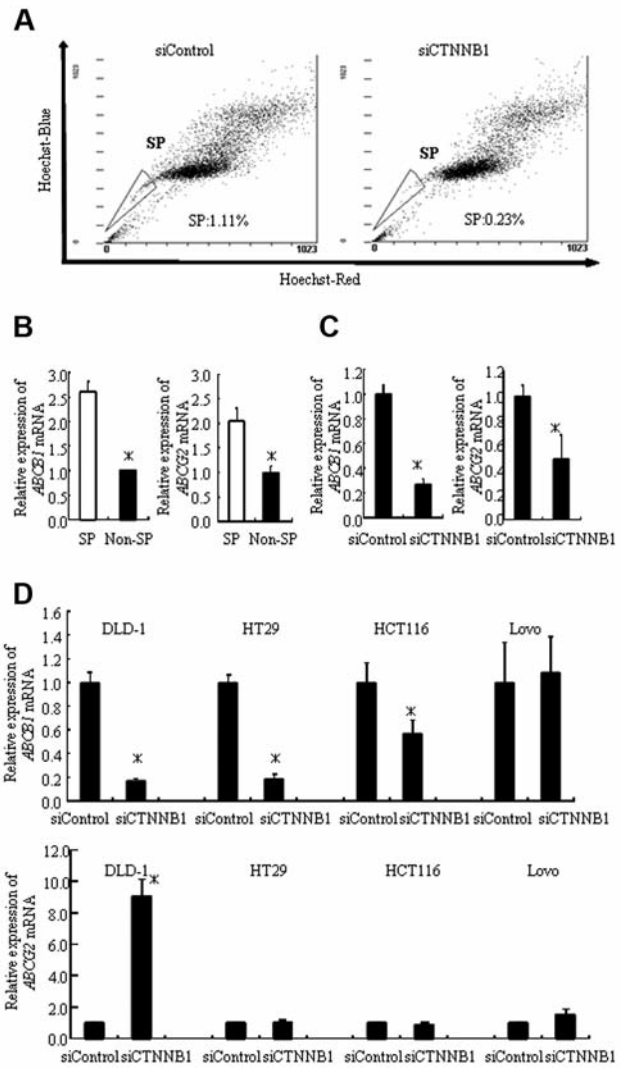

Figure 5. *Inhibition of β-catenin decreases mRNA expression of ABC transporters in SW480 cells. A: SW480 cells were transfected with control or β-catenin siRNA. Forty-eight hours after the transfection, cells were incubated in PBS/2% HICS containing freshly added Hoechst 33342 (5 μg/ml final concentration) for 60 minutes at 37˚C and SP cell ratio was estimated by FACS. B: After SP cells and non-SP cells in SW480 cells were separately collected by cell sorting, ABCB1 (left panel) and ABCG2 (right panel) mRNA expressions in sorted SP cells and non-SP cells were estimated by real-time RT-PCR. Bars show mean±SD. C: ABCB1 (left panel) and ABCG2 (right panel) mRNA expressions in SW480 cells transfected with control or β-catenin siRNA were evaluated by real time RT-PCR. Bars show mean±SD. D: ABCB1 (upper panel) and ABCG2 (lower panel) mRNA expressions in 4 other colon cancer cell lines transfected with control or β-catenin siRNA were evaluated by real time RT-PCR. Bars show mean±SD. *p<0.05.*

decrease in *ABCG2* mRNA expression was not evident in any of the 4 cell lines, while *ABCG2* mRNA expression in DLD-1 was significantly increased (Figure 5D, lower panel). These results indicate that Wnt signaling might partly regulate expression of ABC transporters, especially ABCB1, in colon cancer cells.

Silencing of β-catenin increases sensitivity of SW480 cells to chemotherapeutic drugs. ABC transporters play crucial roles in the efflux of several kinds of chemotherapeutic drugs, including paclitaxel and irinotecan. Here we examined whether silencing *β-catenin* increases chemo-sensitivity of SW480 cells to these drugs. As expected, silencing *β-catenin* increased the sensitivity of SW480 cells to these drugs (Figure 6).

Discussion

Our findings suggest that inhibition of *β-catenin* decreases chemotherapy-resistant SP cells of the SW480 colon cancer cell line. Summarizing the findings of the study presented here, Wnt signaling and *ABC* transporters (*ABCB1* and *ABCG2*) are more highly activated and expressed in SP cells than in non-SP cells. Silencing *β-catenin* decreases

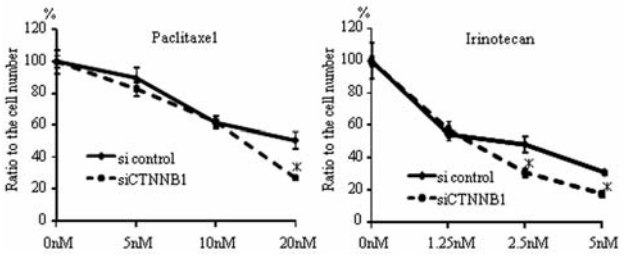

Figure 6. *Silencing of β-catenin increases sensitivity of SW480 cells to chemotherapeutic drugs. SW480 cells were transfected with control or β-catenin siRNA. Right after transfection, cells were co-cultured with the chemotherapeutic drugs, paclitaxel or irinotecan, at the indicated concentrations. Forty-eight hours after the incubation, cells were harvested and counted by the FACS counter. The ratio of live cells to the total cell number at 0 nM of paclitaxel or irinotecan was calculated, respectively. Bars show mean±SD. *p<0.05.*

transcription of these ABC transporters and increases the sensitivity of SW480 cells to paclitaxel and irinotecan. Our present data are consistent with previous studies, which show SP cells to be more resistant to chemotherapeutic drugs than non-SP cells, reported by other investigators using different types of tumors (6, 9-12).

Two decades ago, it was shown that overexpression of P-glycoprotein, the *MDR1* (*ABCB1*) gene product, results in the development of multidrug resistance (30, 31). Based on these findings, clinical trials have been performed using MDR1 inhibitors, but most of them have been unsuccessful (32). However, as it has been recently shown that SP cells exist in some types of tumors and exhibit characteristics, including drug resistance, of so-called stem cells (4, 33-35), we have again begun to pay attention to ABC transporters as therapeutic targets for overcoming drug resistance of cancer stem cells. In the present study, ABCB1 and ABCG2, and paclitaxel and irinotecan were selected as ABC transporters and chemotherapeutic drugs, respectively, because it has been shown that ABCB1 and ABCG2 relate to the efflux of paclitaxel and irinotecan, respectively (12, 36, 37). As speculated, ABCB1 and ABCG2 expression was higher in SP cells than in non-SP cells. Although our data suggest that overexpression of these ABC transporters contributes to drug resistance of SP cells to paclitaxel and irinotecan, the molecules that regulate the overexpression of these ABC transporters, are yet to be identified.

MDR1 has been suggested as a target gene of the TCF4/β-catenin complex (22). Therefore, we hypothesized that Wnt signaling regulates transcription of *ABC* transporters. As expected, silencing of *β-catenin* decreased transcription of both *ABCB1* and *ABCG2*, and increased drug sensitivity to paclitaxel and irinotecan, indicating that Wnt signaling in SW480 cells may regulate drug sensitivity to paclitaxel and irinotecan by controlling ABCB1 and ABCG2, respectively. To further confirm the possibility that Wnt signaling regulates ABC transporter expression, four different colon cancer cell lines were analyzed. Silencing of *β-catenin* significantly decreased transcription of *ABCB1* in 3 out of the 4 cell lines. This result suggests that Wnt signaling commonly regulates the transcription of *ABCB1* in colon cancer cells. On the other hand, silencing of *β-catenin* did not affect *ABCG2* expression in 3 of the 4 cell lines and increased expression in only one cell line. Therefore, it is unclear whether Wnt signaling also regulates transcription of *ABCG2* in colon cancer. Importantly, a link between Wnt/β-catenin signaling pathway and *ABCB1* transcription has recently been shown in other types of cells such as rat brain endothelial cells (24) and human neuroblastoma cells (25).

The finding that silencing *β-catenin* decreases numbers of SP cells raises an important question: Is the decrease in SP cells following the silencing of *β-catenin* a result of fewer SP cells, or a result of SP cells converting to non-SP cells by inhibition of *ABC* transporter transcription? It has been shown that SP cells can generate non-SP cells (33, 38). In this study, SP cells also rapidly generated non-SP cells after the initial culture. When purified SP cells were used as target cells, *β-catenin*-silenced SP cells seemed to generate non-SP cells more quickly than non-targeting siRNA-transfected SP cells. This phenomenon suggests another possibility: that silencing of *β-catenin* may enhance the differentiation of SP cells to non-SP cells. Therefore a conclusive answer cannot be given.

As mentioned above, most clinical trials using MDR1 inhibitors have been unsuccessful (12). Therefore, new therapeutic strategies that reverse or prevent drug resistance need to be developed. siRNAs, generated from double-stranded RNA, can silence homologous gene expression by inducing degradation of the complementary mRNA (39). Reportedly, expression of both endogenous and transfected *MDR1* genes can be effectively inhibited using RNA interference approach (40). In conclusion, inhibition of Wnt signaling or ABC transporters by RNA interference may be a valuable therapeutic strategy for overcoming drug resistance in colorectal cancer.

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Conflicts of Interest Statement

The Authors declare that they have no competing financial interests.

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